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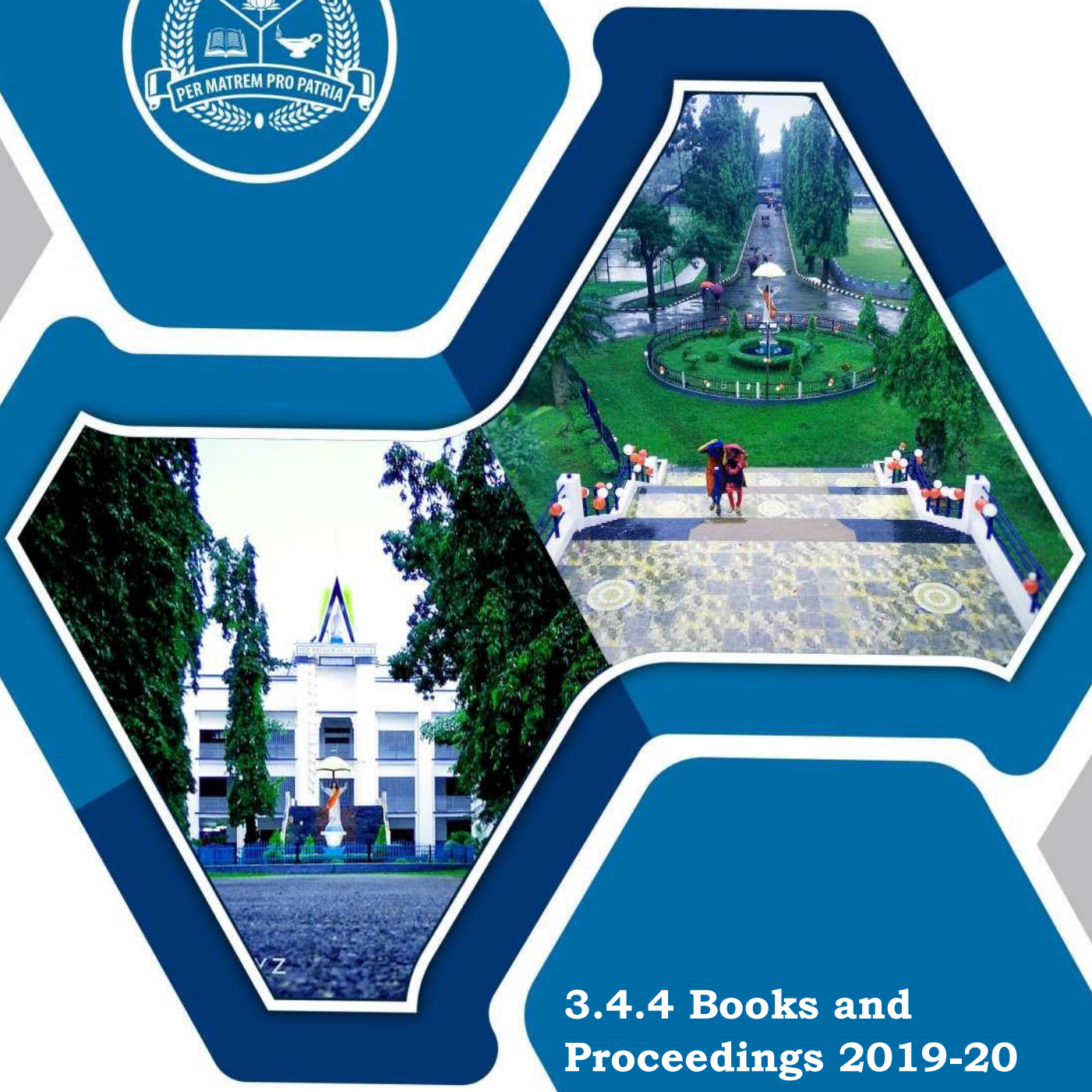

3.4.4 Books and Proceedings 2019-20

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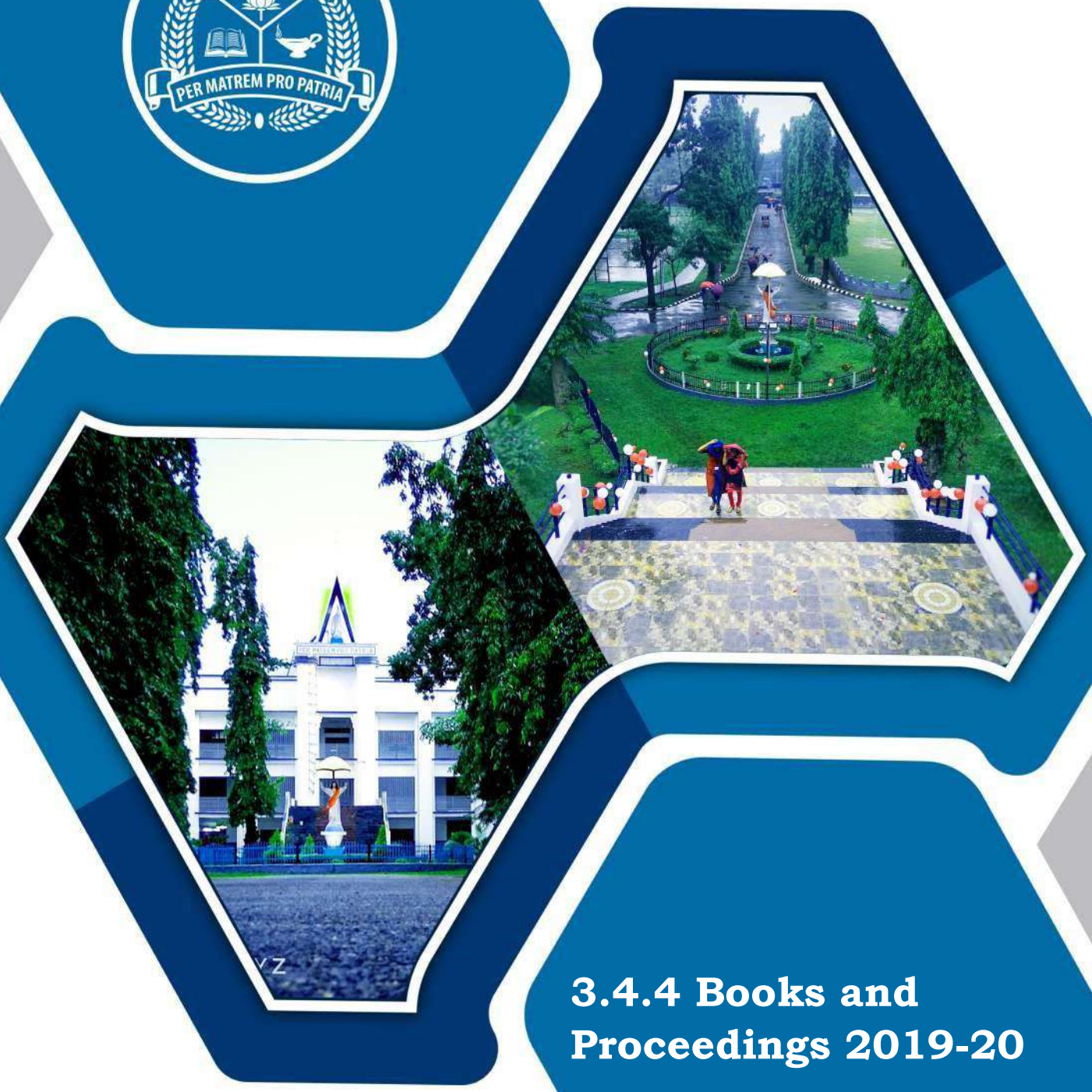

3.4.4 Books and Proceedings 2019-20

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Proceedings of the International Seminar
on
BLUE GROWTH INITIATIVE: SUSTAINABLE FISHERY DEVELOPMENT STRATEGIES AND ADVANCED TECHNOLOGIES FOR AQUACULTURE (BISFAA)

7th - 9th AUGUST, 2019

Organized
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PG & Research Department Of Zoology
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Fatima Mata National College (Autonomous), Kollam, Kerala, India

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PROCEEDINGS OF THE INTERNATIONAL SEMINAR ON

BLUE GROWTH INITIATIVE: SUSTAINABLE FISHERY DEVELOPMENT STRATEGIES AND ADVANCED TECHNOLOGIES FOR AQUACULTURE (BISFAA 2019)

August 7th -9th, 2019

Editors

Dr Sherly Williams E
Prof. Nisha Thomas P
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Dr Vijayasree AS
Mrs. Lekshmi Priya V

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OUR PATRON'S MESSAGE

"The humble and persevering investigator of the secrets of nature is being led, as it were, by the hand of God in spite of himself, for it is God, the conserver of all things, who made them what they are." (Saint Charles Borromeo)

It's my privilege to felicitate all the members of the Zoology department of FMNC for conducting an International Seminar on "Blue Growth Initiative: Sustainable Fishery Development Strategies and Advanced Technologies for Aquaculture."

"We must plant the sea and herd its animals using the sea as farmers instead of hunters. That is what civilization is all about farming replacing hunting" (Jacques-Yves Cousteau). As we progress in our day to day life, our needs are also increasing. In order to meet the vast and multifarious needs of our society there must be a serious and planned change in our culture of consumption. The studies regarding the how of supplying the demands for sea foods and other products must lead us to probe how to protect and conserve the sea, lake, river and the life in them and how to develop the techniques to harvest the fruits of such activity for providing better human life.

The fisheries sector in particular makes a crucial and growing contribution to food nutrition and livelihood security. I understand more people than ever before rely on fisheries and aquaculture for food and income. Even if there are significant successes, there is a decreasing overall trend in the proportion and strategies of aquaculture. In this context, let us draw out some valuable ideas out of this seminar to form apposite aquaculture without affecting our environment.

God to bless you all abundantly with his graces.

✠ H.E Rt. Rev. Dr Paul Antony Mullassery,
Bishop of Quilon

OUR MANAGER'S MESSAGE

His Holiness Pope Francis in his Message for the World Day of Prayer for the Care of Creation (1 September 2018) expressed his optimism in safeguarding the ecosystems: "It is our duty to thank the Creator for the impressive and marvellous gift of the great waters and all that they contain (cf. Gen 1:20-21; Ps 146:6), and to praise him for covering the earth with the oceans (cf. Ps 104:6) … Let us pray that waters may not be a sign of separation between peoples, but of encounter for the human community. Let us pray that those who risk their lives at sea in search of a better future may be kept safe … Let us pray too, for all those who devote themselves to the apostolate of the sea, for those who help reflect on the issues involving maritime ecosystems, for those who contribute to the development and application of international regulations on the seas in order to safeguard individuals, countries, goods, natural resources…"

Servant of God Bishop Jerome M. Fernandez, our founder, in instituting Fatima Mata National College, had the dream of uplifting the posterity of fisher folk of the Kollam coast through education. Our college is always in this pursuit, while expanding and extending the possibilities to others as well. The Department of Zoology should always have the social commitment in this line. Decline in fish catch and depletion of natural resources due to over exploitation of coastal fisheries, unscientific management of aquaculture, increase in the number of endangered species and various other factors affect the life of the population depending solely on the oceanic certainties and uncertainties.

The International Seminar on Sustainable Fishery Development Strategies, in continuation of last year's academic initiative on Blue Economy, I hope, will definitely help the participants to explore strategies beneficial also to the common man and fisherfolk.

God bless everyone connected to this great initiative.

Rev. Dr Rolden Jose Jacob
Manager

FOREWORD

Oceans, Seas, Lakes and Water bodies are inevitable for life on earth. The blue frontiers open vast expanse of improved human well being, constitute key resources for global tourism, open vast potential for renewable blue energy and impact economy so much so that we even speak of blue economy. Marine ecosystems give a third of the oxygen we breathe, form a treasure trove of protein and pharmaceutical products, provide aesthetic and visionary feasts, and sustain the flora and fauna in one way or other.

The term "Blue growth" signifies a sustainable approach to the use of these natural resources with the focus on reducing environmental hazards and ecological balance. It is a long term strategy to support growth in the marine sector as whole.

The ever increasing pressure exerted on the coastal and marine ecosystems have led to irreversible degradation of the water bodies, unbearable and unforeseen climatic changes, and ecological imbalances. Major threats to blue ecosystems include overfishing, coastal development, global climate change, invasive species, and pollution.

It is high time that we address the issue in sync with blue biotechnology to prevent extinction of life on earth. Marine based growth should be founded on the principle of eliminating waste, controlling pollution and guaranteeing efficient use of resources. Several measures have been adopted and agreements signed including Deep Sea Mining Agreement, Fish Stocks Agreement. Marine protected areas have been earmarked and National Biodiversity Targets are on the anvil. So far so good, but much has to be done and done with urgency to make the world safer and better.

The International Seminar "Blue Growth Initiative: Sustainable Fishery Development Strategies and Advanced Technologies For Aquaculture" organized by the PG and Research Department of Zoology, Fatima Mata National College (Autonomous), Kollam is an effort in the area to bring together various stakeholders including academicians, scientists, students and responsible members of society for ensuring sustainable blue growth. I hope this seminar would provide a platform for a take off to a better and safer planet.

Congrats and regards for the great initiative and academic exercise.
Dr Vincent B Netto,
Principal.

PREFACE

Fatima Mata National College (Autonomous), Kollam provides value added quality education to students by giving special emphasis to research activities. It supports all aspects of student life and encourages their academic, sporting, cultural and spiritual pursuits. The college also provides a stimulating, safe and supportive environment in which students aim for success. The Postgraduate Department of Zoology, offering specialization in 'Fisheries and Aquaculture', frequently conducts National / International seminars on topics of relevance.

It is a matter of pride to organize an international seminar on 'Blue growth initiative: Sustainable fishery development strategies and advanced technologies for aquaculture', at Fatima Mata National College, Kollam during 7^{th} – 9^{th} August 2019. The aim of the seminar is to disseminate knowledge on sustainable fish capture and efficient breeding techniques which will lead to faster growth in fish population. The topic of the seminar is most relevant in today's context and it is indeed relevant and appropriate to revitalize the time-tested wisdom to the present day need of the society. The deliberations of the seminar will certainly go a long way in helping the student, faculty, as well as scientific community and contribute to the rapid socio- economic development of the nation.

I hope that the interactions in the sessions with special emphasis on sustainable fisheries will promote and nurture scientific education, research, technological developments and popularize the research findings/innovations. It will provide a platform for young researchers, students, and faculties to interact with senior scientists in their respective areas. On behalf of the organizing committee, I appeal to all the participants and budding scientists to utilize this platform for initiating steps to the advancements in the field of Fisheries and Aquaculture. I wish the seminar a grand success.

Dr. Sherly Williams E

Convenor
Dean, Faculty of Science & HOD Department of Zoology

From the Editors Desk.....

Marine ecosystems have served as a cradle of evolutionary life since their inception. These indispensable creations of nature have also provided nutritious and adequate sustenance for all of its interdependent inhabitants. Equally as important, marine ecosystems have also generated valuable sources of food for mankind who have recently come to rely upon wild fish and other marine resources for much more than mere sustenance.

Despite various efforts to sustainably manage marine resources, more than 75% of the world's fish stocks are now fully exploited, overexploited or depleted according to the United Nations Food and Agriculture Organisation. Long-standing fisheries management policies have routinely neglected decades of scientific recommendations to refrain from overfishing, and they have failed to recognise the value of marine habitats which are indispensable to the production of marine food resources but have been severely damaged by certain types of fishing gear such as bottom trawlers.

A conventional idea of a sustainable fishery is that it is one that is harvested at a sustainable rate, where the fish population does not decline over time because of fishing practices. Sustainability in fisheries combines theoretical disciplines, such as the population dynamics of fisheries, with practical strategies, such as avoiding overfishing through techniques such as individual fishing quotas, curtailing destructive and illegal fishing practices by lobbying for appropriate law and policy, setting up protected areas, restoring collapsed fisheries, incorporating all externalities involved in harvesting marine ecosystems into fishery economics, educating stakeholders and the wider public, and developing independent certification programs.

We hope that the outcome of the deliberations emerging during the three-day International Conference on "Blue Growth Initiative: Sustainable Fishery Development Strategies and Advanced Technologies For Aquaculture" will further enrich the beneficiaries of the seminar and steer their attention towards the development of techniques and practices in enhancing the fish fauna of our water bodies.

Prof. Nisha Thomas P
Organizing Secretary & Chief Editor

CONTENTS

ENVIRONMENTAL IMPACTS OF AQUACULTURE AND DEVELOPMENT OF NEW AQUACULTURE SYSTEMS

V.R. Prakasam, Professor, Mekelle University, Ethiopia
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ABSTRACT

Aquaculture is the culture of aquatic organisms which includes fish, mollusks, crustaceans, algae and plants It, also known as aqua farming , involves cultivation of fresh water and salt water populations under controlled conditions. When it applies to fish it is called fish farming. Worldwide the most important fish species used in fish farming are, in order, carp, salmon, tilapia and catfish. It is the fastest growing food producing sector and now accounts for 50%of the world's fish that is used as food. The global aquaculture production was 79 million tones, 1950-2010, as reported by FAO with China, Indonesia and India leading, in order, in terms of production. Based on its dynamic performance over the last few decades, and with fairly stable catches from capture fisheries, it is likely that the future growth of the fisheries sector will come mainly from aquaculture.

Aquaculture has given rise to a number of environmental problems. The environmental impacts are seen in, for example, increased soil salinity, reduced agricultural production, decreased live stock production and destruction of mangrove forests. Aquaculture has also caused negative impacts on biodiversity through destruction of trees, grass and crabs (biodiversity loss) in the area of production. Environmental problems are also observed in terms of displacement of wild population, genetic impacts(loss of genetic diversity),parasites and diseases, effects on wild life, aquaculture waste, chemicals/pesticides and antibiotics (water pollution), fish feeds and feed conversion ratio. Besides, aquaculture has impacts on human health. The human impacts of farmed Salmon, for example, are due to high fat level, existence of various contaminants and use of antibiotics. In spite of all, there are also positive environmental effects associated with increase in aquaculture production, the most important being increased food production making healthy and affordable food available to more people at lower prices, in addition to reducing fishing effort and pressure on wild life stocks. Besides environmental issues, the present aquaculture system faces many challenges, mainly in water quality management, harmful diseases and epizootics, development of appropriate feeds and feeding practices, hatchery as well as grows -out technologies. These provide considerable scope for the development of new aquaculture systems. Some of the eco-friendly developments for enhancing aquaculture production are integrated farming, integrated multi-trophic aquaculture, aquaponics, recirculation aquaculture system, mono sex culture, neo female technology, bio floc technology and compensatory growth technology. Other new developments are in areas of culture based capture fisheries, sea ranching, capture based aquaculture, biotechnology and genetics based aquaculture, ultraviolet system for disinfection and so on. Based on these developments discussed in the paper, it is concluded that the closed system of aquaculture production, which is possible with most species, can be environmentally sustainable.

ARID LAND AQUACULTURE PRACTICE IN SAUDI ARABIA

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The Kingdom of Saudi Arabia on the Arabian Peninsula is surrounded on three sides by water, with the Arabian Sea to the southeast, the Red Sea to the west, and the Persian Gulf to the east. The nation's lengthy coastline and proximity to water moderates portions of the climate, but most of the country is hot and arid, with summer temperatures reaching 46°C (115°F), average rainfall of only 12 cm per year and water shortages and desertification are pressing environmental problems[1,]. Significant seasonal and even diurnal fluctuations in temperature result in extreme climatic conditions in many areas of the country[2]. Aquaculture development in Saudi Arabia began in 1980, with the establishment of the Fish Culture Project at the Saudi Arabian National Centre for Science and Technology (now called the King Abdulaziz City for Science and Technology) in Riyadh. Currently there are 125 aquaculture farms in Saudi Arabia, 56 of which are fully operational, producing marketable tilapia and shrimp. There are two large freshwater farms, in Qassim and in Dammam and several large shrimp farms on the Red Sea coast [3]. World's largest fully integrated aquaculture project, National Aquaculture Group (Naqua) situated at the Red Sea coast produces Pacific white shrimp, *Litopenaeus vannamei* by following quality and environmentally sustainable methods [4]. Over the past few years, special attention has been directed at boosting commercial aquaculture production and government has been encouraging the aquaculture projects and also supporting the aquaculture project investors by providing free land, interest-free loan for 10 years, and subsidies for fish farming equipments and instruments.

Strategy for sustainable aquaculture production

In order to achieve goals in sustainable aquaculture practice, following strategies are taken in coastal deserts.

1. Integrated approach

Limited water is the biggest constraint for aquaculture in arid/semi-arid regions. Before taking an approach to desert aquaculture, we should identify sites where aquaculture and agriculture can be integrated [6,7]. We should evaluate local environmental conditions including water quality and quantity, soil condition, topography, and climate. In arid regions, aquaculture will need modifications to become a promising production system according to each environmental situation. Aquaculture practices should be based on a balanced ecosystem management approach, the basic premise is to incorporate the biological and environmental functions of a diverse group of organisms into a unified system. Good management is often considered to be the same as practical experience in the application of aquaculture technologies in the field. Proper and timely maintenance of the farm and its installations, successful methods of brood stock management, selective breeding, seed production, pond preparation, stocking, feed and feeding, water quality management, disease control by biosecurity measures, harvesting, processing and marketing are the major elements of this concept of management. For the purpose of increasing production, an integrated approach would be ideal by connecting the operations viz., brood stocks, hatchery, nursery, grow out, feed production and processing phase.

2. Brood stock and Hatchery Management

The foundation of a successful hatchery is the use of high quality disease free brood stock. In order to minimize stress, damage, mortality and infection of brood stock with pathogenic diseases, the collection, holding, preparation, transportation, maturation and spawning of brood stock should be done as carefully and efficiently as possible [8,9]. Hatcheries should be designed to ensure biosecurity, efficiency, cost- effectiveness and should implement good management practices aimed at producing high quality seeds. The success of seed production depends to a large extent on the quality of brood stock selected for maturation [10]. Every effort should be made to ensure that only large, productive, healthy, disease-free animals are selected. Each brood stock should be checked to ensure that it is free from disease (Viral, bacterial and fungal etc.). Brood stock must be maintained, spawned and hatched individually so that any infected brood stock cannot infect the others in the facility [11]. To optimize water quality and reduce disease and stress levels for the growing larvae, it is important to stock the correct number of larvae and exchange sufficient water to maintain optimum water quality conditions throughout the larval rearing process [12].

Routine assessments of shrimp health are important to ensure that any potential problems are recognized early and solutions employed to rectify the underlying causes and thereby increase productivity [13]. Larval growth and survival and the water quality of the larval rearing tanks depend to a large extent on the quality and quantity of food offered to the larvae. Optimization of feeding regimes based on live feeds helps maintain good water quality, whilst promoting fast growth and high survival of the larvae and hence optimal production from the hatchery. Specific *Artemia* egg hatching procedures should be used to obtain the highest number of *Artemia* nauplii from each can of cysts hatched. These techniques are necessary to produce clean *Artemia* for feeding the larvae at the lowest possible cost. For sustainable production, antibiotics should not be used in hatchery. The use of effective probiotics is recommended than using antibiotics [14,15]. Implementing standard operating procedures is advisable for sustainable production of juveniles in hatchery.

3. Grow out operation

Grow out requires large amounts of clean water to support the farmed animals, replenish oxygen and remove wastes [16]. The culture ponds especially in areas of poor (sandy/loam) soils or high temperatures, evaporation and seepage is increased and as much as 1-3% of the pond volume may be lost in this way each day [17,18]. The site chosen should take into consideration tidal amplitude in order to utilize tidal energy for water exchange or harvesting. The grow-out ponds must be of simple design, rectangular in shape with size about 1-10 hectare, and depth 1.2 -1.7 meters. Each pond must have its own inlet and outlet gates to facilitate water exchange, pond preparation and harvesting [19,20]. Two weeks before stocking, the pond must be thoroughly drained and sun-dried until the mud in the pond bottom cracks. In the meantime, both the inlet and outlet gates are to be enclosed with a layer of fine nylon netting of mesh size 0.5 mm to prevent escape of fry and entry of predators or other undesirable aquatic organisms. The purpose of water exchange in addition to maintaining water quality is also to stimulate molting of the animals, resulting in acceleration of growth [21]. However, this procedure also drains out some of the natural food and reduces pond fertility. Hence, in order to maintain natural productivity, the pond water is regularly enriched with organic manure after the last day of water exchange. Supplementary feed shall be given only after 60 days of culture. Formulated pellet feed with 35-38% crude protein may be used to feed the growing juveniles in the pond. Feeding strategy should be based on the higher food intake after molting [22]. Natural food production can be maintained through continuously fertilizing the pond. This would helps to minimize the use of supplementary feed. The feeding rate on the third month may be 6% biomass and 4% from the fourth month until harvest. Efficient feeds improve performance, reduce production costs, improve water quality and reduce use of scarce marine proteins [23]

4. Harvest and shrimp processes

Harvest machine with drain harvest collector, specially designed pump which can transfer live shrimp with no damage, dewatering tower to separate shrimp from pond water, and insulated totes with ice water to receive shrimp is ideal [24]. Mechanical harvesting transfers live shrimp from the pond drain to insulated totes filled with ice water in a matter of seconds. This chill-kill process assures that top freshness and quality is delivered to the processing plant. Transfer of harvested shrimp from ice water totes directly into ice water wash tank at processing plant [25,26]. Ideally, processing plants are to be located near the ponds such that shrimp/fish can be received within minutes of harvesting. Use of the mechanical harvesting systems and ice water totes eliminate all handling until the product enters the plant. Mechanical grading within the plant further minimizes handling, temperature increase, and product deterioration. This assures premium freshness and quality for discriminating markets that demand optimum flavor and texture.

5. Data management and Marketing

A comprehensive database would help to store daily operating parameters of breeding facility, grow out, feed mill, diagnostic lab, hatchery and processing plant. The database allows trace back of each processed lot to the pond, raceway, nursery tank, larval tank, spawner, and breeder from which it originated. It also tracks water treatments, feed raw materials, feed production, harvest and stock of products. This minimizes risk of operation and allows complete traceability [27]. Product needs to be sold directly to long term clients according to predetermined specifications and prices. Long term relationships with premium buyers allow both the supplier and the buyer to negotiate reasonable prices, target preferred product sizes and forms and work toward mutually acceptable goals.

6. Biosecurity Umbrella

In aquaculture, biosecurity can be defined as “an essential group of tools for the prevention, control, and eradication of infectious disease and the preservation of human, animal, and environmental health” [28]. Therefore, excluding infectious agents and reducing stress are important in preventing disease outbreaks. Biosecurity can be applied to aquaculture production systems through a variety of management strategies and by following internationally agreed upon policies and guidelines. In addition, there are a variety of risk assessments that can be used for aquatic animal diseases of finfish, molluscs, and crustaceans [29]. The key elements of biosecurity can be summarized as reliable sources of stock, adequate diagnostic and detection methods for excludable diseases, disinfection and pathogen eradication methods, best management practices, and practical and acceptable legislation. Nevertheless, it is almost impossible to determine the economic benefits of a biosecurity program if there is no disease outbreak and aquaculture producers may be reluctant to adopt biosecurity measures that appear to be an additional cost. A disease outbreak in one area, however, in addition to its economic consequences in that area, may cause unintended consequences in other parts of the world. Early detection of a pathogen incursion into a farm system allows for more effective control of the establishment or spread of a pathogen for more effective response. Surveillance to detect incursions can be either targeted to specific pathogens or more general [30]. For example, for pathogens of particular concern and for which there are diagnostics tests available, animals can be periodically tested, ensuring the number of animals sampled are enough to provide an acceptable level of confidence in detecting the agent were it to be present. The health status of farm animals can also be monitored more generally through gross and microscopic examination including histology. Both targeted and general surveillance requires a degree of specialised expertise, although much can be done with minimal training. An effective disease response plan has three key aspects like surveillance/monitoring, containment and eradication. Each of these is associated various activities aimed at eliminating the pathogen of concern from the system.

7. Environment conservation and waste management

The rapid development of intensive fed aquaculture (finfish and shrimp) is associated with concerns about the environmental impacts of such often mono specific practices, especially where activities are highly geographically concentrated or located in suboptimal sites whose assimilative capacity is poorly understood and, consequently prone to being exceeded [31]. One of the main environmental issues is the direct discharge of significant nutrient loads into coastal waters from open-water systems and with the effluents from land-based *sys*tems [32]. In its search for best management practices, the aquaculture industry should develop innovative and responsible practices that optimize its efficiency and create diversification, while ensuring the remediation of the consequences of its activities to maintain the health of coastal waters. To avoid pronounced shifts in coastal processes, conversion, not dilution, is a common-sense solution, used for centuries in Asian countries. By integrating fed aquaculture (finfish, shrimp) with inorganic and organic extractive aquaculture (seaweed and shelfish), the wastes of one resource user become a resource (fertilizer or food) for the others [33]. Such a balanced ecosystem approach provides nutrient bioremediation capability, mutual benefits to the cocultured organisms, economic diversification by producing other value-added marine crops, and increased profitability per cultivation unit for the aquaculture industry [34]. Moreover, as guidelines and regulations on aquaculture effluents are forthcoming in several countries, using appropriately selected seaweeds, molluscs and sea cucumbers as renewable biological nutrient scrubbers represents a cost-effective means for reaching compliance by reducing the internalization of the total environmental costs [35]. (Chopin et al., 2001). Performance of treatment systems for farm discharge water may be improved by incorporating active nutrient removal strategies such as planting of mangrove forests, culture of bivalves, macroalgae, fish and nitrifying bacteria [36-40].

One of the most difficult tasks of resource managers and policy advisors is understands the assimilative capacity of coastal ecosystems under cumulative pressure as competing anthropogenic activities increase in the coastal zone (sewage effluents, urban/ rural effluents, precipitation, agricultural/industrial runoffs, aquaculture, etc.). Most impact studies on aquaculture operations have typically focused on organic matter/sludge deposition because they are easily noticeable and measurable[41]. Inorganic effluents, such as nitrogen and phosphorus, which are neither visible nor easily measured, have generally received much less attention because of the common human attitude of "out of sight, out of mind." Moreover, it is difficult to measure small long-term changes, and past studies, focusing on local measurements, have often failed to document dispersal patterns of dissolved nutrient fractions. The inorganic output of aquaculture is emerging as a pressing issue as nutrification of coastal water is a worldwide phenomenon [42-44].

Impact of arid land aquaculture

Although aquaculture is considered to be the solution for the declining fish stocks in the kingdom, its ecological impacts are posing threats to the sustainability of environment [45-47]. These impacts have been mainly associated with discharge of suspended solids, nutrient and organic enrichment of recipient waters resulting in the build-up of anoxic sediments, changes in benthic communities of Red Sea. For example, large-scale shrimp culture has resulted in physical degradation of coastal habitats through conversion of mangrove forests and destruction of wetlands [48]. These farms are not helping to reduce poverty levels of the coastal zones as the income directly goes to elite shrimp farmers. Evidence shows that in many parts of the world, these shrimp farms cause high salinity levels in agricultural and drinking water supplies [49]. Most of the local poor agricultural farmers who have no way to avoid polluted drinking water from ground water sources are further affected [48]. The damage to mangrove forests in the coastal zones will have a chain effect, not only on available fish, but also damaging the sustainable coastal ecosystem through alteration of seabed fauna and flora communities [50]. Further, misapplication of husbandry and disease management chemicals, collection of

seed from the wild and use of fishery resources as feed inputs are also causing concern in the coastal zones [51].

Aquaculture, like any other food producing sector, uses natural resources and interacts with the environment. Aquaculture is increasingly confronted with issues of environmental protection, compared to other sectors 47. However, aquaculture is considered to be the only way to satisfy the increasing demand of fish products in a situation where ocean fish stocks are deteriorating at an alarming rate 52. The challenge for the next decade is to produce more fish in aquaculture with increasing efficiency in resource use and minimising adverse environmental interactions. This will be the major goal in aquaculture development which will require commitment and willingness to collaborate by all those involved. Much of the current controversy is centred on resultant environmental degradation, in some cases, from inadequate coordination and management of development. Methods are needed to measure irresponsible aquaculture practices and to avoid negative externalities of aquaculture production which is the key to aquaculture sustainability53. Environmental degradation is causally linked to problems of poverty, hunger, gender inequality and health. Protecting and managing the natural resource base is essential for economic and social development. Similarly, the changing consumption and production patterns, particularly in wealthy nations, are directly linked to the environment 54. Integrating the principles and practices of environmental sustainability into country policies and planning programmes is therefore the key to success.

Arid land or desert aquaculture has been able to play a key role in transforming the economies of the Kingdom. Apart from meeting the increasing demands for sea food, this method has been able to contribute towards some social benefits as well. A lot of job opportunities have been created and the dietary needs of the people have also been taken care of due to this development. Due to the large amount of production, shrimp and fish is becoming more and more affordable and are within the reach of the common masses. The aquaculture-agriculture system in the desert produces quality sea food all the year round. Apart from the social benefits, there are a lot of environmental benefits which are being offered such as utilization of barren coastal deserts, conversion of sabha areas to productive agriculture lands and minimize the disease spread due to extremes of climatic factors. There has been a considerable amount of decrease in the pressure on wild fisheries. Desert aquaculture could prove to be less harmful to the eco system than a few other traditional fishing techniques and is therefore a significant source in combating poverty in rural areas of coastal deserts.

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CHALLENGES IN BRACHYURAN LARVAL CULTURE

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ABSTRACT

Brachyuran larvae are cultured for taxonomic studies and commercial purposes. Taxonomy of brachyuran larvae is the first step in validating a taxon and understanding the phylogenetic relationships of brachyurans. In India, mud crabs (Scylla sp.) and swimming crabs belonging to the genus Portunus are mainly reared commercially. Rearing of brachyuran crab larvae is a very challenging exercise as it requires the management of various abiotic factors, and controlling cannibalism, diseases, parasites, etc. Various methods to manage such challenges are widely discussed in this paper.

TOWARDS SUSTAINABLE MARINE FISHERIES: THE WAY FORWARD

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The concept of blue economy is gaining even further visibility and importance within the framework of the post-2015 Sustainable Development Goals (SDGs), in particular, Goal 2 (end hunger, achieve food security and improved nutrition, and promote sustainable agriculture) and Goal 14 (conserve and sustainably use the oceans, seas and marine resources for sustainable development). It signifies a strategy for utilization of marine resources by incorporating the principles of social inclusion, environment sustainability with innovative and dynamic business models, thus remaining as a catalyst for greening the blue growth. Marine fisheries is vital to the economies, food security and livelihoods of thousands of coastal populace in India. However, the sustainability issues related to marine fisheries management are often challenged by diverse political interests, increased thrust for coastal development and increasing population growth coupled with increasing pressure on marine fishery resources from o pressures from over-exploitation, habitat degradation, pollution and climate change. Ensuring productive and sustainable fisheries involves understanding the complex interactions between biology, environment, politics, management and governance.

Fisheries are faced with a range of challenges, and without robust and careful management in place, levels of anthropogenic disturbance on ecosystems and fisheries are likely to have a continuous negative impact on biodiversity and fish stocks. Ensuring productive and sustainable fisheries involves understanding the complex.

Increased awareness and social recognition of issues has led to the implementation as well as discussion on ecosystem-focused approaches to management, variously termed the Ecosystem Approach to Fisheries (EAF), Ecosystem-Based Fisheries Management (EBFM) or cross-sectoral Ecosystem-Based Management (EBM). The paper discusses the major challenges for fisheries management, which include overfishing, climate change, habitat destruction, pollution, ecosystem shift, ocean acidification, IUU fishing, coastal development and invasive alien species, and common tools in fisheries management such as seasonal closures, total allowable catch, minimum legal size, marine protected areas, ecosystem based fisheries management, closed seasons, mesh size regulations, individual transferable quota, bycatch reduction devices, and marine spatial planning. The paper discusses issues related to contemporary neoliberal policies in ocean governance and fisheries development, which prioritizes and recognizes only economic paradigms, keeping aside ecological sustainability.

MANGROVES AND THEIR ECOSYSTEM SERVICES

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ABSTRACT

Mangrove ecosystems are regarded as a multivalued ecosystem with remarkable biological productivity along the continental margins. This coastal ecosystem forms an ideal ecological asset that sustains a rich spectrum of floral and faunal community. In a broad sense, the importance of mangrove forest can be assessed by ecological sustainability (pollutant detoxification, sediment control, organic carbon flux, nutrient cycling), environmental security (climate mitigation, natural calamity mitigation) and economic prosperity (fishery and other goods, honey, firewood, medicines). Mangrove ecosystem forms the backbone of the coastal economy providing various benefits to coastal population particularly through fishery and aquaculture.

Based on the studies conducted by the authors, this contribution presents the distribution and diversity of mangroves of India. The services rendered by the habitats for sustainable productivity of the pelagic and benthic environments are also discussed. Indian mangrove forest harbours 38 true mangrove species out of total 73 species of the world. Twenty seven true mangrove species were identified from selected islands of Indian Sundarbans and 28 species from Andaman islands. The nutrient-rich alluvial soil along the deltaic coast facilitates higher diversity and richness of mangroves in east coast compared to west coast. The mangroves in Kerala comprised of 18 species of true mangroves of which *Ceriops tagal*, *Avicennia alba* and *Sonneratia alba* were very rarely seen. Phytoplankton in mangrove habitats was represented by 59 species under five classes namely

Bacillariophyceae, Chlorophyceae, Myxophyceae, Euglenophyceae and Dinophyceae of which Bacillariophycean members dominated (37 species).

The zooplankton fauna comprised of eleven groups of which calanoid copepods (62%) formed the dominant population, followed by crustacean nauplii (22%) and mysids (12%).The benthic fauna was represented by 48 species with maximum species richness (17 species) and density of crustaceans (55%) followed by polychaetes, molluscs and other groups such as oligochaetes, platyhelminthes, benthic fishes etc. The diversity and species richness in mangrove habitats have declined due to the human encroachment and pollution impacts.

The detritus driven mangrove ecosystem fulfills the nutritional requirements of fishes, shrimps and molluscs of economic value and regarded as world's most potential nursery. Studies from across the world, estimate mangroves contribution to fisheries in the range of 10–32 percent. The marine fish production in India was 3,443 thousand tonnes in 2013–14, which accounted for 36 percent of total fish production of which Gujarat and Kerala are the leading marine fish producers in the country. Mangrove-dependent fish catch was 1.86 tonnes per hectare per year that accounted for 23% compared to total marine fish catch in 2014 with a rupee value of Rs. 68 billion in India. West-coast regions produce a significantly higher proportion to mangrove fishery with maximum catch at Gujarat (59 %). Kerala recorded 32 % of fish catch with higher contribution of demersals such as nappers, catfishes, pomfrets and croakers followed by crustaceans and molluscs.

SUSTAINABLE SHRIMP FARMING AND SPECIES DIVERSIFIED AQUACULTURE

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Shrimp farming is an aquaculture practice that exists in either a marine or brackish water environment, producing shrimp for human consumption. Coastal aquaculture in India is practiced from the ancient times traditionally in the coastal tide fed lands. Scientific shrimp farming started in the 1980s and the species farmed in India is predominantly *P.monodon*. The production steadily increased upto the year 1994 and from 1995, stagnation due to disease outbreak. The introduction of new candidature species, specific pathogen free *L.vannamei* contributed majority of export quantity.

Disease problems have repeatedly impacted the shrimp production negatively and opened new way aqua farmers through diversification. Diversification is presented as an option for achieving that sustainable development in terms of species and systems. This would entail increasing the production of those species that are currently farmed in small quantities, or reducing the production of those that contribute most to production.

In order to boost the seafood export from Kerala, MPEDA Regional division, Kochi motivated the farmers and some of the abandoned brackish water areas in the state were renovated and started culture of *P.monodon* and *L. vannamei.* For popularizing the diversified aquaculture in Kerala, Regional division, Kochi conducted field level demonstrations on culture of export oriented species like Seabass, Mud Crab, Tilapia (GIFT) and Cobia.

RECENT TECHNOLOGICAL ADVANCEMENT IN DEEP SEA FISHING

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ABSTRACT

Advancement in technology has helped mankind in optimising the fish capture techniques over the years. Larger and faster boats with higher endurance have increased many folds the potential of deep sea fishing. Radars and Global Positioning Systems are vastly responsible for the efficient and accurate deep sea navigation of fishing vessels. Satellite Phones and Internet have been instrumental in efficient communication for deep sea fishing vessels. Remote Sensing Satellites have revolutionised deep sea fishing by identifying the Potential Fishing Zones. Drones, UAVs and Sonars are also being used to accurately pinpoint the Potential Fishing Zones and the fish species. These technological advancements have effectively resulted in larger fish capture at much lesser cost and time.

CHANGING SCENARIO OF FISH CATCH FROM RIVER GANGA AT PRAYAGRAJ: NEED FOR SUSTAINABLE FISHERY

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The change of fisheries scenario is a significant phenomenon drawing attention of fish biologists across the world. India is known to have a rich source of fresh water fisheries since ages. Prayagraj is one of the cities blessed to have two rivers with a biodiversity of freshwater fishes. River Ganga has given sustenance to a variety of economically important fish species, including Indian major carps. However, a major change in fishery scenario has been recorded decade after decade. The decline in fishery is a common problem in almost all rivers/ streams. The gradual erosion of commercial fish stocks due to overexploitation, water abstraction, industries and private use, habitat destruction and defragmentation, pollution level, introduction of exotic species and impacts of global climate change, are the factors which have cumulative effect on the fishery scenario at present. Hence, there is an urgent need of special attention to this sector.

The investigation undertaken on fish population in the Ganga river at Prayagraj has revealed that the small sized fishes which used to be included among "miscellaneous catch" categories have increased by percentage in fish catches and populations of indigenous carps have been replaced by exotic carps *Oreochromis niloticus* and *Cyprinus carpio* were recorded in the Ganga river. Attention is therefore shifted to small sized fishes making a bulk of miscellaneous group of fish catches at Gangetic regions. It is also reported that fishery of Ganga river is essential for the livelihoods and food security of millions of people around the region. The change in scenario is alarming as there is a threat on our indigenous species. Therefore, there is immediate need of fishery biologists, managers, and conservationists to initiate early management strategies and regulations for the sustainable conservation of the existing stocks of fish species in the Ganga river and enhance these by culture practices to save these for future

MICROINJECTION OF DISEASE RESISTANT GENE TO PRODUCE TRANSGENIC ZEBRA FISH

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ABSTRACT

In any aqua cultural operation animal disease accounts for a major loss of revenue. Lysozyme is a major candidature for the production of transgenic fish resistant to a wide variety of bacterial pathogens. op – AFP rt -LYZ (disease resistant gene) carries the rain bow trout lysozyme gene was microinjected into the zebrafish eggs before first cleavage. Their integration was analysed by slot blot and southern blot analysis. F1 progeny was produced by crossing transgenic females with non transgenic males. Slot blot analysis indicated that only one batch of F1 showed integration. However no sign of integration was observed in Southern blot. There was no significant difference in the fecundity and growth rate of control and transgenic fish.

INRODUCTION

'Transgenesis' may be defined as the introduction of exogenous DNA into the host genome resulting in its stable integration, expression and transmission (Khoo, 1995). Techniques such as direct microinjection (Nottle et al., 2001), electroporation (Baer et al., 2000), sperm-s mediated gene transfer (Marialuisa et al., 2012 and Chaparian 2016), retrovirus infection (Baer et al, 2000) and particle gun bombardment (Tucker et. al., 2000) have been widely used to introduce foreign DNA into animal cells. In any aqua cultural operation animal disease accounts for a major loss of revenue. Lysozyme is a major candidature for the production of transgenic fish resistant to a wide variety of bacterial pathogens. op – AFP rt -LYZ (disease resistant gene) carries the rain bow trout lysozyme gene. There fore this gene was selected for the present study

MATERIALS AND METHODS

op – AFP rt -LYZ (disease resistant gene) was linearised with Kpn 1 enzyme for 2 hours at 37^0c. Microinjection was carried out in zebra fish eggs before 1st cleavage using an inverted microscope fitted with micromanipulators (Narishige, Japan). Different concentrations of DNA ranging from 5ng/µl to 200ng /µl was used for microinjection. Their hatching rate and fecundity rate was observed. Integration was analysed by slot blot and southern blot analysis. F1 progeny was set by breeding transgenic females with non transgenic males.

RESULTS

The hatching rate and survival rate of zebra fish eggs injected with various concentrations of linearised disease resistant plasmid DNA (op AFP-rt-LYZ) was observed (Table 1). Maximum survival rate was observed at a concentration of 20 ng

/µl. Therefore this concentration was selected for further experiments. Twenty experiments were conducted with 37-54 eggs per experiment. The integration was assessed by slot blot analysis of genomic DNA from gastrula, newly hatched fry and adults. From each batch half of the surviving fry were sacrificed for DNA extraction and other half were allowed to grow. Seven batches of fishes showed integration which ranged from 4 to 54%. Transgenic females were mated with non transgenic males to produce F1 progeny and fecundity of F_0 was noted (Table 2). Slot blot analysis indicated that only one batch of F1 showed integration. However no sign of integration was observed in Southern blot. There was no significant difference in the fecundity and growth rate of control and transgenic fish (Table 3).

Table 1: Effect of different concentration of linearised op-AFP-rt –LYZ on survival of microinjected zebra fish eggs

DNA concentration (ng /µl)	No of eggs injected	Hatching rate (%)
5	160	60.2±10
10	180	59±11.1
15	280	60.3±8
20	300	62.8±8.3
25	150	50.32±7.1
50	260	55.36±7
100	100	48±8.2
200	55	40.45±6.3

Table 2: Survival and integration of zebra fish eggs microinjected with linearised op-AFP-rt –LYZ

Batch No	No of eggs injected	Survival(%) at		Integration(%)
		Hatching	Feeding	
1	38	54	49	0
2	40	58	38	12
3	37	69	54	0
4	38	70	60	50*
5	42	59	50	54*
6	48	72	62	0
7	44	64	60	0
8	52	70	58	22*
9	50	73	64	-
10	54	68	38	30*
11	48	64	52	-
12	42	60	49	0
13	42	59	45	-
14	37	62	48	-
15	40	68	54	0
16	44	68	50	4*
17	41	50	44	-
18	47	60	48	0
19	53	47	32	28*
20	49	44	40	0

- These were bred to form F1 progeny

Table 3: Fecundity in F_0 generation and integration of linearized op-AFP-rt –LYZ in the F1 progeny of microinjected fish

Batch No	No of Eggs produced	Survival% at 1 week	Integration(% F1)
4	890	72	0
4	780	70	0
5	688	70	0
8	900	71	2
8	714	62	0
10	870	68	0
16	780	75	0
19	728	72	0

DISCUSSION

Lysozyme is a widely distributed hydrolase which likely plays an important role in bio defence system. When microinjection was done there was not much integration in zebra fish. Mosaicism is generally more pronounced in fish presumably a reflection in the late integration of the injected DNA, (Fletcher and Davies 1991). Southern blot analysis of DNA showed no hybridization indicating that the injected op-AFP-rt-LYZ gene cannot integrate into the genome. The hybridization with the probe in slot blot analysis may be due to the persistence of extrachromosomal integration which may get destroyed or failed to replicate in the subsequent developmental stages and F1 generation. Extra chromosomal integration and its subsequent degradation in zebra fish was observed by Pandian et al., (1991). Most of the DNA constructs are lost during the first ten days after delivery into host embryos and only a few are replicated in either as extrachromosomal state or an integrated form during chromosomal DNA replication (Fingerman and Nagabhushanam 2000). The lack of genomic integration may be due to lack of a strong promoter. In the present study the gene construct AFP promoter was from ocean pout, a cold water fish, and it may not be a right promote for warm water fish like zebra fish. There for it is better to use another promoter with lysozyme gene construct for further study to increase resistance to disease in aquatic animals.

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CONCENTRATION OPTIMIZATION OF CLOVE OIL FOR THE TRANSPORTATION OF *HALUDARIA FASCIATA, RASBORA DANDIA* AND *DEVARIO MALABARICUS*

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Abstract

Some degree of sedation with standardized concentration of anaesthetics can be a useful tool for reducing stress during transportation. The effective concentration of clove oil for the transportation of *Haludaria fasciata, Rasbora dandia* and *Devario malabaricus* were determined. The optimum and effective concentration of clove oil for the transportation of *H. fasciata,* and *R. dandia* were determined as 10 mg l^{-1}, whereas16 mg l^{-1} for *D. Malabaricus.*

Key words: Anaesthesia, Sedation, Transportation, Fish

1. Introduction

For preventing transportation stress and morality, fish should be sedated with appropriate dose of most suitable anaesthetic (Husen and Sharma, 2015). Consequently, determination of safe and effective anaesthetic and dose is a significant necessity of aquaculture (Trushenski et al., 2013). Dose of each anaesthetic agent varies noticeably along with species and size (Chambel et al., 2013). Hence the study focuses to optimize the effective dose of clove oil as anaesthetics in the simulated transportation of Melon barb (*Haludaria fasciata*), Slender rasbora (*Rasbora dandia*) and Malabar danio (*Devario malabaricus*) as model species in the closed bag system (LDPE cover) for 24 hr.

2. Materials and Methods

Clove oil (Micro Fine Chemicals, India) was used at different concentrations such as, 6, 8, 10, 12 and 16 mg l^{-1}. The minimum and maximum concentration of each anaesthetic for the experiment was selected on the basis of published references for cyprinids.

2.1. Experimental design

Ten numbers of starved fishes were taken in the polyethylene bags contains 1 L of potable water and known amount of anaesthetic stock solution, medical grade oxygen is then bubbled into the water and tightened with rubber bands. Six bags were similarly maintained for each concentration to 24 hr simulated transportation and bags were placed in Styrofoam box for thermal insulation to prevent the sudden changes in the water temperature. Non anaesthetized control group also maintained similarly. Their behavioural responses were noted every 30 minutes of experiment and mortality after 7 days of experiment. Anaesthetic concentration with desired level of sedation which reduces the reaction to external stimulus and reduces swimming ability without loss of equilibrium (Cooke et al., 2004) is considered as the optimum concentration of anaesthetic for transportation.

3. Results and Discussion

In the present study, low doses of clove oil applied in the simulated transportation condition of plastic bag system found to improve survival rate of *H. fasciata, R. dandia*and *D. malabaricus* without any adverse effect than compared with control (without anaesthetic). The optimum and effective concentration of clove oil for the transportation of *H. fasciata,* and *R. dandia* were determined as 10 mg l^{-1}, whereas16 mg l^{-1} for *D. Malabaricus.*

There are a number of factors including fish size, gender and water temperature (Woody et al., 2002) that may affect time and dose required for anaesthesia. Inoue et al. (2005) reported that 5 mg l^{-1} clove oil reduces stress response in matrinxa (*Brycon cephalus*) during transportation. For sub adult largemouth bass 5 to 9 mg l^{-1} clove oil effective for transportation and quick recovery (Cooke et al., 2004). Alam et al. (2012) reported that the clove oil concentrations from 0.25 to 0.50 ml l^{-1} effective dose for handling transportation of *Anabas testudineus, Channa punctatus* and *C. orientalis.*

4. Summary and Conclusion

The present finding suggested that clove oil could be a safe anaesthesia for *H. fasciata, R. dandia* and *D. malabaricus*. Prior to the transportation of these species with clove oil as anaesthesia, further study is needed to evaluate the physiological response, stress, mortality and water quality parameters during transportation in closed container with oxygen.

5. References

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A PRELIMINARY STUDY ON THE DIVERSITY OF MOLLUSC AT ST.MARY'S ISLAND, KARNATAKA

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ABSTRACT

The studies were conducted about the diversity of mollusc in St.Mary's Island area near Malpe beach,Uduppi,Karnataka. The mollusc were collected by hand picking method .Since this area is rich in mollusc diversity, it is also observed that its mollusc diversity has decreased due to pollution and climatic changes. This low mollusc species composition denotes that St.mary's Island is threatened *ie* undergoing degradation in its diversity

INTRODUCTION

St.Mary's Island are known for their distinctive geological formation of columnar basaltic lava. Distinct variation in distribution and abundance of mollusc diversity in the St.mary's Island were observed during the present day. There is a lack of information on mollusc diversity and the present study was undertaken to study the biological diversity at St Mary's island.

MATERIALS AND METHODS

The mollusc were collected during march 2019. The molluscs were collected by hand picking method

RESULTS

The following Mollusc species were identified :

Anadara sp

Anadara is a genus of saltwater bivalves, ark clams, in the family Arcidae. It is also called Scapharca.This genus is known in the fossil record from the Cretaceous period to the Quaternary period.

Cardites bicolor

Cardite is a genus of marine bivalve mollusc, in the family Carditidea. The family Cardites attains a maximum size of 15-50 mm. Shell creamy white externally with brown blotches and white internally .

Gafrarium divaricatum

Gafrarium divaricatum can be quite common on shores, among coral rubble or hidden under stones near the low water marks. Elsewhere, they are found on intertidal shores with coarse sand and gravel.

Saccostrea cuculla

Saccostrea cullata, the hooded oyster or Natal rock oyster, is a species of rock oyster found mainly in the Pacific Ocean.

Spondylus sp

The genus *Spondylus* appeared in the Mesozoic era, and is known in the fossil records from the Triassic Cassian beds in Italy (235 to 232 million years ago) onwards. About 40 extinct species are known.

Turritella attenuate

Turritella attenuata is a species of sea snail, a marine gastropod mollusc in the family Turritellidae. Slender long conical shaped shell which is elogated than T. acutangula.

Cypraea sp

Cypraea is a genus of medium-sized to large sea snails or cowries, marine gastropod mollusks in the family Cypraeidae, the cowries.

Trochusradiates

Turritella attenuata is a species of sea snail, a marine gastropod mollusc in the family Turritellidae. Slender long conical shaped shell which is elogated than T. acutangula

Planaxissulcatus

Planaxissulcatus is a species of marine prosobranch gastropod, commonly found in the rocky intertidal environments throughout the Indo-Pacific region

DISCUSSION

A detailed description of the natural flora and fauna of the islands and the DeriaBahdurGhur (the islands north of the port of Malpe, named after the cross set up by Vasco da Gama), have been compiled in a manual by John Sturrocks, the district collector of Mangalore in 1894 (Rao, 2003). Colonies of gulls, Scolopacidae (sandpipers) and a few crows have been sighted on the Islands. But on the approach to the Islands from the Malpe beach, brahminy kites (Haliasturindus)), great white egrets, grey egrets (breeding plumage) and groups of large green bee-eaters have been recorded (Raffaelli *et al.*2003). Since this area

is rich in mollusc diversity it is also observed that region has decreased its mollusc diversity due to pollution and climatic changes. This low mollusc species composition denotes that St.mary's Island is threatened that is the place is undergoing degradation in diversity.

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PLATE- 1

Anadara sp ***Carditesbicolor*** ***Gafrariumdivaricatum*** ***Saccostreacuculla***

Spondylus sp ***Turritellaattenuate*** ***Cypraea sp*** ***Planaxissulcatus***

Babylonia spirata ***Trochusradiatus***

STUDIES ON THE FISH BIODIVERSITY AT THANNERMUKKOM BUND

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Abstract

Vembanad Lake is under increasing pressures from anthropogenic and climate factors and in the threat of decline in many earlier reported fish species. The study evaluated the fish diversity at Thanneermukkom bund area in order to find out the present status of fish diversity in that area. Fish samples were collected during February to March 2019 from local fishers at the fish landing centre using different types of nets like cast net and gill net. A total of 14 species belonging to 10 families were recorded from the study area during the present study.

Introduction

Biodiversity studies on icthyofauna have gained recent attention. Conservation of fish diversity assumes topmost priority under the changing circumstances of gradual habit degradation. The information of diversity helps us to understand the need to conserve rare species and prevent exploitation for a sustainable environment. Conservation of fish diversity assumes top most priority under the changing circumstances gradual habit degradation.

The present study was conducted about the diversity of fishes at Kattachira near Thaneermukkom bund. Decline in estuarine diversity is the result of overfishing, insufficient management practices and habit degradation, which reduces the chances of its sustainability. Therefore, knowledge on the status and trends of backwater fisheries is the key to sound policy development, better decision making and responsible fisheries management.

Materials and methods

Study area

A study on the diversity fishes of Thannermukkom, Cherthala (9°40'29.0"N 76°23'58.6"E) was carried out for a period of one month Februarytomarch2019.

Collection

The present study was conducted at Thaneermukkom panchayath, Alappuzha district. Comparatively less polluted area was selected. The fish were collected during summer (February 2019-March 2019) and during the afternoon. The fishes were collected with the help of fishermen using fishing nets. The collections were made three times during the period and observed for morphological parameters such as colour, shape, size and patterns etc. They were all photographed and preserved in formaldehyde. Identification of the fishes was done based on morphometric and meristic characters following Munro (1995).

Results

A total of 14 species of fin fishes were identified from the Thannermukkom Bund during the study period. All the 14 fishes were edible. A checklist of the species collected from the study area is given below :

Sl.no	Name of the fishes
1	*Etroplus suratensis*
2	*Siganus canaliculatus*
3	*Dawkinsia filamentosa*
4	*Elops machnata*
5	*Xenentodon cancila*
6	*Arius subrostratus*
7	*Otolithes ruber*
8	*Etroplus maculatus*
9	*Leiognathus decorus*
10	*Megalops cyprinoides*
11	*Channa marulius*
12	*Lutjanus argentimaculatus*
13	*Tilapia mossambicus*
14	*Lates calcarifer*

Etroplus suratensis

Xenentodon cancila

Etroplus maculatus

Otolithes ruber

Leiognathus decorus

Lates calcalcarifer

Siganus canaliculatus

Lutjanus argentimaculatus

Dawkinsiafilamentosa

Elops machnata

Tilapia mossambica

Channa marulius

Megalops cyprinoides

Arius subrostratus

Discussion

Vembanad lake is considered to be rich in fish diversity but recent years have observed a decline in fish diversity due to pollution and climatic changes. During the study a total of 14 species of fishes were collected and identified upto species level. Although the collected fishes were the most common species available the number seems to decrease. This low fish species composition could denote that the area is undergoing degradation in diversity.

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MYCOFLORA IN DRIED FISH AND PRAWN FROM TWO DISTRICTS OF KERALA

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ABSTRACT

The quality of salted sun dried fishes are adversely affected by the occurrence of microorganisms. The mycoflora of salt dried fish and prawn from markets in Kottayam and Ernakulam were studied. Samples are cultured on Sabouraud Dextrose Agar (SDA) to identify fungi growing on the sun dried sole fish. Fungus like Aspergillus sps and Abscidia sps were found in the sample.Moisture content of fish plays an important role in spoilage and lowering of moisture retards the spoilage.

INTRODUCTION

Fish is a rich substratum for the growth of microbes especially fungi. Fungal growth on dried fish indicates spoilage and deterioration of the product. This results in the negative effects such as discoloration, rotting and the production of off odour making the food unmarketable(Bennett and Klich,.2003). Another risk of fungal contamination in dried fish is the production of mycotoxins. Consumption of mycotoxins results in the carcinogenic and mortality effects (Eaton and Grooman, 1994).). The main objectives of the study include to isolate and identify the mycoflora present in dried fish and prawn by using dilution plate technique, to find the quality of dried fish available in markets from two districts and recommendations to prevent/retard fungal contamination which has public health risks .

MATERIALS AND METHODS

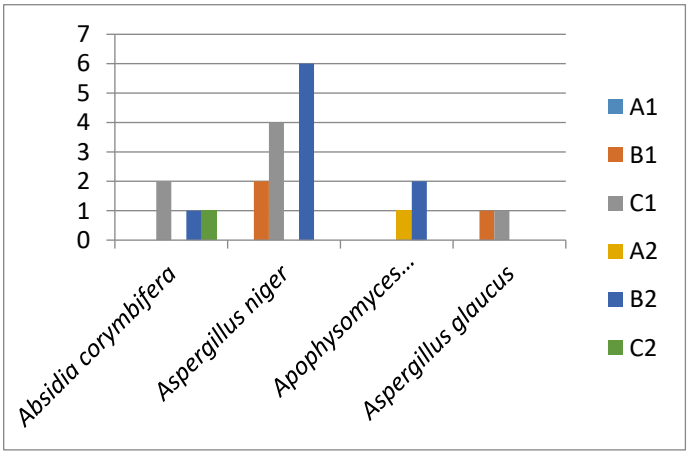
The selected fish species were sole fish (*Cynoglossus macrostomus*). Mycological assay used including enumeration technique of fungal CFUs, isolation and identification which largely involved microscopy.Pour plate method was used in quantifying and detection of fungi (Pitt and Hocking,2009) in the sun dried sole fish. After serial dilution the samples incubated in SDA medium at a temperature of 37℃ for 3-5 days. From the fungal growth on primary culture were sub cultured using piece of fungal mycelia on to fresh media of SDA. The mould was inoculated near the centre of the plate to best colony development and sporulation. Taxonomic identification of the genus was carried out according to macro and microscopic characteristics of the colonies using identification key from guide to clinically significant fungi. Moisture content determined . The percentage of moisture content of the sample from each market was recorded.Staining of fungal isolates done by Lacto phenol Cotton Blue, used in staining fungal mounts. A drop of 95% alcohol was applied on the slide and fungal isolates were placed on it. The isolates were teased out gently with a needle then spread on the slide allowing the alcohol to evaporate.

RESULT AND DISCUSSION

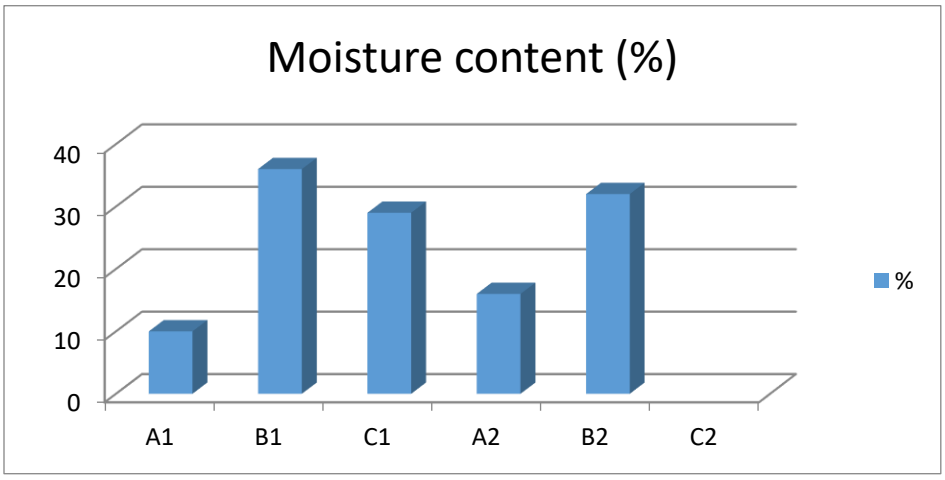
Four different types of fungi were isolated from sole fish and two different types were isolated from prawn. The fungi isolated from sole fishes are;*Absidiacorymbifera, Aspergillusniger, Apophysomyceselegans and Aspergillusglaucus.*In prawn sample, there are two different types of fungi. *Aspergillusniger and Aspergillusversicolor*.

Fungal species	A1	B1	C1	A2	B2	C2
Absidiacorimbifera	-	-	2	-	1	1
Aspergillusniger	-	2	4	-	6	-
Apophysomyceseleg ans	-	-	-	1	2	-
Aspergillusglaucus	-	1	1	-	-	-

A1,B1,C1 denote Stall 1, stall 2, stall 3 of Kottayam and A2, B2, C2 denotes stall 1, stall2, stall 3 of Ernakulam.



SAMPLE	Moisture content (%)
A1	10
B1	36
C1	29
A2	16
B2	12
C2	12



Abila, (2003) reported that fishes are prone to microbial attack due to unhygienic methods of handling, transportation and storage. It is reported that several species of yeast and aspergillus produce toxic substances which if consumed will cause health problems. A study carried out in Uyo state, Nigeria on the mycoflora of smoked dried fish indicate that *Aspergillusniger, Aspergillusflavus, Aspergillusterus, Aspergillus fumigates, Absidia sp., Mucor sp., Cladosporium sp.,penicillium sp., and Rhizopus sp.*, where associated with smoked sp., (Bukola and Adebayo, 2008).

Appropriate handling and storage conditions are important in minimizing the growth of fungi on sundried fish. Bukola and Adebayo, (2008) have observed that good hygiene practices (GHP) and hazard analysis critical control point (HACCP) should be made operational along the harvesting, processing and distribution line of smoked fish. If this is done with sundried sole fish will ensure that the fish is produced under hygienic condition so as to address possible risks associated with contamination. Recommendations as based on findings are;1.Both the wholesalers and retailers should always ensure that they sun-dry the fish properly to reduce the moisture content. This will also reduce the growth of fungi and reduce the risks associated with consumption of mycotoxin contaminated sun dried fish.2. During the storage period the fish should be kept in water proof bags or gunny bags with a polythene lining from inside to prevent the fish from absorbing moisture during the storage period. 3. Fish sellers should observe both personal and environmental hygiene when handling and displaying the fish for sale. 4. Pre-cooking treatments like washing can help to reduce the fungal load on fishes.

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STUDIES ON DIFFERENT MATERIALS USED FOR IMAGE NUCLEI PRODUCTION

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Introduction

The discovery and refining of the cultured pearl production practice was sophisticated by the beginning of 20th century. The Akoya pearl oyster *Pinctada fucata* (Gould, 1850) belonging to the family Pteriidae is one of the pearl producing bivalves which produces gem quality pearls namely Akoya pearls of 3 mm to 10 mm having a great market value. In India, technology has been recently perfected for the production of quality image pearls (mabe pearls) which brings a fine value ranging from $20 - 50 per pearl (Rinju *et al.* 2018). Image pearls are those that are produced by implanting a nucleus or mould prepared by shell based cement; using metal templates of requisite shape against the inner shell rather than the soft tissue of an oyster. Oyster then secretes layers of lustrous coat over the image nuclei producing an image pearl which is cut off from the shell, polishes and used in pendants, studs, brooches or rings (Anil *et al*. 2007). In case of cultured Akoya pearls, purity and colour is significant as often they are typified by lesser nacre thickness, and the nuclei must be invisible for a pearl to remain commercial (Ward, 1995). Current study deals with the effect of different materials *viz*, white cement material, alginate impression material (chromatic geltrate) and hot glue material (Ethylene-vinyl acetate (EVA) copolymer) in the rate of nacre coating of *P. fucata* in Vizhinjam waters and the results were elucidated.

Materials and methods

Different materials including white cement material, acrylic repair material and hot glue material were used for preparing the nuclei. The nuclei used for implantation had a diameter of 5 mm and a thickness of 2 mm. Samples were obtained from the hatchery bred stock and adult oysters of *P. fucata* with an average size of 60 mm (DVM), 56 mm (HL), 28 mm (THK) and 27g (WGT) were selected for the present study. Each study trials were attempted in oysters of 25 numbers and after implantation the oysters were kept in box cages of 50 X 50 X 12 cm dimensions with a mesh size of 25 mm, hung from

the wooden raft which was moored in Vizhinjam bay. After 50 days of implantation, the implanted oysters were sacrificed and the nacre coated nuclei was cut using a saw. The nacre was separated from the nuclei and the thickness was measured using an Axiocam camera fitted to Carl Zeiss Lab. A1 microscope (ERC 5s). Trials were conducted for a period of two years (September 2016 - October 2018).

Methods involved in preparing different types of nuclei.

(1) White cement material

Nucleus was prepared using white cement material and molten wax was used as mould. And the method involves;

- Making an impression of the image on a metal sheet
- Making wax mould
- Making white cement paste
- Making white cement nuclei
- Curing the white cement bead
- Removing the nucleus from wax mould
- Grinding the nuclei by which the final outer shape, size and thickness of the nucleus is decided

(2) Acrylic repair material

DPI - RR cold cure (powder : liquid) was used for preparing nuclei and the mould used was molten wax. The method involves;

- Making an impression of the image on a metal sheet
- Making wax mould
- Mixed dpi - RR cold cure (powder) and dpi - RR cold cure (liquid) in 1:1 proportion to form a suspension
- Filling the suspension inside the wax mould
- Keep the nuclei undisturbed till it sets
- Remove the nucleus from wax mould and shape it using fine scissors or cutter by which the final outer shape and size of the nuclei is decided.

(3) Acrylic repair material and alginate impression material (chromatic geltrate)

Nuclei was prepared using DPI - RR cold cure (powder : liquid) and alginate impression material (chromatic geltrate) was taken as mould. The method involves;

- Making an impression of the image on a metal sheet.
- Alginate powder was dissolved in tap water in a 1:1 proportion until the mix changes its colour from creamy to pink and soon after mixing; the metal template was pressed into which it produces a print of image (mould for acrylic material to fill)
- Mixed DPI - RR cold cure (powder) and DPI - RR cold cure (liquid) in 1:1 proportion to form a suspension and fill it into the chromatic geltrate mould
- Keep it undisturbed for 20 minutes.
- After setting, remove the nuclei from the alginate mould, shape it using fine scissors or cutter in which the final outer shape and size of the nuclei was decide.

(4) Hot glue material (Ethylene-vinyl acetate (EVA) copolymer)

In this method nucleus was prepared by using hot glue (Ethylene-vinyl acetate (EVA) copolymer) and metal templates were used as mould. The method involves;

- Making an impression of the image on a metal sheet.
- Filling the metallic mould using hot glue material.
- Within 5 -10 seconds hot glue sets which gives the shape of the metallic mould.
- The image then separated from the metallic mould; which was further used as the nuclei for implantation.

Results

Nuclei prepared by DPI-RR cold cure material showed maximum rate of nacre thickness and lustrous quality. An average thickness rate of 513.67 µ/50 days (10.2 µ/day) was recorded for the nuclei prepared by DPI-RR cold cure and alginate impression material. Correspondingly, the nacre coat thickness recorded from the nuclei prepared by DPI-RR cold cure and molten wax was 507.11µ/50 days (10.1 µ/day). Nacre coat thickness over shell cement nuclei was measured as 379.96 µ/50 days with a per day coating rate of 6.7 µ. In all the three methods a uniform mode of coating was observed over the nuclei

except hot glue material. Average rate of nacre coating over the hot glue material was measured as minimum with a per day coating rate of 5.94 µ/day (297.34 µ/50 days).

Conclusion

Current study reveals the suitability of different materials in image pearl production. Results showed a maximum rate of nacre coating over nuclei prepared by DPI cold cure material and the nuclei prepared using alginate impression material as mould showed slightly higher nacre thickness rate than those prepared by molten wax as mould. Nuclei prepared by means of shell cement material also showed better coat rate and lustrous quality. Whereas, nuclei prepared by the hot glue material gave the least rate of nacre coating.

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CYTOMORPHOLOGY OF BLOOD CELLS IN THE MAJOR IMMUNE ORGANS OF *SCOLIODON LATICAUDUS*

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ABSTRACT

The spade-nose shark scoliodon laticaudus a marine elasmobranchii. The spade nose shark, the only member of the genus scoliodon, is a shark of the family Carchiarhinidae . The present study was aimed to investigate the cytomorphology of blood cells in the major immune organs of S.laticaudus. Both mature and immature cells were seen in the liver imprints. The mature cells were predominated by …BCs, a few mature lymphocytes and occasionally, granulocytes and platetes. The stricking features of liver imprints was the occurance of large number and variety of immune cells of both erythrocytic and leucocytic series. The most dominant cells in the spllen imprints also were RBCs and lymphocytes. The immature cells in the spleen were predominated by lymphoblastis followed by granuloblast, developmental stage of macrophages were also more abundant than in liver imprints. A better understanding of the fish immuno system will enhance our ability to develop vaccines and immune-stimulatory molecules can better direct the immune system to prevent in aquatic animals. The present study aimed at comparing the cell types found in the major immune organs of an Scoliodon Laticaudus

Keyword : Cytomorphology, Scoliodon laticaudus, Carcharstrinidae, erythrocytes, leucocytes.

INTRODUCTION

The immune system of Elasmobranchi) as usually represented by the sharks, has only been partially investigated and available data largely reflect studies at the molecular level that is,antibody gene structure. Morphologically, all elasmobranchs exhibit thymus and spleen having characteristic structure. The encapsulated thymes is clearly separated into a thymocyte filled cortex and a less dense medulla region . The elasmobranchii thymus also involves rapidly upon maturity as mouse and human thyme. Shark possess differentiatable red blood cells and white blood cells, macrophages capable of phagocytosis, eosinophils and a second type of granulocyte that is lymphocyte is extremely important B-cells and T cells are found within the general lymphocyte population (Evans, 1998).

The thymus is primary lymphoid organ responsible of the generation of 'educated' Tcells (Jane Way *etal*;2001). Presentation and processing of antigen and antigen complexes occur in secondary organs such as the spleen and GALT (Gut Associated Lymphoid Tissue). In contrast to the mamalian system, frustratingly little is known about the shark T –cell/ MHC education procedure, or even whether shark T – cells can discriminate between self and non self in the same manner as human T-cells. This knowledge may be hampered by the difficulties in establishing and maintaining shark T-cell, B-cell and macrophage cell lines. Morphologically the shark and ray thym consist of multiple labes. Containing separated cortical and

medullary regions as in mammalian lymphoid organ that filters the blood and allows the B-cells that percolate through the white pulp to interact and respond to blood borne antigens. Since the clasmobranches are benefit of bone marrow, the chondrichthyan lymphocytes are putatively derived from the epegonal and heydog organs and while the spleen is heavily populated by lymphocytes (Evans, 1998). The adult nurse and leopard sharkes have a lymphoid thymes, spleen and intestine associates lymphoid tissue. The thymes is a distinct organ having a cortex and medulla. Circulatory smears reveal lymphocytes, macrophages and granulocytes. (Good etal,1966). In the point of view, the importance of Blood cells in the major tissue organs of elasmobranchii S. Laticaudus present work was attempted to study the cytomorphology of blood cells in the major immune organs of *Scoliodon lesticaudus* specimen were collected from the fish landing centre at Neendakara,Kollam.

MATERIALS AND METHODS

Specimens of these species were collected from the fish landing centre at Neendakara, Kollam . As soon as collected, the fishes were dissected out and the spleen liver and kidney of the *Scoliodon lesticaudus* were carefully exised and the imprints of each organ were prepared. The imprints were fixed in absolute methanol and stained with. Giema's stain properly stained imprints were made into temporary, xylene mounts and examined under oil immersion objective of a research microscope. Cell types present in each organ were studied for their shape and staining properly.

RESULTS AND CONCLUSION

In the present study imprints of the spleen of S.laticaudus showed the presence of mature and immature blood cells. Immature stages almost all cell types were discernible and specially presence of haemocytoblasts undoubtly confirm the active involvement of this organ in the haemopoiesis of the elasmobranchii species and attests the general consensus that is elamobranchii.Spleen is primary lymphoid organ. Barring the presence of erythrocytes, the immune organs/sites examined of the elasmobranchi, S. laticaudus revealed in the presence of several cell types which in fishes are known to play crucial role in immunology such as lymphocytes, neutrophil, eosinophil and monocytes in the latter. In the present study of S. laticaudus, the lymphocytes are small cells with variations in shape and size. There was no significant difference in the appearance of the lymphocytes. In S. laticaudus the neutrophils are spherical to subsperical; oval cells were rarely not within the imprints of liver and spleen. The nucleus may be whole or segmented. The cytoplasm is nearly colourless to faint pink and the nucleus deep purple.

Eosinophils are comparatively large and round with eccentric nuclie that occupy nearby 2/3 of the cytosome. The morphology of the eosinophil of S.laticaudus confired to that described for other elasmobranch species. In S.laticaudus thrombocytes are various shapes such as kidney-shaped, subspherical or spherical. They are very small cells. The cuytoplasm is hardly visible. Erythroblasts were frequently met with in the liver imprints of S.laticaudus. In S. laticaudus, basophilic erythroblasts were frequently met with in the imprints of the liver so were basophilic erythrocytes that are advanced in maturity than basophilic erythroblasts (Lekshmi, 1992). The lymphoblast of S.laticaudus found copiously in liver imprints, were large, round cells,

with very little distinction between cytoplasm and nucleus. The development stages was noted only in S. laticaudus during the present study and it was noted both in the spleen and liver imprints of this fish (Fijan and Bluff,2005). Promonocytes were not abundant thereby attesting a good heakth status of the individuals of S.laticaudus examined during the study.

CONCLUSION

The liver imprints of the S. laticaudus contained mature and immature cells. The mature cells were erythrocytes, lymphocytes, granulocytes and platelets and the immature erythroblasts, haemocytoblsts and lymphoblasts. In the spleen, mature cells present were erythrocytes, lymphocytes and eosinophils and immature cells, lymphoblsts, granuloblasts and promonocytes . The present study was concluded that a wide veriety of structurally and functionally dissimilar cells partake of the immune system and a number of morphologically and functionally diverse organs and tissues serves as cradle for development of cells of the immune system.

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THE EFFECT OF ADDITION OF PROPIONIC ACID STABILISED FERMENTED FISHERY WASTE AS BIO-FERTILIZER IN THE CULTURE OF ORNAMENTAL FISH - GUPPY (*POECILLA RETICULATA*)

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ABSTRACT

The economic as well as ecological impact of unconsumable or trash fish waste is very significant. The discarded and deteriorated fish waste can be recycled as potential source of highly nutritional animal feeds especially as fish food. The effect of different levels of propionic acid (0.25, 0.5 & 1.0 ml/100g) on the Total Heterotrophic Bacterial (THB) Count, mycotic flora, p^H, carbohydrate, protein, and amino acid concentration of fermented fish waste was estimated. Results of the present study revealed that addition of 0.5 to 1% propionic acid to the fishery waste was effective in controlling the growth of mould and fungus during the fermentation process. Addition of a fermented product as bio-fertilizer, augmented the growth in terms of body length in ornamental fish *Poecilia reticulata*. The survival data showed that the acidified fermented product don't have any toxic effect on the guppy fish (*Poecilia reticulata*). From this study, it is clear that, the preparation of fermented fish silage using propionic acid as preservative is a good supplementary source of fertilizer/food for ornamental fish culture. It can be utilized as organic bio-fertilizer in aquaculture especially in homestead aquaculture activities like freshwater ornamental fish and/or food fish cultivation.

(Key words: Fishery waste, fermentation, Guppy, propionic acid, Total Heterotrophic Bacterial Count, Mycotic Flora, *Poecilia reticulata*)

INTRODUCTION

Fermentation has been applied to fish for many years (Han-Ching *et al*., 1992) and represents a low level (artisanal) and affordable (neither capital nor energy intensive) technology for tropical developing countries. Fermented fishery products are susceptible to spoilage through mould or bacterial decay, insect infestation or fragmentation. Various chemical preservatives have been used to prevent spoilage when silages are exposed to air, thus enhancing the aerobic stability of silage. Of the short chain fatty acids, propionic acid has the greatest antimycotic activity. During acid silaging of poultry and fish offal propionic acid was effective in suppressing yeasts and moulds (Mahendrakar *et al*., 1991). At present, large amounts of fishes were discarded from various fishing activities/industries and have been creating a lot of ecological problems. Under these circumstances, an attempt was made to transform the fishery waste into a stable feed ingredient or as a bio-fetilizer. The growing interest in aquarium fishes has resulted in steady increase in aquarium fish production the world over and at present it is the sunrise industry in the aquaculture sector. The objective of the present study is to investigate the effect of addition of

different levels of propionic acid as a preservative agent in the fermentation process of fishery waste and also to study the efficacy of the fermented product as bio-fertilizer in the culture of ornamental fish, Guppy (*Poecilia reticulata*).

MATERIALS AND METHODS

Samples of fishery wastes were collected from Sakthikulangara Fishing Harbour, Kollam. Propionic acid was added to the fish waste at varying levels 0.25, 0.5, 1.0 (w/w) and designated as T1, T2, T3. In each treatment, minced fish waste was mixed thoroughly with 10 % w/w jaggery (Cane sugar) as fermentable sugar and placed in uniform sized plastic bottles and placed the lids air tight. A control (C) was maintained without addition of propionic acid. p^H of the fermented materials was determined on weekly basis. Soluble protein was estimated following Lowry's method (Lowry *et al*; 1955). Glucose/Carbohydrate estimation was done by Anthrone method (Jayaraman, 1992). Free amino acids were determined by Ninhydrin method (Yemm & Cocking, 1995). Pour Plate Method (APHA, 2005) was used to estimate the Total Heterotropic Bacterial (THB) and Mycotic Count and results were expressed in Colony-forming units (*cfu*/mL). An indoor experiment was conducted to study the effects of fermented organic material as bio-fertilizer source on the growth of guppy (*Poicela reticulata*) in an outdoor rearing system. Commercially available fish feed was added to tanks. Fermented fish waste as bio-fertilizer was applied to the treatment tanks at 1g $week^{-1}$. No water was exchanged during the whole experimental period. Fishes were harvested using a hand net and total body length of fishes was recorded. The growth experiment was terminated on 60^{th} day. The survival rate was calculated from the number of fishes survived.

RESULTS AND DISSCUSSION

Presence of propionic acid lowered the pH to an acid range which further reduces the possibility of mycotic growth. In present study, the decrease in protein revealed the dissociation of proteins during the process of fermentation. Degradation of nitrogen components proceeds during storage and is manifested an increase in free amino acid and peptides. These substances increased the pH in the reaction mixture. The present study revealed that the free amino acid content was increased with increase in addition of propionic acid concentration. Proteins are hydrolyzed to free amino acids, thus making silage the most available amino acid source for protein biosynthesis (Espe *et al*., 1992). The reduction in the protein content from the initial value showed the degradation of most of the protein into amino acids, which was indicated by the final increase in the concentration of amino acids. The carbohydrate was effectively utilized by the bacteria as the final concentration decreased from the initial. Results of the present study revealed that addition of 0.5 to 1% propionic acid to the fishery waste was effective in controlling the growth of mould and fungus during the fermentation process. Treatment, T_3 which has the highest concentration of propionic acid (1%) showed the least count in mycotic biota. In the case of mycotic biota, there is not much difference between 0.5 and 1% level of propionic acid inclusion.

The result of the present study revealed that the addition of fermented fish waste to the water column improved the growth performance in terms of body length gain in *Poicilia reticulata*. The growth data indicated that the fermented fish product with 1% level of propionic acid added to the water column doesn't have any growth inhibition in *Poicilia reticulata.* Zynudheen *et al.* (2008) studied the effort of dietary supplementation of fermented fish silage on egg production in Japanese quail. The fermented product may provide nutrients, minerals, vitamins or even lactobacillus to the

fish culture system. Survival rate was not affected by the addition of fermented fish waste to the water column. 90% and 95% survival rates were recorded in the control and treatment tanks respectively. The fish survival data of the present study revealed that the addition of propionic acid at 1% level does not have any toxic effect on *Poecilia reticulata*.

CONCLUSION

The results of the present study revealed that a stable fermented product can be prepared by the addition of preservative, proponic acid at an inclusion level of 0.5-1% to the fermenting fish waste. Addition of fermented product as bio-fertilizer augmented the growth in terms of body length in ornamental fish *Poecilia reticulata*. The fish survival data of the present study indicated that a fermented product prepared by the addition of 1% propionic acid level to fish waste doesn't have any toxic effect on the guppy fish (*Poecilia reticulata*). Effective utilization of fishery waste for the preparation of fermented products and its use as potential bio-fertilizer is a rational approach in ornamental fish culture. Application of the fermented fish silage as a supplementary fish feed/fertilizer can reduce the operational cost. This approach can be adopted by the self-help groups to generate income using the locally available recourses in a sustainable way.

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EFFECT OF AMMONIA EXPOSURE ON NITROGEN EXCRETION IN AIR-BREATHING PERCH (*ANABAS TESTUDINEUS* BLOCH)

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ABSTRACT

Fishes tolerate ammonia and are able to maintain their plasma ammonia levels within a range. Freshwater fishes excrete ammonia whereas ammonia is converted into urea in seawater fish. Ammonia formed in the liver and other tissues of fish and is cleared from the blood at the gills where it rapidly diffuses into water. With the help of ureogenic enzymes some freshwater fishes can synthesize urea or can store ammonia as biochemical adaptation to face osmotic challenges. Consequently, euryhaline fish possess the ureogenic capacity to tackle the problem of ammonia toxicity.

INTRODUCTION

Fishes tolerate ammonia and are able to maintain their plasma ammonia levels within a range (Mommsen and Walsh, 1992). Freshwater fishes excrete ammonia whereas ammonia is converted into urea seawater fish (Wright, 2002). With the help of ureogenic enzymes some freshwater fishes can synthesize urea or can store ammonia as biochemical adaptation to face osmotic challenges.

One of the major path ways for detoxification of body ammonia is the ornithine-urea cycle (O-UC). The presence of a functional urea cycle with significant activity of enzymes of urea cycle has been reported in some species of freshwater teleosts including climbing perch (Saha and Ratha 1987, 1989). This ureogenic capacity exists throughout the life cycle as adaptation to unusual environmental circumstances including high ambient ammonia. Similarly high levels of urea cycle enzymes and enhanced urea excretion rates has been reported in *Heteropneustes fossilis* (Saha and Ratha 1987, 1989), walking catfish *Clarias batrachus* (Saha and Das, 1999; Saha *et al*., 2000).

MATERIALS AND METHODS

Anabas testudineus (Bloch, 1795) commonly called the climbing gouramies or climbing perches comes under the order Anabantidae and family perciformes. Adult climbing perch, *Anabas testudineus* (35 ± 5 g body mass) collected from a local supplier were maintained in the laboratory in glass tanks. Fishes were acclimated to tap water at 28 ± 2ºC under natural photoperiod (12L/12D) for two weeks prior to the experiment. Fish were fed with commercial feed at the rate of 1-5% of body mass/day.

Experimental protocol

Freshwater (FW) were exposed to varied concentration of ammonia. In this approach twenty-four FW fish were grouped in to four of six each and kept in 100 L glass tanks. The untreated group of fish was the control. Two fish groups were exposed to 10 and100 μ*M* of ammonium sulphate $(NH_4)_2SO_4$ for 48 h. A group of fish were first exposed to 100 μ*M* ammonia for 48 h and then kept for 96 h recovery in fresh water, which were compared with100 μ*M* treated fish.

Sampling and analysis - All fish were anesthetized briefly in 0.1% 2- phenoxyethenol (Sigma, St. Louis) solution. Blood was drawn from the caudal vessels using heparinised syringe and centrifuged at 5,000g for 5 min at 4 ºC and the plasma was separated and stored at -20ºC until analysed.

Ammonia and urea estimations

Plasma urea was estimated by DAM method using commercially available kit (Span Diagnostics, New Delhi). Plasma ammonia was estimated according to the method of Bergmeyer and Beutler (1985).

RESULTS

Effects *of ammonia exposure on FW perch*

Plasma ammonia decreased significantly (P<0.001) with increasing concentration of waterborne ammonia in FW fish. Plasma urea concentration increased significantly (P<0.05) only at high dose of water-borne ammonia in FW fish and its level returned to basal level (P<0.05) in the fish kept for recovery in freshwater for 96 h.

Status of FW fish	Plasma ammonia	Plasma urea
0	0.223±0.02	0.611±0.01
10 µM $(NH_4)_2SO_4$	0.106±0.04***	0.962±0.013
100 µM $(NH_4)_2SO_4$	0.094±0.003***	0.962±0.013*
100µM $(NH_4)_2SO_4$+ 96 Hr R	0.101±0.04	0.848±0.013*

DISCUSSION

The climbing perch *Anabas testudineus* is ammonotelic in fresh water. Saha and Ratha (1987; 1989) reported that *Heteroneustes fossilis, Clarias batrachus* and *Anabas testudeneus* have the full complement of OUC enzymes in both liver and kidney tissues; however another air breather *Channa punctatus* does not have this machinery.

The substantial decline in plasma ammonia and the rise in urea level in the FW fish after ammonia challenge indicates that these fish convert its body ammonia into urea by inducing ureogenesis. Climbing perch though rely on ammonotelism in their normal condition changes their mode of nitrogen excretion to ureotelism. This compensatory shift of perch into ureogenism points to the possibility of disturbed ammonia excretion through gills. The presence of ambient ammonia may not permit the gills to eliminate the ammonia, thus favouring these fish to convert ammonia into urea. Ammonotelism is

possible because ammonia is highly soluble in plasma and water and ammonotelism requires unlimited access to fresh water. In salinity-acclimated fish ammonia formed cannot be easily washed out into the surrounding as it requires a large amount of water.

When exposed to ambient ammonia, fish either excrete the ammonia or converted it into urea depending on the water availability. Teleosts fish *Oreochromis alcalicus grahami* in Lake Magadi (pH10) excretes urea and not ammonia (Randall *et al*., 1989; Wood *et al*., 1989:Chew and Ip 2014). As an osmolyte urea is retained in elasmobranches (Wright, 1995). Indian air breathing catfishes *H. fossilis* and *C.batrachus* are hardy and capable of living in derelict water bodies and can tolerate temporary water deprivation (Saha and Ratha 2007). These species are potentially ureogenic teleosts expressing the complete cycle of ureogenesis, not only in hepatic tissue but also in certain non hepatic tissues when exposed to high environmental ammonia. In walking catfish *C. batrachus* it appears that the OUC enzymes are expressed in early life stages at relatively high levels and remain expressed all through the life stages with a potential of stimulation of ureogenesis through out the life cycle as a sort of physiological adaptation to survive and breed successfully under hyper ammonia and various other environmental stresses (Zaiba *et al*., 2006). African lungfish *Protopterus aethiopicus* when confronted with high concentrations (30 or 100 mmol l^{-1}) of environmental ammonia significant increases in urea contents occurred in various tissues of fish (Loong *et al.*, 2007). Ching *et al* (2009) in their study reported that exposure to a sublethal concentration of ammonia would induce oxidative stress in gills and brain of the mudskipper *Boleophthalmus boddarti* which has high tolerance of environmental and brain ammonia.

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IMPORTANCE OF WETLAND QUALITY ASSESSMENT ON VELI LAKE

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ABSTRACT

The wetlands all over the world faces severe ecological crisis and are depleting in an ever increased rate. The loss of wetland ecosystem is accompanied by irreversible loss in the valuable ecological and environmental functions. Ramsar Convention provided the frame work for the conservation of wetlands. The present study was conducted in the Veli Lake.The study helps to understand various environmental aspects of Veli Lake related with wetland ecological degradation. The Veli Lake is the smallest estuarine wetland in the southwest coast of Kerala (08° 31 and 08° 31 ´ NL and 76° 522 30´ to 76° 532 30´ EL) situated at Thiruvananthapuram.

Key words;-Wetland, Estuary, Veli lake, Ecology, Ramsar convention.

INTRODUCTION

Wetland is the general term applied to the land areas that are seasonally or permanently water logged including lakes, rivers, estuaries and fresh water marshes. Now days, the wetlands are depleting in an ever increased rate (Acharya and Adak, 2009). Proper understanding of wetland's ecological status is a pre-requisite in the management and conservation of ecosystem.

. The present study helps to understand various environmental aspects of Veli Lake related with wetland ecological degradation. The Veli Lake is the smallest estuarine wetland in the southwest coast of Kerala (08° 31 and 08° 31 ´ NL and 76° 522 30´ to 76° 532 30´ EL) situated at Thiruvananthapuram. In the close vicinity of the lake are two factories, the English Indian clay factory in the southern bank of the lake and the Travancore Titanium Products in the eastern side.

MATERIALAS AND METHODS

General ecological parameters i.e., environmental and physico chemical parameters of the Lake were carried out for a period of two years. The analysis of parameters was performed by the use of standard analytical techniques followed by APHA (1995). Data analysis was carried out with the help of commercially available software SPSS.

RESULTS AND DISCUSSION

The influx of sea water and fresh water output in the estuary may be the significant reason of higher water temperature (Nandan, 1997). Higher concentration of Phosphate, Nitrate and Ammonia may be due to the fertilizers, pesticides, decomposition of organic matter and input of sewage and similar observations were reported by Vass et al. (2015). Indication of organic and industrial pollution can be attributed with the elevated values of BOD and COD; similar results were obtained by Ambelu et al. (2013).

The Lake also has been deteriorated because of the pollution load due to the industrial discharge from Travancore Titanium Products and English Indian clay factory. Higher water temperature and sediment temperature values may probably due to the shallowness of estuary and meteorological characteristics (Patra et al., 2010).The place is becoming the point of waste disposal from hotels, hospitals and industries. It is noted that the parts of Veli Lake had been reclaimed for economic benefit by draining water for agricultural crops and building sector.

CONCLUSION

Nowadays the rate of wetland reduction is becoming accelerated which adversely affects the quantity and quality of Veli Lake. Management strategy of this area should address the key issues like retention of water quality. Wetland ecology studies may help to conserve natural habitat in standard quality.

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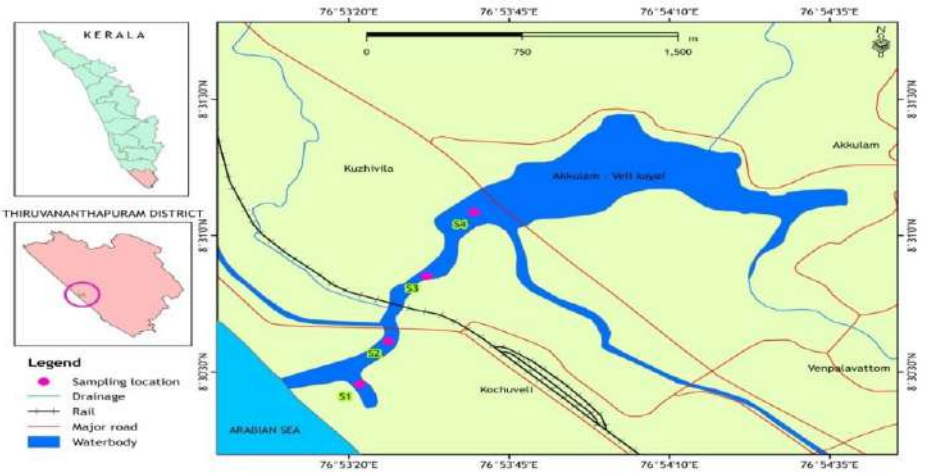

Fig 1. Map of Veli Lake showing the selected sites for sampling

Table 1. Descriptive statistics of Hydro-ecological parameters of Veli Lake

Variable	Maximum	Minimum	Mean± S.D
Atm. temp. (°C)	38	30	32.989±1.933
Water temp. (°C)	37	27	31.797±2.229
Sed. temp. (°C)	38.5	28.5	32.366±2.144
Water pH	9.5	7.7	8.681±0.402
Sediment pH	9.5	7.3	8.429±0.429
TDS (µ/l)	1580	600	962.5±211.97
EC (µ/l)	1900	840	1214.375±228.407
Salinity(ppt)	7.8	4	5.897±0.969
Phosphate (mg/l)	0.9	0.2	0.665±0.164
Nitrite (mg/l)	3.9	0.4	1.243±0.616
Nitrate (mg/l)	100	32.7	71.731±16.298
Ammonia (mg/l)	8.4	1.9	4.640±1.2554
Copper (µ/l)	190	60	129.916±31.811
Silicate (mg/l)	2	0.28	0.798±0.4091
Hardness (mg/l)	186	110	147.145±19.423
Alkalinity (mg/l)	115	65	82.739±9.685
DO (mg/l)	9	3.5	5.514±1.242
Organic matter (mg/l)	7.8	4.2	5.585±0.824
BOD (mg/l)	4.4	1.5	2.651±0.719
COD (mg/l)	14	1.5	9.35±2.623
Rainfall (mm)	411.1	27.4	180.525±118.251

PREMONSOON HEALTH STATUS ASSESSMENT OF KILLIYAR, A TRIBUTARY OF KARAMANA RIVER USING ODONATE POPULATION AS BIOMONITORING TOOL

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ABSTRACT
An aquatic ecosystem has a major role in regulating the life existing on earth. Purity and quality of water is essential for this process. The present study was conducted using aquatic odonates as bioindicators in Killiyar, a tributary of Karamana River to analyze the health status of the river. The investigation was conducted during the month of January 2019. The study adopted rapid bioassessment protocol to assess the impact of anthropogenic pressure in Killiyar. Odonata population showed variations in reference site and test sites. A total of 7 families of insects under Order Odonata were recorded from the reference site and reductions in the number of Odonata families were noticed on test sites. The decline in the number of sensitive families and significant increase in the number of tolerant families of Odonata in the test sites reveals considerable ecological degradation during study period.

Key words: Killiyar, Biomonitoring, Aquatic insects, Odonates

INTRODUCTION

Global population is increasing, modernization and rapid expansion of industrial and urban activities and unscientific agricultural practices results in the accumulation of waste materials in water bodies causing its gradual degradation (Subhendu, 2000). River water pollution is a major global issue and it is severely affects the aquatic biodiversity. Several stream ecosystems are heavily polluted by anthropogenic activities like dumping of sewage, agricultural runoff, urban waste and industrial effluents (Trivedy and Goel, 1985; Kumar, 2001). Biological approach to river water quality monitoring involves the use of the river organisms as a basis for assessing the intensity of pollution.

MATERIALS AND METHODS

Killiyar is the main tributary of Karamana River, originated from the ottakompu kunnu and karimchathi mala in Theerthankara in Nedumangadu taluk. It flows north- south direction for about 35 km through Kalliodu, Panavoor, Anad, Karakulam, Kodappanakunnu and Vattiyoorkavu panchayaths and it joins with the Karamana River at Pallathukadavu near Thiruvallam. Collection of entamofauna was conducted at four sites along killiyar, the study sites were Theerthankara (River origin), Vazhayila, Jagathy and Pallathukadavu respectively. The study sites were categorized into reference site and test sites. River origin was taken as reference site, where anthropogenic activity is minimum and was near

natural condition during the study period. The present investigation adopted rapid bioassessment protocol for sampling aquatic insects. Premonsoon sampling was done during the month of January 2019.

RESULTS AND DISCUSSION

Premonsoon collection of odonates from the reference site showed a total of 33 individuals under 7 families. Among the collected Odonata families Gomphidae constitutes the highest percentage it is 24.2%. and a least percentage of 6% was represented by family Coenagrionidae. In Vazhayila a total of 28 individuals of odonates under four families were recorded. The highest percentage was represented by Coenagrionidae is about 39.2% and Gomphidae constitute a least percentage of 10.7%. In Jagathy segment of Killiyar a total of 38 individuals under 2 families were collected a highest percentage of 55.2% is contributed by Coenagrionidae. A total of 47 individuals of odonates under two families were collected from Pallathukadavu segment 68% is dominated by family Coenagrionidae. Reduction in number and absence of some sensitive families of odonates in the test site indicates that pollutants are highly increasing in Premonsoon season especially due to anthropogenic activities. Aquatic ecologists show an enthusiasm towards relationship between river water quality and diversity of aquatic insects (Bonada *et al.*, 2007). Advantage of Biomonitoring is they give exact data about the anthropogenic effect on aquatic ecosystem about prolonged time period but the physico chemical data is only momentary evidence (Camargo *et al.*, 2004).

Table:1 Number and Tolerance values of insects under Order Odonata collected from reference and test sites of Killiyar during Premonsoon

S.l NO	Taxa			Number of insects			
	Order	Family	Tolerance value	Theerthankara (River origin)	Vazhayila	Jagathy	Pallathukadavu
1	Odonata	Calopterygidae	5	4	8	-	-
2		Corduliidae	4	6	6	-	15
3		Macromiidae	3	5	-	17	-
4		Gomphidae	1	8	3	-	-
5		Chlorocyphidae	0	4	-	-	-
6		Coenagrionidae	9	2	11	21	32
7		Euphaeidae	0	4	-	-	-
Total				33	28	38	47

CONCLUSION

River is a major source for humans. It must be properly protected to maintain its quality. Killiyar showed a significant level of pollution load during study period. Survival of aquatic insect population is severely affected due to anthropogenic pressure. Immediate attention is needed for the protection of river.

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MORPHOLOGICAL AND DNA BARCODING ANALYSIS FOR THE IDENTIFICATION OF COMMON FRESHWATER PRAWN SPECIES FROM TANEERMUKKOM, ALAPUZHA

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INTRODUCTION

DNA barcoding is emerging as an essential supportive tool for morphology based species identification, characterization and discovery of new species. Thaneermukkom river have a wide range of fresh water prawn species of economic and medical importance and their genetic diversity is still unknown. In this present study DNA barcodes were generated for four freshwater prawn species during off season and conducted their DNA isolation, Morphological Identification, DNA Sequencing, Genetic Divergence Analysis , DNA Barcoding and Phylogenetic tree construction. DNA barcoding done in fish species will help in the identification and further classification of fishes. (Nicolas *et al*., 2015)

MATERIALS AND METHODS

Samples were collected from Thaneermukkom river during the off season and was morphologically identified with the help of previous morphological description provided by jhingram,1991. DNA isolation was done by using Phenol-Chloroform-Isoamyl alcohol: SDS/ PCI, then stored in -20°C and runed in 0.8 % Agarose Gel electrophoresis, and photograph was taken using Bio-Rad Gel Documentation unit. PCR was carried out using co1 fish forward and reverse primers under specific thermoprofile. DNA Sequencing was done by sanger method and performed Homology search for obtained sequences using Blast n Algorithm. BLAST is one of the most widely used bioinformatics programs for sequence searching.(Casey *et al*., 2005) . Conducted Phylogenetic Analysis using Neighbour Joining Algorithm and Multiple Sequence Analysis using Clustal w Algorithm.

RESULTS AND DISCUSSION

Actual length of trimmed sequences where 638, 658, and 576 base pairs for *Macrobrachium rosenbergii, Penaeus monodon*, and *Macrobrachium malcolmsonii* respectively. Multiple sequence alignment followed by phylogenetic analysis placed our query sequences in the *M. rosenbergii* cluster. Thus confirmed the isolated sequences as *M. rosenbergii* with an alignment of 98.9%, *M. malcolmsonii* having 99.48% and *P. monodon* of 99.54%. Blast resulted in same sequences for *M. rosenbergii* male and female. No insertion, deletion were observed in any sequences. Multiple sequence analysis

resulted in consensus length of 650 sites including base pairs and gaps. In the phylogenetic tree it is revealed that *M. rosenbergii* and *P. monodon* belongs to closest cluster compared to *M. malcolmsonii*. The average nucleotide composition for co1 was 27.4% A, 31.4% T, 22.4% C and 18.8% G. The inter specific distance o

1.11 % and intra specific distance of 0.31% observed between obtained species. From the present study DNA barcoding is considered to be a suitable method for differentiating species in a group when interspecific variation exceeds intraspecific variation by one order of magnitude, which is called the _barcoding gap' (Ward *et al* ., 2005). the resulted genetic variability further compel more extensive sampling and generation of more DNA barcodes data of studied species from different geographical localities to resolve the genetic dissimilarity.

Samples collected

Tiger prawn, Bull male, Naaran konju, Mottakonju.

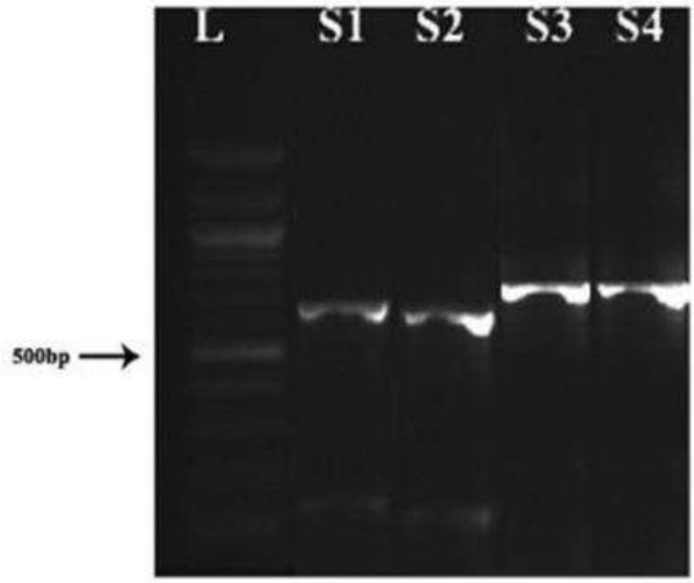
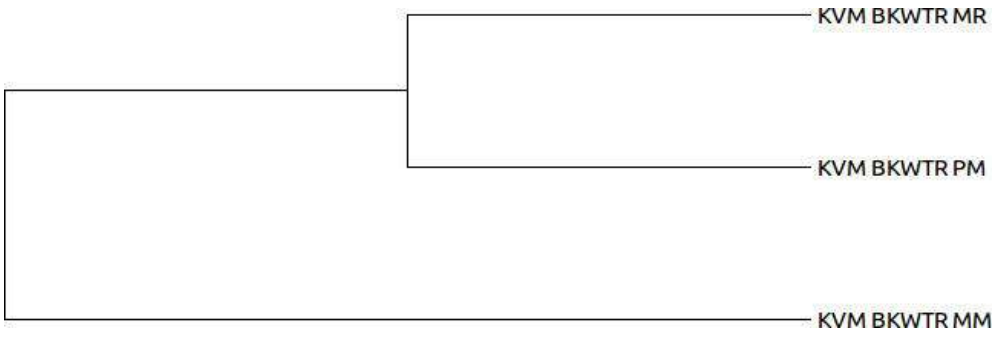


Figure 6: 1% AGE showed ~500bp of genomic DNA.

L-100bp ladder; S1- *Macrobrachium malcolmsonii*, S2- *Penaeus monodon*, S3- *Macrobrachium rosenbergii*(bull male), S4- *Macrobrachium rosenbergii*(berried female).

Phylogenetic tree topology

Phylogenetic significance of COI gene sequences generated in different species of freshwater prawns,



Neighbour-joining phylogram showing the relationship between CO1 haplotypes from *M. rosenbergii, P. monodon* (PM) and *M. malcolmsonii* (MM) species.

CONCLUSION

In this present study the sequences *M. rosenbergii M. malcolmsonii* and *P. monodon* showed considerable degree of variation in the amino acid sites which indicate that they were very well discriminated. Phylogenetic significance of co1 gene generated in different species of freshwater prawns revealed that *M. rosenbergii* and *P. monodon* belongs to the closest cluster compared to *M.malcolmsonii*. Hence it implies that there is no accurate sequence of this species alignment in NCBI and can be a new strain. Thus further identification and studies are required for its validation.

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STUDY ON THE WATER QUALITY AND PLANKTON DIVERSITY OF SURFACE WATER IN AN URBAN AREA

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ABSTRACT

Protecting surface water for health is a challenge set forth in Sustainable Development Goal which ultimately contribute to environmental and public health protection. The present study is aimed at understanding the status of the physico-chemical parameters and the plankton diversity in the Parvathy Puthen aar, the fresh water surface body flowing through Chackai, the urban area in Thiruvananthapuram city. Water samples were collected for analysis during the South West monsoon, North East monsoon and Post monsoon period of study during August-February. Twenty two physico-chemical parameters of the samples were determined using standard analytical methods prescribed by APHA, 2005. Bacteriological analysis was done to determine Total and Faecal Coliform bacteria. The phytoplankton and zooplankton diversity were studied. The results show that most of the physico-chemical parameter values were not within the desirable limits. Iron content ranged from 0.19-0.27mg/L and showed significant difference at $p<0.001$. The value is very near the permissible limit which indicates the chances of metallic contamination and bioaccumulation if not seriously looked upon. Zinc and Copper content were detected in the water source in minor quantity of 0.02mg/L and 0.01mg/L respectively in all seasons. More number of bioindicators of pollution point to the threat existing to other communities. The present study indicates the importance and need of conservation of aquatic biodiversity and maintenance of habitats in addition to safeguard sustainable fresh water resources.

Key words: Physico-chemical parameter, Bioindicators, Urban area, Sustainable development

INTRODUCTION

Surface water naturally carries sediments, debris and pathogens. Natural leaching of organic matter and nutrients from soil, by hydrological factors that lead to runoff and by biological processes within the aquatic environment can alter the physical and chemical composition of water **[1].** Manmade contaminants like household chemicals, agricultural chemicals and other organic and inorganic impurities leach in to the surface water and affect the aquatic biodiversity and destroy the critical habitats. The highly polluted surface water can contaminate the main fresh water resources, mostly used as potable water. Water quality thus impacted negatively by both natural and human causes need to be protected from quality degradation and conserved for human use since the demand for water resources is increasing and the availability is declining due to poor water quality of surface sources and loss of sources like ponds and springs. Scientists have studied the planktons as an index of water quality with respect to industrial, municipal and domestic pollution **[2].** The physical and chemical characteristics of water bodies affect the species composition, abundance, productivity and physiological conditions of aquatic organisms. These stressed systems support an extraordinarily high proportion of the world's biodiversity **[3].** The present study is

aimed at understanding the status of the physico-chemical parameters and the plankton diversity in the Parvathy Puthen aar, the fresh water surface body flowing through Chackai, the urban area in Thiruvananthapuram city.

METHODOLOGY

Water samples were collected directly from the Parvathy Puthen aar flowing through Chackai region during morning hours between 6.00 and 10.00 A.M during South West monsoon, North East monsoon and Post monsoon period of study during August-February. Samples for physico-chemical analysis were collected in clean polythene cans and for bacteriological analysis in pre-sterilised bottles. Plankton was collected using plankton net and preserved in 4% formaldehyde solution **[4].** Analysis was done as prescribed by APHA, 2005 **[5]** to understand whether the values were within the tolerance limit. The parameters studied were colour, odor, turbidity, pH, conductivity, total hardness, total dissolved solids, BOD, COD, DO, alkalinity, chloride, phosphate, sulphate, nitrite, sodium, potassium, iron, carbonate, copper, zinc and lead. Color, odor and turbidity were checked to understand the grade of water quality. Turbidity was measured using Nephelometry Turbidity meter. pH was measured using digital pH meter and electrical conductivity using conductivity meter. Alkalinity was measured by titrimetric method. Sodium and Potassium was measured by Flame photometer. Sulphate and Nitrate values are measured by UV-Visible spectrophotometer. COD, BOD and DO, the most important water quality parameters was also done. Statistical analysis was done using SPSS version 10 and were compared with the BIS water quality. Plankton was studied under microscope and was identified using standard keys**.**

RESULTS AND DISCUSSION

The physico-chemical parameters of the samples showed significant seasonal variation in the water quality. pH showed a slightly acidic tendency during all the seasons. Kaul and Handoo **[6]** found that increased surface pH in water bodies is due to increased metabolic activities of autotrophs, because in general they utilize the carbon dioxide and liberate oxygen thus reducing H^+ ion concentration. The water was partially stagnant due to the deposition of sediments and suspended particles and degradation of organic matter. The water was highly turbid and had a bad odor during the seasons. The colour ranged 40-50 Hazen Units. Conductivity value, a important physical water quality parameter showed a significant difference ($p<0.0001$) during seasons with the highest value of 1527.33µS/cm in North East monsoon which was beyond the tolerance limit. Total hardness value of the water sample was high in South west monsoon and was beyond the permissible limit. The value showed significant seasonal variation ($p<0.0001$). Calcium and Magnesium values were beyond the permissible limit of BIS in all the seasons. COD value was higher than BOD in all the seasons indicating the presence of both organic and inorganic mater in the water source. DO value was maximum in monsoon months and below 1mg/L in the post-monsoon period. DO concentration is a key parameter for characterizing natural and wastewaters and for assessing the global state of the environment in general **[7].**Alkalinity value showed significant seasonal variation ($p<0.0001$). Chloride value was maximum (244.67mg/L) in the South West monsoon and showed a significant difference ($p<0.0001$). Chandrasekhar et al **[8]** studies showed the presence of chloride concentration in a water source is used as an indicator of organic pollution by domestic sewage. High sulphate value was seen in the water sample in all the seasons and a bad odour indicating high contamination. Nitrite value showed a significant difference ($p<0.0001$). Sodium and Potassium content in the water body was within the desirable limit and showed significant difference at $p<0.002$ and $p<0.01$. Iron content ranged from 0.19-0.27mg/L and showed significant

difference at $p<0.001$. The value is very near the permissible limit which indicates the chances of metallic contamination and bioaccumulation if not seriously looked upon. Zinc and Copper content were detected in the water source in minor quantity of 0.02mg/L and 0.01mg/L respectively in all seasons. Lead content was not detected in the water body during the study period. Bacteriological results indicate the surface water source not good for domestic use **[Table.1]**. Phytoplankton diversity was more in the water body compared to zooplankton. Fluctuation in diversity was seen during the study **[Table.2].** Bhade et al. **[9]** observed positive correlations of phytoplankton and total hardness of freshwater systems. This might be the reason for the high productivity seen in this water source. A remarkable evidence of Chlorophyceae and Cyanophyceae species, well known to be tolerant to organic pollution were seen in this habitat. Phytoplankton population observation may be used as a reliable tool for biomonitoring studies to assess the pollution status of aquatic bodies **[3].** Zooplanktons are delicate microscopic organisms and are found to be diverse and play a key role in the pelagic food web. Zooplankton like Euglena, Paramecium and Brachionus were noted. Zooplanktonic organisms are bioindicators of water quality because of their growth and distribution are closely correlated to ecological environmental parameters **[10]**

CONCLUSION

The analytical result gives a clear supportive evidence of the pollution status of the water body. Plankton diversity in the water source forms the basis for food webs in the freshwater ecosystem. The frequency of more number of bioindicators of pollution point to the threat existing to other plankton communities. The study thus clearly point to the need to maintain good water quality needed to encourage the proliferation of all class of phytoplankton and zooplankton that is essential to maintain a good ecosystem. The study concludes with the need to stop the unhealthy anthropogenic activities necessary to conserve a fresh water resource and aquatic biodiversity for sustainable development.

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Table 1. PHYSICO-CHEMICAL PARAMETERS OF THE SURFACE WATER OF PARVATHY PUTHENAR

Parameters	August		November		February		Total		F	Sig.
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
pH	6.47	0.21	6.60	0.10	6.57	0.15	6.54	0.15	0.565	0.596
Conductivity	997.00	102.09	1527.33	6.43	631.00	10.54	1051.78	393.66	172.849	0.000
Turbidity (NTU)	34.00	1.00	34.33	2.08	33.33	0.58	33.89	1.27	0.412	0.680
Total hardness (mg/L as CaCO3)	654.33	32.81	589.33	11.50	572.00	3.61	605.22	41.45	13.880	0.006
Total Dissolved Salts (ppm)	988.00	42.46	902.00	16.70	754.67	2.08	881.56	104.71	60.068	0.000
Ca. Hardness (mg/L as $CaCO_3$)	506.67	28.87	475.67	5.86	470.67	1.15	484.33	22.42	3.939	0.081
Mg. Hardness (mg/L as $CaCO_3$)	147.67	5.13	113.67	6.43	101.33	4.73	120.89	21.32	57.581	0.000
BOD (Mg/L)	6.90	0.95	24.00	2.00	10.33	1.53	13.74	7.95	101.675	0.000
COD (Mg/L)	43.00	7.55	63.67	1.53	46.00	3.61	50.89	10.56	15.516	0.004
D O (Mg/L)	1.40	0.26	0.10	0.10	0.37	0.06	0.62	0.61	50.920	0.000
Alkalinity (mg/L as $CaCO_3$)	101.00	1.00	129.33	3.51	106.67	3.21	112.33	13.21	85.479	0.000
Chloride (mg/L)	244.67	9.87	228.67	5.03	98.67	2.08	190.67	69.58	454.394	0.000
Phosphate (mg/L)	0.18	0.02	0.17	0.02	0.21	0.01	0.19	0.02	4.826	0.056
Sulphate (mg/L)	35.00	1.00	32.67	1.53	32.00	1.00	33.22	1.72	5.154	0.050
Nitrite (mg/L)	2.24	0.02	2.33	0.02	2.02	0.04	2.20	0.14	107.922	0.000
Sodium	38.37	0.38	37.53	0.35	36.73	0.15	37.54	0.76	20.701	0.002
Potassium	12.90	0.80	11.29	0.47	12.93	0.15	12.37	0.94	8.966	0.016
Carbonate	60.56	0.60	77.55	2.11	63.96	1.93	67.36	7.92	85.479	0.000
Iron (mg/L)	0.19	0.02	0.21	0.02	0.27	0.01	0.22	0.04	30.059	0.001

Total Coli form (TC/100ml)	87333.33	2081.67	78000.00	2000.00	64166.67	1040.83	76500.00	10210.29	129.850	0.000
Faecal Coli form (FC/100ml)	39000.00	1000.00	32333.33	577.35	10766.67	873.69	27366.67	12800.78	934.831	0.000

TABLE 2. PLANKTON DIVERSITY IN THE SURFACE WATER OF PARVATHY PUTHENAR

Types	Presence	Class	Types	Presence	Class
PHYTOPLANKTON					
Anabaena	++	Cyanophyceae	Isochrysis	++	Pryminisiophyceae
Actinastrum	+++	Chlorophyceae	Nodularia	+++	Cyanophyceae
Biddulphia	++	Bacillariophyceae	Navicula	+++	Bacillariophyceae
Chaetophora	+++	Cyanophyceae	Nitzschia	+++	Bacillariophyceae
Eunotia formica	++	Bacillariophyceae	Oscillatoria	+++++	Chlorophyceae
Chlorococcum	++++	Chlorophyceae	Planktosphaeria	+++	Chlorophyceae
Coelastrum	+++	Chlorophyceae	Scenedesmus	++++++	Chlorophyceae
Chlorogonium	+++++	Chlorophyceae	Sphaerocystis	++	Chlorophyceae
Chlorella	+++	Chlorophyceae	Spirulina	++++	Cyanophyceae
Closterium	+++++	Chlorophyceae	Spirogyra	+++	Zygnematophyceae
Cyclotella	++	Cosinodiscophyceae	Stauroneis	++++	Bacillariophyceae
Gomphosphaeria	++++	Cyanophyceae			
ZOOPLANKTON					
Brachionus	++++	Monogononta			
Euglena	+++++	Monogononta			
Paramecium	++++	Ciliata			
Lepocinclis	++	Euglenophyceae			

ANALYSIS OF BIOCHEMICAL CONSTITUENTS IN THE MUSCLE OF LIZA PARSIA (HAMILTON, 1822)

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Introduction

Fish and fishery products are generally regarded as important part of a healthy diet. Apart from being a source of cheap animal protein, they are widely consumed since they have high quality proteins, other essential nutrients, low in saturated fat and contain omega 3 fatty acids (NSPFS, 2005).

Fish is a favorite foodstuff for the majority of societies. Fish meat contains most significant nutritional components and serves as a source of energy for human beings (Ojewola and Annah, 2006; Sutharshiny and Sivashanthini, 2011). Fish is also a vitamin and mineral rich food for young as well as old age people (Edem, 2009; Moghaddam et al., 2007). Fish protein occupies an important place in human nutrition. It has high digestibility, biological and growth promoting value. Biochemical composition of fish tissues are of considerable interest for their specificity in relation to food values of fish and evaluating their physiological needs at different periods of life. Fish exhibit large variations in their biochemical composition from species to species. Hence, the knowledge of proximate compositions of fish is of paramount importance to evaluate it in regard to nutrient value and physiological condition (Brown and Murphy, 1991).

Biochemical analysis is an index of nutritive value. Body composition illustrates the nutritional quality of the food because analysis of biochemical composition including protein and fat is very important in assessing food value (Kamal et al., 2007). So, biochemical evaluation is necessary to ensure the nutritional value as well as eating quality of fish (Azam et al., 2004). In general, the biochemical composition of the whole body indicates the fish quality. Therefore proximate biochemical composition of a species helps to measure its nutritional and edible value in terms of energy units compared to other species. The spawning cycle and food are the main factors responsible for the variation (Love, 1980). Nutritional value of the fish can be calculated by its chemical composition which includes protein, lipid, moisture, and ash content (Moghaddam et al., 2007; Zafar et al., 2004).It was found to be varied from species to species,

within the same species (Fawole et al, 2007) and also by feeding habit, sex and seasonal variation (Islam et al., 2005). The Knowledge of chemical composition is essential in order to compare its value as food with other protein foods. It is also necessary to have data on the composition of fish in order to make the best use of them as food and in order to develop the technology of processing fish and fish products.

A number of studies have been carried out on the chemical, proximate composition and caloric energy value of several fish species *Cyprinus carpio* (Sivakami et al., 1986); *Clarias batrachus* (Sinha and Pal, 1990); *Channa striatus* (Jyotsna et al., 1995); Cat fish (Abdullahi, 2001); Silver carp (Ashraf et al., 2011); *Glossogobius giuris* (Islam et al., 2005); *Scylla serrata* (Zafar et al., 2004); *Liza ramada* (Mustafa et al,2008); *Tilapia mosambicus* (Adefemi,2011); *Schizothorax* (Ghulam et al., 2012); *Labeo niloticus* (Elagha et al., 2013). The biochemical analysis of fishes of Asthamudi lake especially that of mullet is scarce. So the present study was undertaken to evaluate the biochemical composition of muscle, liver and ovary of *L. parsia* of Ashtamudi lake.

Material and methods

L. parsia were collected from Ashtamudi lake , the Ramsar site from December 2010 to November 2011. The collected fishes were cleaned and the tissues were taken from three different regions viz, muscle, liver and ovary. The tissue extract was obtained by homogenization and subsequent centrifugation. The supernatant was used for the estimation of lipid whereas the precipitate was utilized for the estimation of carbohydrate and protein.

Estimation of the moisture content was carried out by drying the pre-weighed wet samples at 60 °C until a constant weight was obtained. The difference in weight was calculated and expressed as percentage moisture content of the sample. Total protein was assayed by the folin-ciocalteu method using Bovine Serum Albumin (BSA) as standard (Lowry et al., 1951). The total glycogen content was estimated by the method of Dubois et al. (1956). The total lipid content was estimated by the method of Folch et al. (1957). Ash content was determined by muffling the sample at 6000-7000°C to dry ash. The energetic values were determined indirectly- using Rubner's coefficients for aquatic organisms: 9.5 kcal g-1 for lipids, 5.65 kcal g-1 for proteins (Winberg, 1971), and expressed in kJ g-1 wet mass as described by Eder and Lewis (2005). One way analysis of varience (ANOVA) was done to check the significant

variation among the three sites and post hoc multiple comparison (LSD) was done for the parameters which showed variation among the sites.

Results

The monthly percentage of proximate composition of carbohydrate, protein, lipid, moisture and ash in the muscle of *L. parsia* from of Ashtamudi lake are shown in table 1. The monthly percentage of carbohydrate in the muscle varied from . It showed that the carbohydrate percentage found to be more during the month of May and June where the fishes are in the immature stage. Monthly percentage of protein in the muscle of *L. parsia* from three sites ranges from. The muscle protein was found to be higher in the month of December (32.81%) in site 1. Matured fishes from all the three sites showed higher percentage of protein. Lowest value of muscle protein was shown by *L. parsia* during the month of May, June and July where the fishes are in the immature stage. The monthly percentage of lipid content in the muscle of *L. parsia* ranged from Lipid content was found to increase from immature to maturing fishes and found to decrease during the month of November to March in which the fishes reached the sexual maturity. The highest percentage was shown by site 2 (2.98%) during the month of May followed by site 3 and site 1.

Monthly concentration of moisture in the muscle varied from The mean concentration of moisture in the muscle was found to be 71.37% ± 2.90, 61.34% ±1.77 and 69.27% ± 2.84 at site 1, 2 and 3 respectively. The moisture content showed inverse relationship with maturity because it gradually reduces when the fishes attaining maturity. Moisture content in the muscle was found to be low in the month of December, February and November in all the three sites.

Monthly percentage of muscle ash showed an inverse relationship with maturation at all three sites and it varied from 0.39% to 0.81% at site 1 and 0.29% to 1.61% at site 2 and 0.35% to 1.72% at site 3 (Table 6.10 and Fig 6.13). Ash content decreased with the maturity of fish. The highest percentage of ash in the muscle was shown by site 1 during the month of May (1.81%) where the fishes are in the immature stage. Mean percentage of ash in the muscle of *L. parsia* at site 1, 2 and 3 are 0.9 ± 0.49, 0.77 ± 0.47 and 0.84 ± 0.47 respectively.

Values of biochemical composition of the tissues of *L.parsia* from three sites of Ashtamudi lake were pooled together to determine the biochemical constituent of *L. parsia* of Ashtmaudi

lake. Results are depicted in Fig 6.16 to Fig 6.18. Results showed that in muscle, moisture account for 68 % followed by protein (27%), lipid (2%), carbohydrate (2%) and ash (1%).

Proximate composition in the muscle of *L.parsia*

Month	Carbohydrate %	Protein %	Lipid%	Moisture %	Ash%
December	1.30	30.62	1.74	64.02	0.35
January	1.23	29.62	1.76	68.21	0.51
February	1.31	30.42	1.80	64.37	0.35
March	1.43	28.2	1.84	65.64	0.76
April	2.16	26.78	2.45	65.92	0.88
May	2.51	24.12	2.67	67.32	1.71
June	2.56	22.64	2.39	71.51	1.59
July	1.75	24.74	2.56	70.28	1.35
August	1.71	25.54	2.40	70.38	0.89
September	1.51	27.88	2.56	68.15	0.82
October	0.99	28.62	2.69	66.25	0.42
November	1.12	29.50	2.19	65.91	0.37

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Discussion

Proper knowledge on the biochemical composition of fish finds application in several areas. According to Kingston and Venkataramani (1994), knowledge of biochemical composition of fish is of great help in evaluating its nutritive value. The major constituents in the edible portion of the fish are moisture, protein, lipid, carbohydrate and ash. The analysis of these constituents in the tissues of fish is often referred as proximate analysis. Fish and fish products are found to be important source of protein in the human diet. As this protein contains all the ten essential amino acids, the fish are superior to any other protein source (Srivastava, 1999). The factors responsible for the variation in the biochemical composition comprise spawning cycle, food supply, fishing ground, fishing season, age and sex of the individual and reproductive status (Love et al., 1980).

In the present study carbohydrate forms a minor percentage of total composition in muscle. Carbohydrate content in fish is generally very low and practically considered zero (Payne et al., 1999; Anthony et al., 2000). Low values of carbohydrate in muscle were that in fishes it does not contribute much to the total reserve material of the body (Jayasree et al., 1994). Muscle carbohydrate percentage in the present study showed variation as the fish matures. Muthukaruppan (1987) also observed the same trend in *L. parsia.* It is discerned that carbohydrate content decreased with the translocation of carbohydrates from deposit site to where the energy prompt is required. Sivakami et al. (1986) observed gradual increase of muscle carbohydrate content with the maturation of gonads in the *C. Carpio.*

Fish protein contains all essential amino acids which are easy to digest. The protein digested and assimilated is mostly incorporated in the muscles of the fish (Dabhade et al., 2009). Ali and Qaiser (2001) reported that protein content, which is a vital component of living cells, tends to vary comparatively little in healthy fish unless drawn upon during particular demands of reproduction or during food deprivation periods. In the present study protein was found to be the one of the most dominant biochemical constituent in the tissues of *L. parsia*. Muscle, rich in proteins, forms mechanical tissue intended for mobility and do not participate in metabolism. In *L. parsia,* muscle protein percentage showed increase with gonadal maturation. Muthukaruppan (1987) also observed increase of muscle protein in *L. parsia* during gonadal maturation. Ando and Hatano (1986) found a positive correlation between gonado somatic index and muscle protein content in *chum salmon*. Sivakami et al. (1986) found that muscle protein percentage increased from the second stage of gonadal maturation in *C. Carpio*. The study showed that the *L. parsia* has high protein content in its muscle Gokhan and Hikmet (2011) in their study in the mullet species had found a protein percentage of 16.19%, where as Elagha Haji (2013) found that Nile fishes in the Sudan water has a protein content of 31%-76% in their muscles. In the present study the muscle protein was the highest. Kumaran et al. (2012) got a protein value of 18% in *Mugil cephalus* and Mustafa et al. (2008) got protein parentage of 17% in *L. ramada*. As the protein forms the major biochemical constituent in the tissues of *L. Parsia,* it revealed the nutritious value of this fish. The change in the protein content in the body of fishes depends on time of year, environmental condition, stage of maturity of the gonads, state of nutrition and age (Mustafa et al., 2008).

According to Ackman (1989), generally fish can be grouped into four categories according to their fat content: lean fish (<2%), low fat (2–4%), medium fat (4–8%), and high fat (>8%). In

the present study *L. parsia* comes under the category of low fat fish in as the lipid in the muscle ranges between 2- 4 in all the three sites. In the present study, it has been observed that muscle lipid content of *L. parsia* got depleted with the maturation of gonads. The results are in agreement with that of Masurekar and Pai (1979) who observed muscle fat content depletion with the maturation of gonads in *C. Carpio.* Nelson and Mc Pherson (1987) who observed decrease in lipid content in muscle and viscera of brook char (*Salvelinus fontinalis*) with the progress of reproduction. Earlier works in *Sparus auratus* (El-sayed et al., 1984), *pelagic sculpins* (Kozlova, 1997), *chum salmon* (Ando and Hatano, 1986) and *L. parsia* (Muthukaruppan, 1987) have also shown correlation of muscle fat depletion with the maturation of gonads. However, muscle lipid content increases with the maturation of gonads in *C. Carpio* (Sivakami et al., 1986) and in the *common dentex* (Chatzifotis et al., 2004). The correlation in the lipid content in different tissues of *L. parsia* varied with the maturation of gonads. It was observed that liver lipid content drastically decreased as the fish matures and that consequently ovary lipid content increased with maturation of gonads. This is suggestive of the mobilization of liver and muscle lipids to the ovary with the progress of vitellogenesis. Mobilization of lipids to the gonads takes place during the spawning period to the gonads.

In the present study moisture was found to be the major component of the biochemical composition in the tissues of fishes from all the three sites and throughout the investigation period. Water is essential for all living systems. Body fluid acts as the medium of transport for all the essential metabolites and nutrients. It is required for the normal functioning of many biological processes. In *L. parsia* the moisture content was found to decrease in all the tissues with the maturation of gonads. The low muscle moisture percentage in matured fish may be due to its replacement with proteins. The results are in agreement with Sivakami et al. (1986) who recorded decrease in muscle moisture percentage in *Cyprinus carpio* with the advancement of maturation where major energy reserves in the muscle are mobilized for the growth of gonads. Masurekar and Pai (1979) noticed fluctuating muscle water content during the maturation of gonads in *Cyprinus carpio.* In the present study, ash content was less in the muscle while more in ovary when the fish gets matured. This indicates that of mobilization of minerals from muscle to ovary takes place with the maturation of gonads. Sivakami et al. (1986) observed decline of muscle ash content from stage III to stage V in the female *C. carpio*.

The present study may provide an insight in rate of turnover of various biochemical constituents due to the influence of pollutants from various sources. Results of biochemical analysis showed

that moisture, protein and carbohydrate were found to be higher in the fishes at site 1 followed by site 3 and site 2. Lipid was found to be higher at site 2 which may be due to relatively more pollutants in comparison to other sites. The study also revealed the fluctuating trend in biochemical constituents associated with gonad maturation. Further, the study revealed that *L.parsia* from Ashtamudi lake is highly proteinacious, but if anthropogenic influence persists it will tend to decrease the quality of the fish.

HISTOLOGICAL ASSESSMENT OF *RASBORA DANDIA* EXPOSED TO CLOVE OIL

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ABSTRACT

Histopathological changes have been widely used as biomarkers for evaluating degree of pollution or toxic effect of an organism. In the present study, gills and liver histological integrity of *Rasbora dandia* exposed to clove oil anaesthesia in simulated transportation condition for 24 hr were clearly distinguished with control fish group. Histological alterations observed in gills and liver were considered as defensive mechanism of fishes. The secure margin of anaesthetics does not affect the normal condition of *R. dandia* gills and liver.

Key words **:** Clove oil, Gills, Histology, Liver

Introduction

Histological study widely used as the biomarkers for assessing the health, degree of pollution, toxicity of contaminants and immune response (Khoshnood et al., 2010). Histology represents a useful tool to assess the degree of effectiveness of anaesthetic dose. The aim of the present study was to assess the optimal concentration of clove oil anaesthetic with the degree of expression of histological changes in gills and liver tissues of *Rasbora dandia* (Slender rasbora) and compare with the non anaesthetized group. Fish gills considered as the most sensitive organ to physical and chemical alteration of the aquatic medium. Liver plays primary roles in the metabolism and excretion of compounds with alterations occurring in some toxic conditions (Rocha and Monteiro, 1999). Histological alterations were noticed after 10 min and 24 hr exposure and they were compared with non-anaesthetized fishes.

Material and Methods

Ten numbers of *R. dandia* (6.03±1.1 g) were taken in the polyethylene cover contains 1 L of water mixed with 10 mg Clove oil (Micro Fine Chemicals, India) for the 24 hr simulated transportation experiment. 420 numbers of *R. dandia* (6.13±1.15 g) were used for histological study after anaesthetic treatment. Fish were divided into fourteen groups includes six anaesthetic groups and common control groups for 10 min and 24 hr experiment. In each treatment groups, three replicates with 10 numbers of fish were maintained. For histological study, fish were dissected and their gill and liver were extracted from the first seven groups after 10 min exposure. The remaining groups were dissected after 24 hr for the study. Each of the samples was fixed with 20% formalin and then tissue processing steps were done according to the standard histological techniques (Lakshmi Narayanan, 2011). Glass slides were examined by light microscopy. Microphotographs of the changes induced by anaesthetic treatment in the gill and liver tissues were taken using an OLYMPUS CH 20i Microscope with Olympus E 420 Camera.

Result and Discussion

After 10 m and 24 h of simulated transportation, control group of Slender rasbora gills stands without any histopathological changes (Fig. 1. A and B).

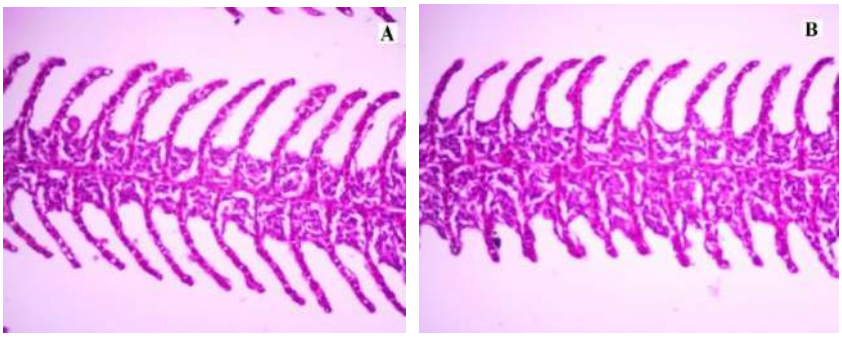

Figure 1. Section through the gill of *R. daniconius* after 10 min (A) and 24 hr (B) of simulated transportation without anaesthetics, H&E stain; 400x magnification

Histological alterations in the gills of *R. danconius* after 10 min of clove oil treatment included lamellar fusion (LF), mild epithelial lifting (EL) and edema (E) (Fig.2. A). After 24 hr of simulated transportation fish gills (Fig. 2. B) noticed with mild epithelial lifting (EL), epithelial hyperplasia, edematus (E) secondary gill lamellae.

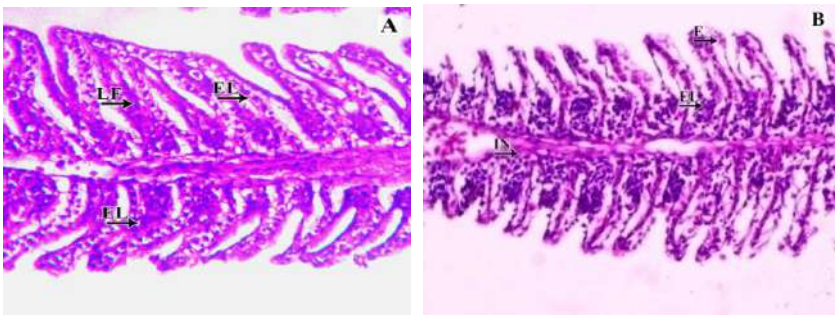

Figure 2. Section through the gill of *R. daniconius* after 10 min and 24 hr clove oil (A and B) H&E stain; 400x magnification (EL- Epithelial lifting; LF- Lamellar fusion; E- edema)

Separation, lifting up of the epithelium and edema might be defense response of the fish (Van Heerden et al., 2004) in response with surrounding environment could serve as mechanism of defense. Osman et al., (2010) observed as edematous changes in gill filaments are probably due to increased capillary permeability.

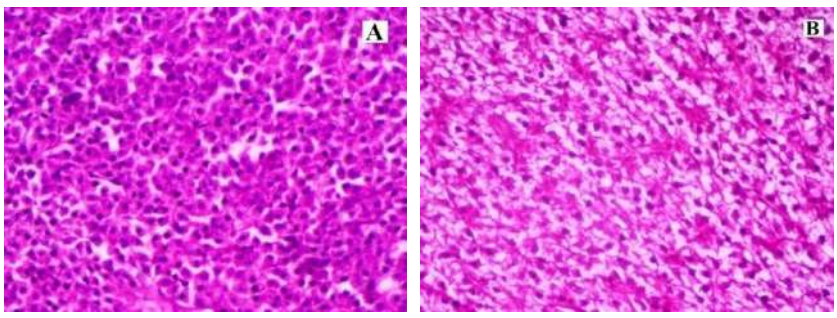

Figure 3. Section through the liver of *R. daniconius* after 10 min (A) and 24 hr (B) of simulated transportation without anaesthetics, H&E stain; 400x magnification

Control fish (without anaesthetics) liver after 10 min and 24 hr of simulated transportation (Fig. 3 A and B) were normal hepatocytes in cords and traberculae. In clove oil treatment moderate dilated sinusoid were observed after 10 min and 24 hr exposure (Fig. 4. A and B).

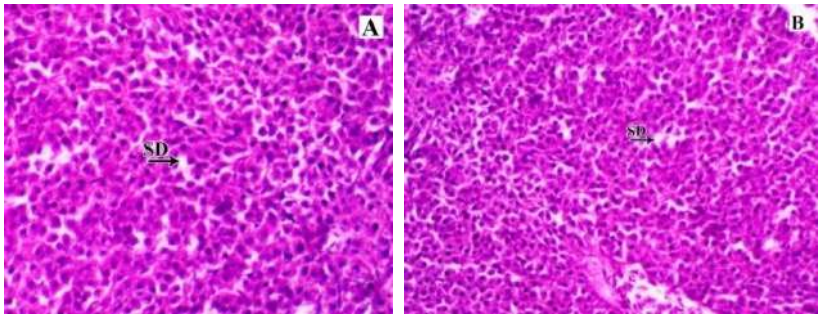

Figure 4. Section through the liver of *R. daniconius* after 10 min and 24 hr on Clove oil (A and B) anaesthesia, H&E stain; 400x magnification
(SD- Sinusoid dilation)

Summary and Conclusion

In the present study, it is concluded that, gill and liver histological alteration could be due to reaction of the fish anaesthetics intake and an adaptive response, therefore they could serve as defensive mechanism. Progressive anaesthesia with higher dose may leads to sever histological alteration. Results of the present study suggest that the use of anaesthetics at acceptable dose does not cause irreversible damage in the test fishes and the literatures also support the same.

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STUDIES ON PROXIMATE ANALYSIS OF SOME COMMERCIALLY IMPORTANT FISH SPECIES OF KOLLAM DISTRICT, KERALA.

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ABSTRACT

Proximate analysis of Rastrelliger kanagurta (Indian mackerel), Priacanthu sarenatus (Red snapper), Daysciaena albida (Spotted croaker), Mystus gulio (Catfish), Siganus fuscescens (Rabbit fish) and Gerresery throurus (Silver biddy) of Kollam coast were analysed during the present study. The moisture contents of the fishes varied from 80.1 to 87.9 % with Daysciaena albida having the highest moisture content followed by Priacanthus arenatus. The protein content of fishes ranged from 9.9 to 18.3%. All the fish types were found to be less in fat concentration and all the fishes come under the category of low fat fish, as the lipid in the muscle found to be below 2. The mean values of the ash for six species ranging from 0.7 to 1.1%. The six fish species investigated are good sources of protein and the study suggest that the fish species can be recommended for consumption.

INTRODUCTION

Throughout the world, it is well accepted that fishes are good sources of animal protein and other elements for the maintenance of healthy body (Andrew, 2001). Fish flesh contains up to 15-25% protein, 80% water, 1-2% mineral matter (CSIR, 1962). Biochemical composition of fish is a good sign for the fish quality (Hernandez, 2001), the physiological condition of fish and habitat of fish (Aberoumad and Purshafi, 2010; Shamsan and Ansari, 2010). The Knowledge of chemical composition is essential in order to compare its value as food with other protein foods. It is also necessary to have data on the composition of fish in order to make the best use of them as food and in order to develop the technology of processing fish and fish products.

In this study Six commercially important fishes from the Marine and freshwater were selected from the kollam district of Kerala. The fish selected were *Rastrelliger kanagurta* (Indian mackerel), *Priacanthus arenatus* (Red snapper), *Daysciaena albida* (Spotted croaker), *Mystus gulio* (Catfish), *Siganus fuscescens* (Rabbit fish) and *Gerres erythrourus* (Silver biddy).

MATERIALS AND METHODS

Six f*ish* samples were collected from the Kollam coast during the month of March 2019. The collected fishes were cleaned and for biochemical analysis, a portion of the muscle from the widest part of the body (devoid of bones) after removal of skin wastaken and used for moisture, protein, fat and carbohydrate determination. The tissue extracts were obtained by homogenization and subsequent centrifugation. The supernatants were used for the estimation of lipid whereas the precipitates were utilized for the estimation of carbohydrate and protein.The moisture, protein, fat and ash contents of the fish species were determined using loss in weight, Lowry *et al* method, Barnes and Blackstock method and dry-ashing.

RESULTS AND DISCUSSION

Proximate compostion of selected fishes along the Kollam coast was represented in Table 1. Moisture forms the major component of the biochemical composition with 86.1% in *Rastrelligerkanagurta*,86.9% in *Priacanthus arenatus*, 87.9% in *Daysciaena albida*, 81.2% in *Mystus gulio*,83.7 % in *Siganus fuscescens*, and 80.1% in *Gerres erythrourus*. The percentage of protein in *Rastrelliger kanagurta, Priacanthus arenatus , Daysciaena albida, Mystus gulio, Siganus fuscescens, Gerres erythrourus* are 11.5%,10.3%, 9.9%,17.4%,14.8%, and 18.3% respectively. The percentage compostion of ash was found to be 1.1%, 1.0%,1.0%, 1.0%, 0.9% and 0.7% in *Rastrelliger kanagurta, Priacanthus arenatus,Daysciaen aalbida, Mystus gulio, Siganus fuscescens, Gerres erythrourus* respectively. The lipid compostion was found to be 1.3% in *Rastrelliger kanagurta*, 2.0% in *Priacanthus arenatus*, 1.2% in Daysciaena *albida*, 0.4% in *Mystusgulio*, 0.6% in Siganus *fuscescens* and 0.9% in *Gerres erythrourus.* In the present study, moisture was found to be the major component of the biochemical composition in the muscle of all the six fishes. Moisture forms the major component of the biochemical composition with 86.1% in *Rastrelliger kanagurta*, 86.9% in *Priacanthus arenatus*, 87.9% in *Daysciaena albida*, 81.2% in *Mystus gulio*, 83.7 % in *Siganus fuscescens*, and 80.1% in *Gerres erythrourus*.

According to Ackman (1989), generally fish can be grouped into four categories according to their fat content: lean fish (< 2%), low fat (2–4%), medium fat (4–8%), and high fat (> 8%). In the present study all the fishes comes under the category of low fat fish, as the lipid in the muscle found to be below 2. As the protein forms the major biochemical constituent in the tissues of *L. Parsia*, it revealed the nutritious value of this fish. The result agrees with with the report from some fish species obtained from brackish, fresh and marine water (Poulter and Nicolaides, 1985; Ali *et al.*, 2005; and Turan *et al.*, 2007). The result obtained in this present study is lower than that reported for marine *Raja clavata*, (1.38± 0.0%) (Turan *et al.*, 2007).

From the proximate analysis, it was clear that all the fishes are highly proteincious with low fat content. This study has provided baseline information on proximate composition of these commercial six fish species.

Table 1. Proximate Composition in selected fishes of Kollam coast

Fish	Protein (%)	Ash (%)	Moisture (%)	Lipid (%)
Rastrelligerkanagurta (Indian mackerel)	11.5	1.1	86.1	1.3
Priacanthusarenatus (Red snapper)	10.3	1.0	86.9	2.0
Daysciaenaalbida (Spotted croaker)	9.9	1.0	87.9	1.2
Mystusgulio (Catfish)	17.4	1.0	81.2	0.4
Siganusfuscescens (Rabbit fish)	14.8	0.9	83.7	0.6
Gerreserythrourus (Silver biddy)	18.3	0.7	80.1	0.9

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TOXIC EFFECTS OF CHLORPYRIFOS ON HISTOLOGICAL CHANGES IN INTESTINE OF SOUTH INDIAN FRESH WATER MURREL, *CHANNA STRIATUS* (BLOCH-1793)

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Abstract- Histopathological changes in the tissue induced by sub-lethal concentrations of chlorpyrifos in *Channa striatus* was highly reflected in the present study. Fishes were exposed 0.4ppm dose of chlorpyrifos for duration of 30 days. Several histopathological alternations were observed in the tissue section. Damage in serosa, submucosa, mucosa, vacuole formation, necrosis, disturbed in longitudinal muscularis, proliferated mucous.
Keywords: *Channa striatus*, Damage in serosa, mucosa, necrosis, vacuole formation.

Introduction

Aquatic pollutants refer to substances which are capable of making any physical, chemical or biological change in the water bodies. Aquatic pollution is a universal problem and the most important inorganic pollutants are the pesticides of their toxicity, accumulation and biomagnification by aquatic organisms. According to Burger *et al*., 2002 there are two main routes of pollutant exposure. The primary route is intake of pollutants in fish is via gill or transport of dissolved contaminants in water across biological membranes and ionic exchange. The secondary route is through the intestine by food or sediment particles with subsequent transport across the gastro intestinal tract. It is known that fishes accumulate environmental contaminants in their tissues particularly those of digestive tract. The digestive tract is a major organ which maintains a contact with the environment, since this is the site where much of the digestive and absorptive processes take place by absorption and utilization of materials such as food and water. The intestine is an important part of gastrointestinal tract. It plays an important role in digestion process and subsequent absorption of food stuffs. In addition, the intestine serves as potent organ for evaluating toxicity of contaminants as they enter in to the fish body directly via contaminated food (Muniyan, 1999). *Channa striata* is commonly called chevron snakehead, striped Murrel or striated Murrel is one of the most economically important species inhabiting fresh water as well as brackish water (Bloch, 1793). It is also a well-known food fish widely used for medicinal and pharmaceutical purpose. The main objective of the present study was to analyse the histopathological alterations in the freshwater Murrel *Channa striatus* exposed to sub-lethal concentrations of Organophosphate pesticide chlorpyrifos.

Materials and methods

Live specimens of *Channa striatus* (Fig.1) were collected from fresh water habitat of Kottayam (9.5916° N, 76.5222° E) district, Kerala. The length and weight of the fishes were determined with 1mm division measuring scale and 0.1mg accurate electronic balance respectively. The size of the fish varied from 15±3 cm in length and 42±3 gm in total weight.

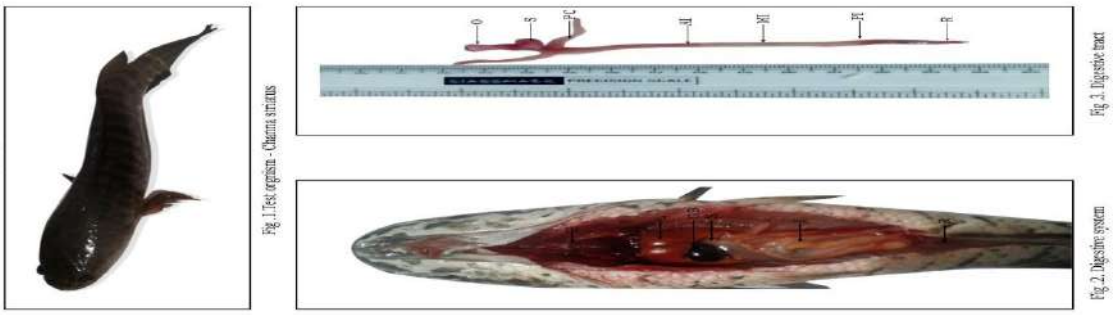
Sample preparation and histopathological analysis

The treatment was given by using chlorpyrifos at a sub lethal dose of 0.4mg/l for 30days. Control set of the experiment was also maintained. Water was changed in every alternate day. Physicochemical quality of experimental water was monitored regularly. Fishes were sacrificed for chronic toxicity test of heavy metals and pesticides. Intestine was removed from the sacrificed fish and prepared for the histopathological analysis. Dissected portion of the tissues were fixed in Bouin's fluid for 24 hrs then washed with water. Then tissues were dehydrated through graded ethyl alcohol embedded in the paraffin. Tissues and sectioned by microtome 3-5µm thickness. Then tissues were stained with haematoxylin and eosin (H&E) to study the histological organization and histopathological variations of the different regions of the digestive tract. Slides were observed under light microscope Labomed (CXR111) at varying magnifications (10, 40 and 100X) and photographs were taken under research microscope supported with software (Olympus) (Fig.2 and 3).

Results and Discussion

In control condition the intestine was composed of four histological layers, mucosa, submucosa, muscularis and serosa. The intestinal mucosal layer was formed of the intestinal villi. The intestinal mucosa was composed of columnar epithelial cells with centrally and basally placed nuclei, mucous cells and leucocytes. Mucous cells were present all over the intestinal mucosa. Intestinal villi were covered by thin layer of tissue matrix. Lamina propria was formed by the loose connective tissue fibers of submucosa layer. Blood cells were present in the lamina propria and submucosa layer. Muscularis layer was formed by the inner circular muscle fibres and outer longitudinal muscle fibres. The serosa layer was composed of a single layer of flat cells with blood capillaries and connective tissue fibers (Fig.4 and 5).

The present study revealed that *Channa striatus* exposed to a concentration of chlorpyrifos, showed severe histological changes such as vacuole formation (V), necrosis (N), distorted submucosa (DSMU) (Fig.5). Similar findings were reported by Nazi Khatun *et al*., (2016) after the exposure of 0.28 ppm of chlorpyrifos exposure for 30 days. The fusion of mucosal fold (FMUF) is also observed (Fig.6). This was agreement with data obtained by Kaoud *et al*., (2011). So, the above changes showed that the severity of damage is dependent upon the dose of concentration.



Intestine- control condition

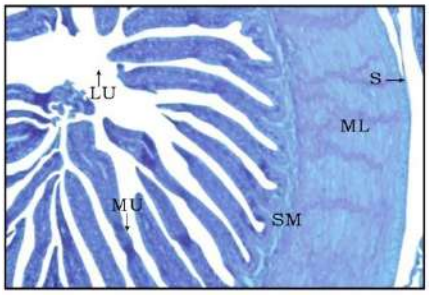


Fig.4

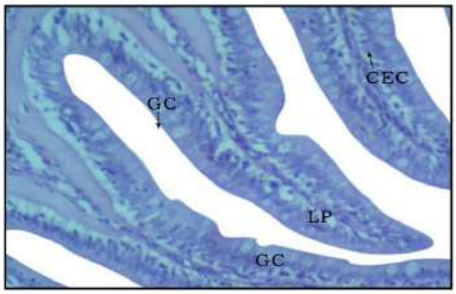


Fig.5

Intestine -treated condition

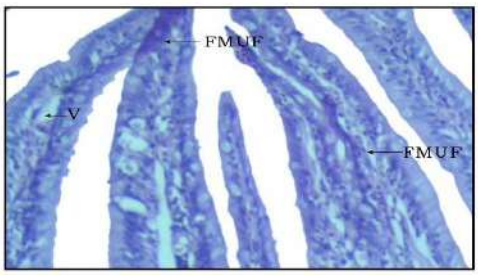


Fig.6

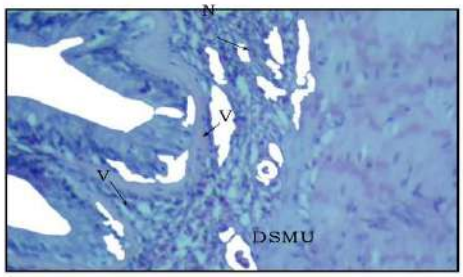


Fig.7

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BENTHIC MICROALGAL DIVERSITY OF AYIRAMTHENGU MANGROVES AND THEIR ECOLOGICAL ROLE

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Abstract: Mangrove ecosystems are one of the most productive and species diverse rich areas on earth. The study site, Ayiramthengu mangroves (Kollam district, Kerala) are fringing on the Kayamkulam backwater ecosystem. This area is the breeding and feeding grounds of a number of fishes and crustaceans. So huge numbers of larval forms of these species utilize this area as nursery grounds and they feed on planktons, micro benthos and organic detritus enriched by the mangroves. Regular samplings were done from three sampling stations for assessing the diversity of benthic microalgae. Fifteen species of benthic microalgae were collected and identified. The benthic micro flora reported from the three stations comprised of benthic algae which belongs to four classes viz. Bacillariophyceae, Cyanophyceae Pyrophyceae and Chlorophyceae. Class Bacillariophyceae dominated in the three sampling station. Pollution indicators were also present among the benthic micro algae. Presence of green alga, Spirogyra was noted from Station-3.

Key words: *Mangroves, Ayriamthengu, larval food, benthic microalgae*

Introduction

Mangroves are very interesting and distinctive communities found in tropical and subtropical land-sea ecotones. Mangroves represent a unique and ecologically important coastal habitat in the tropical and subtropical belts and are frequently seen as pioneer vegetation in coastal areas (Chapman, 1984). They are of the most productive ecosystem with high intrinsic and ecological value (Sunil Kumar, 2002). According to Quasim (1999) mangroves support high primary productivity and form rich nursery grounds for a variety of fishes, shellfishes and crustaceans. They are the feeding and breeding ground for many marine species (Shokita *et al*., 1989; Nayak and Anjali, 2001). The larval forms of fishes and crustaceans often depend upon planktons, benthic microalgae, and detritus materials. Of which, benthic microalgae serve as main carbon source for the higher trophic organisms and macrofauna (Harith and Bojo, 2012). Benthic microalgae are microscopic eukaryotic algae that is abundantly seen in marine and detritus rich brackishwater ecosystems. In coastal waters, both phytoplankton and microphytobenthos, are considered as the prime component of higher trophic level organisms (Gillespie *et al*., 2000). A number of studies have already done for documenting phytoplankton from aquatic ecosystems, but benthic microalgae are understudied. They are not always obvious and called as "secret garden" (Mac Intyre *et al*., 1996). The paucity of the information on the diversity of benthic microalgae from Ayiramthengu mangroves prompted on the investigation on the assessment of diversity of benthic microalgae present in the Ayiramthengu mangrove ecosystem.

Materials & Methods

Ayiramthengu mangrove ecosystem (Kollam district, Kerala) was selected for the present study, which lies between latitudes 9^0 02' and 9^0 16' north and longitudes 76^0 20' and 76^0 32' east. The mangroves are a part of Kayamkulam estuary, which is a narrow stretch of tropical backwater on the west coast of peninsular India. The estuary opens to the sea at Valiaazeekal. Three stations were selected from the Ayiramthengu mangroves for the sampling of benthic microalgae. Station-1 is the part of the estuary and hence there will be regular interaction between the estuary and the mangrove area. This station is 1.5 km away from Ayiramthengu mangroves was fixed in the estuary for sampling. It is 1.5 m deep with sandy bottom. Station-2 is a shallow portion of the mangrove forest and is inundated and subjected to tidal influence. Major portion of the mangroves are found growing on the boarder and also within this zone. Station-3 is inside the mangrove forest, a stagnant shallow pool is formed due to the growth of mangroves. Here tidal effect is in a minimal rate due to bordering of the pool by roots of the mangrove trees. Hence most of the decaying plant parts and leaf litter are trapped here with very little disturbance. Sediment samples were collected from the three stations using a locally designed corer. Three replicates from each sampling site were also taken. The sediment samples were kept in polyethylene bags and preserved in formalin. The samples were sieved out and micro-benthos were separated and preserved in formalin. The micro benthos was examined under a binocular microscope and was identified with the help of standard books (Pennak, 1978; Tonapi, 1980).

Results & Discussions

The benthic micro flora comprised of benthic algae which were represented by four classes: Bacillariophyceae (*Amphora* sp**.,** *Campylodiscus* sp., *Chaetoceros* sp., *Cocconeis* sp., *Coscinodiscus* sp., *Cyclotella* sp., *Hemidiscus* sp., *Melosira* sp., *Navicula* sp., *Pleurosigma* sp., *Triceratium* sp.), Cyanophyceae (*Chroococcus* sp., *Oscillatoria* sp.) Pyrophyceae (*Ceratium* sp.) and Chlorophyceae (*Spirogyra* sp.). Maximum number of species (11) were included under Bacillariophyceae, belonging to two orders, five Pennales and six Centrales. Pennales consisted of two families, Naviculoideae, 4 species (*Amphora* sp., *Campylodiscus* sp., *Navicula* sp., *Pleurosigma* sp.) and Achnanthaceae, one species only (*Cocconeis* sp.). Centrales included four families, Chaetoceraceae, one species (*Chaetoceros* sp.), Coscinodiscaceae, three species (*Coscinodiscus* sp., *Cyclotella* sp., *Melosira* sp.), Eupodiscaeae, one species (*Hemidiscus* sp) and Biddulphieae, one species (*Triceratium* sp.). Class Cyanophyceae consisted of two species in two orders, Chroococcales (Chroococcaceae family, *Chroococcus* sp.) and Oscillatoriales (*Oscillatoria* sp.). Chlorophyceae and Pyrophyceae were represented by only one species each and they were *Spirogyra* sp. and *Ceratium* sp. respectively.

A comparative study on the occurrence of major groups of benthic algae was conducted. A total number of 15 species were predominantly and regularly found in Ayiranthengu mangroves. Maximum number of benthic micro algae (12 species) was reported from Station-2. Station-3 was represented by 10 species and Station-1 by 9 species. Among all the three stations, Class Bacillariophyceae was dominated. Twelve Bacillariophyceae, one Pyrophyceae and one Cyanophyceae were identified from Station-1. Station-2 was represented by nine Bacillariophyceae, two Cyanophyceae and one Chlorophyceae, while seven Bacillariophyceae, two Cyanophyceae and one Chlorophyceae were documented from Station-3.

Pollution indicators were notable members among the benthic micro algae. Presence of green alga, Spirogyra was noted from Station-3. The presence can be justified by the stagnant nature of the station with penetrating sunlight to the bottom. In the estuarine and shallow subtidal systems like mangroves phytoplankton and benthic microflora play an equally significant role by contributing their biomass to the ecosystem (Underwood *et al*., 1998). The biomass of benthic microalgae are considered to be equal or even surpass the biomass of phytoplankton. Pellagic phytoplankton intercept the flux of light from the surface to the bottom, at the same time benthic algae intercept the flux of nutrients from the sediment to the water column. The change in the dominance of these two groups may alter the entire trophic web (Reynolds, 2008). Any disturbance of the benthic regions of mangrove areas and adjacent brackish water areas especially, sand mining and plastic pollution can definitly impart adverse effects on the biomass of microbenthos and in turn the food web of the ecosystem. Thus the intricate food webs in the mangrove areas can be sustainably conserved through the conservation of benthic microflora and benthic floor. This will further increases the productivity of these ecosystem. It is high time to derive sustainable conservation and management policies for the take care of the benthic floors of Ayiranthengu mangroves and adjacent Kayankulam backwaters.

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IMPACT OF BIOFABRICATED GOLD NANOPARTICLE SUPPLEMENTED DIET ON GUT HISTOLOGY OF *OREOCHROMIS MOSSAMBICUS*

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INTRODUCTION

The recent development and implementation of new technologies have led to new era, the nano revolution which unfolds role of plants in green synthesis of nanoparticles which seem to have drawn quite an unequivocal attention with a view of synthesizing stable nanoparticles(Kavitha K.S *et al*;2013).The possibilities of employing plants in the deliberate synthesis of nanoparticles have burgeoning interest as an important source towards reliable and environmentally benign method of metallic nanoparticles synthesis and its characterization.Nanofeed applications could be used to improve the delivery of micronutrients or unstable ingredients. In the present study, the plant selected for biofabrication of gold solution is *Curcuma longa.* The anti-oxidant property of curcumin can facilitate the reduction of gold chloride and the functional groups (hydroxyl, carbonyl, etc.) present in curcumin can stabilize the gold nanoparticles (GNPs)effectively.The objective of the study is to determine the effects of biofabricated gold nanoparticle on *O.mossambicus* with emphasis on gut histology.

MATERIALS AND METHODS

Juveniles of *Oreochromis mossambicus* in the range 7± 0.35cm and 5±0.62gm were collected and stocked at 20 fish/1000L and fishes were maintained at laboratory conditionsExperimental diets were prepared by incorporating 10µl of biogenic gold and chemically synthesized GNP per 100gms of basal feed.Non-treated control diets and aquous extract of *Curcuma longa* incorporated diets too were prepared .The experimental schedule was for eight weeks and the fishes were fed at 2% of body weight twice daily.For histological and histopathological studies, small pieces of the liver and gill were taken from control and treated fish at the end of exposure regimen (30 days). Samples were fixed in 10% buffered formalin for twenty four hours at 4ºC, dehydrated in ascending grades of ethanol, immersed in xylol, and embedded in paraffin wax. Sections of 4-5 µm thick were mounted on clean glass slides, deparaffinized, rehydrated, stained with hematoxylin and eosin and mounted with DPX. Sections were examined using a light microscope (Roberts and Smail, 2001)

RESULTS

The UV absorption spectra of the nano gold solutions in experiment is shown in (Fig:1 and 2). The absorption is a typical gold surface Plasmon vibration excitation for colloidal GNPs when they interact with electromagnetic radiation. In the optical absorption spectrum of the resultant nanoparticles the absorption wavelength of gold nanoparticles were observed at 520 nm

Intestine is the main route through which toxicants enter into the body and thus one of the first sites which come into contact with toxicants. Any damage to the intestinal linning can be a good indicator to the toxicity of the xenobiotics to biological system.Intestinal mucosa is made up of superficial and glandular epithelium.The superficial epithelium composed of compactly arranged columnar epithelium cells with oval shaped nucleus.The glandular epithelium consists of tubular gastric glands with rhomboidal shaped cells with spherical nuclei.The gland are surrounded by thin layer of tunica propia.

No structural damage is seen in control,*Curcuma longa* and nano *Curama longa* treated fishes.(Fig:3,4 and 5) The histological sections exhibited simple columnar epithelium, lamina propria, submucosa, tunica muscularis and serosa as well defined layers in the order from internal to external surface of the intestine. The epithelial cells appeared as long and thin columnar cells and the mucosa showing surface microvilli. The mucosa was highly branched and folded .

Disjointment of layer,loosening of muscularis and vacuolisation were seen in fish exposed to synthetic gold nanoparticulated diet.(Fig:6).Congestion of mononuclear cells and mild degeneration of villi were also observed with synthetic nanogold treatment.Degeneration of the intestinal villi in the treatment groups decreased its absorptive surface,which will ultimately resulted in reduced efficiency of food utilization.The epithelial cell of the outer wall disintegrate which would eventually result in th breakdown of the intestinal function

Fig:1 Fig:2.

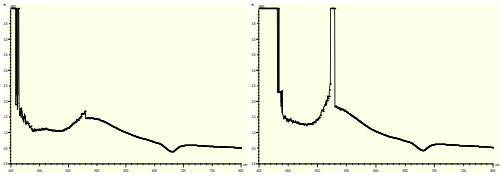

Fig:1.UV-VIS Spectrum of Chemically reduced GNP,Fig:2: UV-VIS Spectrum of biofabricated GNP

HISTOPATHOLOGY OF GUT TISSUE OF *O*REOCHROMIS *MOSSAMBICUS* SUPPLEMENTED WITH BIOFABRICATED GOLD NANOPARTICLE (STAIN : HAEMATOXILIN EOSIN)

Fig:3 Fig:4 Fig:5 Fig:6

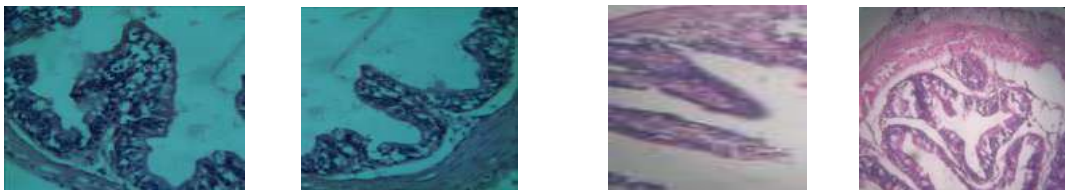

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Fig:3-Control(40x)**,Fig:4.***Curcuma longa*** extract treated(40x),**Fig:5..**nano ***Curcuma longa*** treated (40x),**Fig:6.**Chemically reduced gold nanoparticle treated.(40 x)

DISCUSSION

The synthetic gold nanoparticulated diet, had a deleterious effect on the internal epithelial lining cells and such changes in intestine, probably induce changes in biological functions of the fish that may contribute to lethargic behavioural responses. Toxicity of exposure to nanoparticles may vary with structure and composition of engineered nanoparticles (Griffitt *et. al.,* 2008; Klaine *et.al.,* 2008).No specific damage could be observed in biogenic nanoparticle exposure. Hence in this study, cytotoxicity of biofabricated gold is ruled out and it is confirmed that *Curcuma longa* along with GNPs produce no deleterious effects.These results clearly demonstrate that the phytochemicals within *Curcuma longa* provide nontoxic coating on GNPs .The lack of any noticeable toxicity of biofabricated gold nanoparticles provide new opportunities for the safe application in drug delivery.The results are in agreement with the work of Amit Singh *et.al;*2011.The present study confirms that biogenic GNPs can be looked upon as an environmentally benign replacement to the toxic chemical methods for synthesis of nanostructures and as promising candidates for biomedical applications. Synthesized NPs can circulate in the body for extended periods of time without being rejected by the body's immune system. This can be exploited for benificial results by green synthesis of gold nanoparticle that avoids some reactive functional groups produced in chemically synthesized nanoparticles which can be toxic to biological system.

5.CONCLUSION

The present laboratory investigation confers the risk of fish exposure to the synthetic gold nanoparticles .Risk on this most edible and economically important fish were proved by detecting the histopathological alterations occurring in intestine of fish fed with synthetic GNPs embedded diet. The biogenic nanostructures are better at biocompatibility and, thus, will have immense application for biological and clinical prospects in terms of cytostructural profile of gut.

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PHYTO AND ZOOPLANKTON ABUNDANCE AND COMMUNITY STRUCTURE OF POLACHIRA WETLAND ECOSYSTEM IN SOUTHERN KERALA, INDIA

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ABSTRACT

The incidence of plankton is directly proportional to the quality of water in an ecosystem. In order to study the plankton diversity and abundance of Polachira wetland water samples were collected during February to July 2014. Five algal class belonging to 18 genera were identified. Seven genera of Chlorophyceae was the dominant group followed by Bacillariophyceae with five genera. Cyanophyceae consist of four genera and Zygnemataceae and Dinophyceae comprised of one genera each. The increase in density of phytoplankton observed during February. *Closterium* sp., *Oscillatoria* sp., *Spirogyra* sp. and *Synedra* sp. were the widespread species while *Cocconema* sp. was least (24 cells/L). The phytoplankton was minimum (804 cells/L) and maximum (3024 cells/L) during April and February respectively. A total of 13 genera of zooplankton belonging 5 taxa were recorded; 6 genera of Crustacea, 3 genera of Ciliophora, 2 genera of Rotifera and one each genera belonging to Arachinida and Protozoa. Mysis was reported to be in high count in Crustacean followed by Ciliophora. The occurrence of zooplankton was ranged from a minimum and maximum of 12 cells/L and 624 cells/L during April and July respectively. The investigation revealed the plankton community enjoyed a balanced widespread indicating the dynamic nature of the Polachira wetland.

Key words: phytoplankton, zooplankton, diversity, density, Polachira wetland

INTRODUCTION

Plankton are the microscopic floating organisms of the surface water that move along the direction of water current in the aquatic ecosystem. Phytoplankton forms the primary link of the food web and transfers energy to the higher trophic levels in aquatic ecosystem. The interaction of the plankton with its environment is a reflection of changes of ecological variables. Wetlands are one of the Earth's richest ecosystems, offering ''sanctuary'' (Hazarika, 2013) to a rich biological diversity. Being an integrated system it is always influenced by hydro-ecological consequences. Based on the plankton dominance one can evaluate the hydrological quality of the water. The

present study is the investigation of species composition, distribution and density of plankton around Polachira wetland.

MATERIALS AND METHOD

The present study focused on the quantitative and qualitative investigation on plankton of Polachira Wetland. It is located in the Southern part of Kollam district in Kerala. Polachira wetland is located between 8°50′26.89″N longitude and 76°42′0.3″E latitude. The wetland is formed in the estuaries of the Ithikkara River and Paravur backwaters encircled by small rivulets. The plankton samples were collected from five stations in the second week of every month during a period from February to July 2014. Numerical estimation of phytoplankton was made by Lackey'drop method and zooplankton was done by Sedgwick-Rafter cell method (Trivedy and Goel, 1986). Identification of phytoplankton species were made as per the observations made by Prescott (1962) and Sarma and Khan (1980). Zooplanktons were identified as per the standard taxonomic keys of Battish (1992), and Ward and Whipple (1992).

RESULT AND DISCUSSION

Qualitative and quantitative estimation of phytoplankton and zooplankton of Polachira wetland ecosystem is depicted in Table 1 and 2. During the investigation 5 algal class belonging to 18 genera were identified. Bacillariophyceae with five genera followed seven genera of Chlorophyceae representing the major group. Cyanophyceae consist of four genera and Zygnemataceae and Dinophyceae comprised of one genera each. A total of 13 genera of zooplankton belonging 5 taxa were recorded; 6 genera of Crustacea, 3 genera of Ciliophora, 2 genera of Rotifera and one each genera belonging to Arachinida and Protozoa.

Plankton density and diversity was lower with lesser number of species. The maximum diversity of phytoplankton was attained during the month of July. The Cyanophyceae members included *Ocillatoria* sp., *Mougeotia* sp., *Phormidium* sp. (February and March) and *Arthrospira jermeri* was noticed only during July. Chlorophyceae group was comprised of *Spirogyra* sp., *Oedogonium* sp., *Closterium* sp., *C.didymotocum, C.moniliferum, Desmidium* sp., *Pediastrum boryanum, Ankistrodesmus* sp., and *Cosmarium* sp. All these species were recorded at all months except

Cosmarium sp. which noticed only during July. The genera in Bacillariophyceae *Synedra* sp., *Navicula* sp. and *Fragillaria* sp. were found in all months. *Cocconema* sp. observed only during April and *Nitzchia* sp. was recorded only during June. Zygnemataceae included *Sirogonium* sp. occurred during May and July. Dinophyceae was confined only of *Ceratium longicorne* during May and July may be due to the incursion of saline water from Paravur estuary.

Quantitative analysis of phytoplankton showed that maximum density of phytoplankton (3024 cells/L)) achieved during February. *Closterium* sp., *Oscillatoria* sp., *Spirogyra* sp. and *Synedra* sp. were the dominant species while *Cocconema* sp. was least (24 cells/L) during the study period. The phytoplankton was minimal (804 cells/L) during April. Monthly variation in abundance of each taxa showed fluctuations. Chlorophyceae was the most dominant form.

In the class Chlorophyceae, *Closterium* sp. *Spirogyra* sp and *Ocillatoria* sp. were the most abundant form and its population contributes the major portion in the phytoplankton population. In Bacillariophyceae, *Synedra* sp., *Fragillaria* sp. and *Navicula* sp. were occurred abundantly. Among the five major groups Chlorophyceae constituted the major portion. Bacillariophyceae was the second dominant group. Cyanophyceae contributed with four genera ranked the third dominant group with luxuriant growth. Similar observation was made by Jagadeeshappa and Vijaya kumara (2013) in wetlands of Tiptur taluk. Banu *et al.,* (2014) have also recorded abundance of Chlorophyceae, Bacillariophyceae and Cyanophyceae during summer months in Lake Kolleru, Andhra Pradesh. The abiotic factors together with sewage discharge and outfall from catchment areas contributed nutrient concentrations to the floodplain might have tremendously influenced the quantity and quality of phytoplankton.

Seasonal succession of zooplankton communities in the tropics has been attributed to a number of factors such as the environmental characteristics of the water, predation, quality and quantity of algae and competition (Onwudinjo and Egborge, 1994; Ovie and Adeniji, 1994). Five groups of zooplankton were observed from the study area. Crustacea comprised of *Nauplius, Mysis, Mesocyclops, Daphnia, Leydigia acanthoceroides* and *Oxyurella singelensis.* Ciliophora included *Loxophyllum* sp., *Hexotricha* sp. and *Coleps hirtus.* Rotifera was encompassed of *Colurella* and *Branchionus calciflorus.* Arachnida was comprised of *Hydracarina* and Protozoa included *Centropyxis.* Among the thirteen genera of zooplankton documented, crustacean group was

abundant. Among crustacea *Mysis* was accounted the maximum of 576 ind/L (July). Ciliophora was the second copious group. Highest density of zooplankton in May, June and July could be attributed to the heavy rainfall, which carried optimal nutrients from the catchment areas into the ecosystem enriching the growth of phytoplankton which subsequently increased zooplankton.

Polachira wetland is the most wanted destination for waterfowls and other migratory birds. The current indispensable information of the plankton diversity and abundance would form a helpful implement for further ecological evaluation and monitoring of the ecosystem of Polachira wetland.

Table 1. Qualitative and quantitative analysis of phytoplankton (cells/L) at Polachira wetland ecosystem during the month of February-July 2014

Class	Genera	FEB	MAR	APR	MAY	JUNE	JULY	Total
Cyanophyceae	*Ocillatoria* sp.	276	264	156	204	648	516	**2064**
	Arthrospira jermeri	-	-	-	-	-	132	**132**
	Phormidium sp.	72	100	-	-	-	-	**172**
	Mougeotia sp.	336	60	60	84	12	170	**722**
Chlorophyceae	*Spirogyra* sp.	672	468	36	228	276	12	**1692**
	Oedogonium sp.	144	60	12	84	12	12	**324**
	Closterium sp.	540	696	264	396	360	300	**2556**
	C. didymotocum	12	24	12	12	12	12	**84**
	C.moniliferum	12	12	12	12	12	12	**72**
	Desmidium sp.	192	40	12	12	12	12	**280**
	Pediastrum boryanum	12	24	12	24	23	12	**107**
	Ankistrodesmus sp.	180	150	24	74	48	12	**488**
	Cosmarium sp.	-	-	-	-	-	34	**34**
Bacillariophyceae	*Synedra* sp.	156	377	96	372	120	252	**1373**
	Navicula sp.	324	132	36	24	204	156	**876**
	Fragillaria sp.	96	60	48	12	336	36	**588**
	Cocconema sp.	-	-	24	-	-	-	**24**
	Nitzchia sp.	-	-	-	-	60	-	**60**
Zygnemataceae	*Sirogonium* sp.	-	-	-	36	-	72	**108**
Dinophyceae	*Ceratium longicorne*	-	-	-	48	-	60	**108**
TOTAL		**3024**	**2467**	**804**	**1622**	**2135**	**1812**	**11864**

Table 2. Qualitative and quantitative analysis of zooplankton (ind./L) in Polachira wetland ecosystem during February-July 2014

Taxa	Genera	Feb	Mar	Apr	May	Jun	Jul	Total
Crustacea	*Daphina* sp.	24	-	12	-	-	-	**36**
	Leydigia acanthoceroides	-	-	-	-	12	-	**12**
	Oxyurella singelensis	12	-	-	-	-	-	**12**
	Nauplius	-	-	-	12	-	-	**12**
	Mesocyclops	12	-	-	12	-	12	**36**
	Mysis	-	-	-	256	-	576	**832**
Rotifera	*Colurella* sp.	-	-	-	12	-	-	**12**
	Branchionus calciflorus	-	12	-	-	-	-	**12**
Cilliophora	*Loxophyllum* sp.	-	-	-	-	12	36	**48**
	Hexotricha	-	-	-	-	48	-	**48**
	Coleps hirtus	-	12	-	-	-	-	**12**
Arachnida	*Hydracarina*	12	-	-	-	-	-	**12**
Protozoa	*Centropyxis*	-	-	-	-	12	-	**12**
Total		**60**	**24**	**12**	**292**	**84**	**624**	**1096**

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RELATIVE GUT LENGTH AND GASTRO-SOMATIC INDEX OF *OXYURICHTHYS TENTACULARIS* (VALENCIENNES, 1837) FROM ASHTAMUDI LAKE, KERALA

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ABSTRACT

The study provides an understanding of the feeding habit and feeding intensity of the arrow fin goby Oxyuricthys tentacularis, by analyzing its relative gut length (RGL) and gastro-somatic (GaSI) index. The Fish specimens were collected from Ashtamudi Lake for a period of 2 years, from February 2016 to January 2018. It is seen that he feeding habit of this goby varied with month and season of sample collection and accordingly, the RGL and GaSI also varied. These results provide new knowledge on the feeding habit and feeding intensity of this fish species, which also helps to understand the fish's adaptation and conservation in the study area.

*Keywords: G*astro-somatic index, Gobiideae, *Oxyuricthys tentacularis*, Relative gut length

INTRODUCTION

Oxyurichthys tentacularis (local name Koozhali) is a genus of fish in the subfamily Gobionellinae, known commonly as arrow fin gobies inhabiting the Ashtamudi Lake (Ramsar site No. 1204) on the west coast of India (8o 53' - 9o 02' N; 76o 31' - 76o 41' E),which is the second largest estuary in Kerala. Though its abundance and distribution is noted in other estuaries and backwaters of India, perhaps this is the only estuary in India where there is a commercial fishery for a goboid species (Kurup and Thomas, 2001). Most species live in shallow waters under 10 meters deep over fine substrates such as silt. There is no data on its feeding habit and feeding intensity and its variation with seasons. The relative gut length (RGL) index is helpful for feeding habit determination and gastro-somatic index (GaSI) one is used to examine the feeding intensity. The results will be helpful for understanding of the fish feeding habitat and intensity, being used for fish adaption in muddy habitat knowing.

MATERIALS AND METHOD

The specimens of *O. tentacularis* were collected from Ashtamudi lake (8^o 53'- 9^o 02' N; 76^o 31'- 76^o 41' E) using a modified gill net, locally known as "koozhalivala", cast net and dip net with the help of local fishermen, for a period of 2 years, from February 2016 to January 2018. A total of 720 guts (length range of fish 9cm to 17cm) were examined following the procedures suggested by Windell and Bowen (1978). The total length, weight and fullness of the guts were recorded. The stomach contents were weighed to the nearest 0.1 g to determine the Gastrosomatic Index (GaSI), the method used to estimate the feeding intensity of fish. GaSI values (Desai, 1970) were calculated following the changes in the gut weight in relation to body weight by using the following formula

$$GSI = \frac{\text{Weight of the gut content}}{\text{Total weight of the fish.}} \times 100$$

The Relative gut length is calculated using the formula: $$\text{Relative gut length (RGL)} = \frac{\text{Gut length}}{\text{Body length}}$$

RESULTS AND DISCUSSION

Gastrosomatic Index (GaSI) - Month wise Gastrosomatic index values of *O. tentacularis* are furnished in Fig.1. As illustrated, the GaSI value for the months from February to April and August to November were on lower side, which manifests the very low feeding intensity during the above period. The mean monthly values of Gastro-Somatic Index (GaSI) have been observed to remain high during May to July when the stomachs were full and contained good amount of food. Then it started to drop down from July onwards; reached the lowest value in the month of August and then gradually started to increase from November onwards to reach its peak value during the month of December. The low values of GaSI along with the declining trend from February to April and also from August to November depicting the poor feeding activity in this fish species during this time period which is in correspondence to their intense breeding periodicity (Gupta and Banerjee, 2013). The results correlates with the findings of Remya *et al* (2017), as two peak spawning periods were reported in this species i.e., during February-April and August-November. The Gastrosomatic index is related to feeding intensity of fish. The feeding intensity means the fullness of stomach. The changing trend of monthly GaSI values is in synchronization with the changing percentage of gut fullness. The feeding intensity was improved after spawning period is over. Similar results were obtained by Dinh *et al* (2015).

Relative length of Gut (RLG) - Total length of the fish was measured from the tip of snout to the tip of the tail to the nearest millimeter. Body cavity of each specimen was then carefully opened and the whole alimentary canal was dissected out and spread over a glass plate. Gut length was measured from the esophagus to anus. Most gobiids are considered omnivorous and feed on benthic algae and detritus. *O.microlepis*, *Stenogobius gymnopomus* and *Oligolepis acutipennis* mainly feed on benthic diatoms and detritus (Geevarghese 1983).Monthly variation in the values of Relative length of Gut (RLG) is represented in Fig. 2. The large relative length of gut during May to July and November to January supports the feeding nature of *O. tentacularis*, i.e. the voracious feeding after the breeding season. In the present study, the RLG showed that *O. tentacularis* is omnivorous according to the scale described by Geevarghese (1983). The relative length of gut values of *O. tentacularis* ranged from 0.65 to 1.06 during the study period (Table 2). The maximum values were obtained in December and minimum values were noted in the month of July (Fig.2). The food analysis and study of the alimentary canal show this fish to be an omnivore. In omnivores, the gut length varies between that of herbivores and carnivores. Relative length of Gut (RLG) is a useful index which provides an idea of the nature of food consumed. The details available on the feeding habits of other species of gobioids shows that they may range from near herbivorous to purely carnivorous; feeding on a wide

variety of ingestible organisms from its habitat. The presence of sand grains, and detritus in the diet was a sign of the benthic behaviour of the fish (Serajuddin, and Rustam 2005). If this characterization is to be relied upon, *Oxyurichthys tentacularis* is also a bottom feeder as with most of the gobioid fishes.

SUMMARY AND CONCLUSION

Precise depiction of fish diets and feeding habits also provides the basis for understanding trophic associations in aquatic food network. The Gastrosomatic index is related to feeding intensity of fish. The changing trend of monthly GaSI values is in synchronization with the changing percentage of gut fullness. From the food analysis and relative length of gut values it is inferred that *O. tentacularis* is an omnivorous fish.

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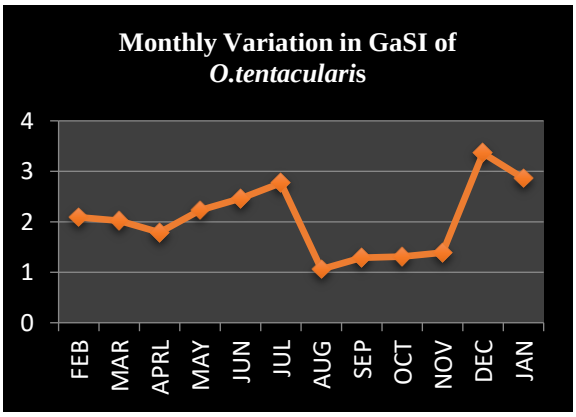
Fig.1


Monthly Variation in GaSI of
O.tentacularis
4
3
2
1
0
FEB
MAR
APRL
MAY
JUN
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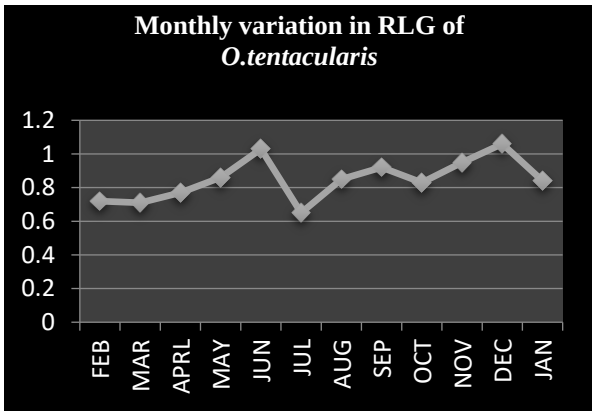
DEC
JAN

Fig .2


Monthly variation in RLG of
O.tentacularis
1.2
1
0.8
0.6
0.4
0.2
0
FEB
MAR
APRL
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JAN

GILLS OF *SCYLLA SERRATA* (FORSSKAL, 1775) - SITE OF ACTION OF HEAVY METAL POLLUTION LOAD OF ASHTAMUDI LAKE (RAMSAR SITE), KOLLAM, KERALA

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ABSTRACT

The bioaccumulation status of selected heavy metals such as Cadmium, Chromium, Copper, Zinc, and Lead were determined in the gill samples of *Scylla serrata* collected from three study sites of Ashtamudi Lake for a period of one year. Statistical analysis (ANOVA followed by Fisher's LSD post hoc test) was performed in order to demonstrate the significant variations in the samples of the study sites. The results reveal that the extent of heavy metal pollution in the gills was more in the specimens collected from the polluted regions (Kureepuzha – site 1 and Perumon – site 2) of the lake when compared with the reference site (West Kallada – site 3). The mean values of heavy metals Cadmium, Chromium, Copper, and Zinc recorded in the gills of *Scylla serrata* at site 1 were 1.517, 7.417, 9.575, 9.550, 19.083 mg/kg respectively and at site 2 the values were 1.550, 4.867, 5.883, 1.358, 13.650 mg/kg respectively.

Key words – ANOVA, Ashtamudi Lake, Gills, Heavy metals, *Scylla serrata*

INTRODUCTION

Ashtamudi Lake is one of the prime ecosystems, which receives the designation 'the second largest backwater in Kerala'. Heavy metal contamination, mainly occurs due to the influx of industrial wastes as well as domestic wates has become a serious threat and a predominant factor in decline of water, sediments and fish quality of Ashtamudi Lake. (Razeena *et al.,* 2012, Sherly *et al.,* 2015). Among the various organs, the gills of fishes are considered the main site of entry to aquatic pollutants as they are in direct contact with the external medium to perform gaseous exchanges and ionic regulations. For the present study the gills of mud crab, *Scylla serrata* were targeted in order to analyze the severity of heavy metal contamination in them.

MATERIALS AND METHODS

Kureepuzha (site 1), Perumon (site 2), and West Kallada (site 3) of Ashtamudi Lake were the three study sites selected for the present study. Sites 1 and 2 were very much disturbed with the influx of heavy metal pollution. (Girish Kumar, 2016., Razeena *et al*., 2012). Input of domestic wastes and discharge from retting grounds and were the predominant sources of heavy metals at site 2 (Girish Kumar, 2016).The region of Kallada lake which is not much disturbed with anthropogenic interferences and urbanization, was selected as the third and reference site (Lekshmi and Sherly, 2018). *Scylla serata* of about 3 to 6 cms in carapace width and 220 to 270 gm weight (60 numbers) were collected from the three study sites for a period of one year from February 2016 to January 2017. Gills were dissected and the heavy metals such as Cadmium, Chromium, Copper, Lead and Zinc were detected using an Atomic Absorption Spectrophotometer (AAS, Pinnacle, 900H) as described by APHA (1998). Data analysis in the tissue samples was performed using statistical package of SPSS 22. Significant differences between heavy metals concentration of various

sites, determined using One Way Analysis of Variance (ANOVA) followed by Fisher's LSD (Least significant difference) post hoc test.

RESULTS AND DISCUSSION

The heavy metal analysis in the gill samples of *Scylla serrata* of three study sites with respect to their mean values was shown in figure 1. The tabulation of statistical results of One-way analysis of variance (ANOVA) was shown in table 1. The results revealed that the heavy metals such as Cadmium ($F = 25.517$), Chromium ($F = 81.889$), Copper ($F = 75.820$), Lead ($F = 64.170$) and Zinc ($F = 34.829$) were found to be different in their values with respect to the sites and showed higher significance at 1% level ($p< 0.01$). The results of the Fisher's LSD (Least significant difference) Post hoc multiple comparisons further reveal that site 1 and 2 significantly differ from site 3 with respect to the accumulation of heavy metal Cadmium. Site 1, 2 and 3 significantly different among each other with respect to the heavy metals Chromium, Lead and Zinc. Site 1 was significantly different from other two sites in the case of Lead accumulation.

An assessment of heavy metal pollution loads into Ashtamudi Lake with reference to the muscles of scylla *serrata* was documented by Lekshmi and Sherly (2018). Their results revealed that the muscles of *Scylla serrata* from the Kureepuzha and Perumon region of Ashtamudi lake was beyond the PMTDI (Provisional Maximum Tolerable Limit for Daily Intake) limit, which was not fit for human consumption. Report of Sherly *et al* (2015) emphasized on the morphological alterations caused by different kinds of aquatic pollutants, especially heavy metals on gills and fins of *penaeus monodon* collected from the study sites, Kureepuzha and Perumon region of Ashtamudi Lake. In the present study also, the samples collected from the study sites Kureepuzha and Perumon were found to bioaccumulated with heavy metals when compared with the reference site.

SUMMARY AND CONCLUSION

The manifestation of heavy metals in the gill samples of *Scylla serrata* collected from site 1 and 2 is an indication of the extent of heavy metal pollution load in those regions. Likewise, *Scylla serrata*, other organisms inhabiting the lake has an equal chance for the accumulation of heavy metals in their tissues. These will in turn pave way for the deterioration of the aquatic organisms inhabiting the lake. Stringent and immediate necessary actions should be taken up by the responsible authorities in order to safeguard the aquatic organisms and thereby the protection of Ashtamudi Lake as a whole from severe pollution.

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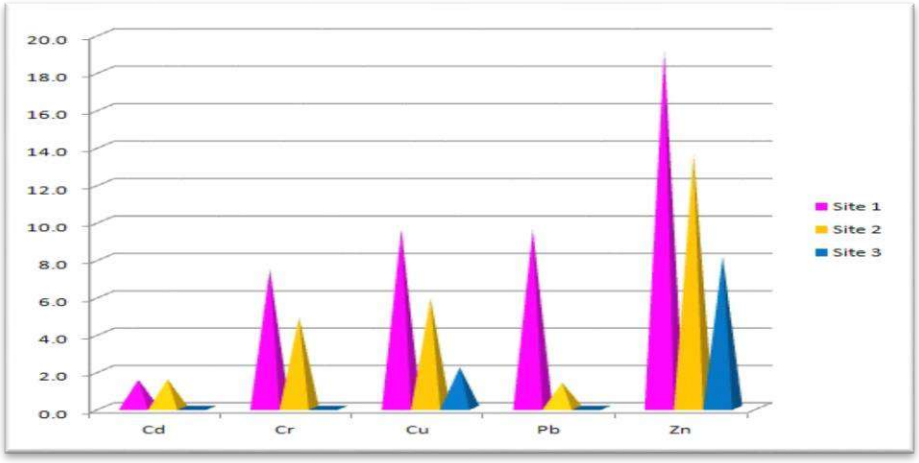

Figure 1: showing the heavy metal accumulation in the gills of *Scylla serrata* of study sites.

Table 1: Analysis of variance (One-Way ANOVA) of heavy metals of the gills of *Scylla serrata* comparing study sites of the Ashtamudi Lake.

	Study sites				
Heavy metals (mg/kg)	Site 1 (Mean ± SD)	Site 2 (Mean ± SD)	Site 3 (Mean ± SD)	F value comparing study sites	P Value
Cadmium	1.517 ± 0.803 [a]	1.550 ± 0.678 [a]	0.0 ± 0.0 [b]	25.517	< 0.001*
Chromium	7.417 ± 1.355 [a]	4.867 ± 2.098 [b]	0.0 ± 0.0 [c]	81.889	< 0.001*
Copper	9.575 ±2.143 [a]	5.883 ± 1.008 [b]	2.183± 0.935 [c]	75.820	< 0.001*
Lead	9.550 ± 3.711 [a]	1.358±1.096 [b]	0.0 ± 0.0 [b]	64.170	< 0.001*
Zinc	19.083± 5.141 [a]	13.650±2.029 [b]	8.117±0.723 [c]	34.829	< 0.001*

** = p < 0 .01, The mean difference is significant at 1% level; SD – Standard deviation;[a, b, c] - Means within rows with differing subscripts are significantly different using Fisher's LSD post hoc test.*

ISOLATION AND CHARACTERIZATION OF MARINE EPIPHYTIC *PSEUDOMONAS* SP. AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT

Marine seaweeds and their associated bacteria produce bioactive compounds which have been found to be important for health promotion and disease prevention. The present study focused on the preliminary screening of the antibiotic producing bacteria associated with seaweeds from the coast of Varkala, Thiruvananthapuram, Kerala. Ethyl acetate extracts of bacterial supernatant were screened for antibacterial activity. From the five isolates, *Pseudomona stutzeri* showed antibacterial potential against plant pathogen *Rhizoctonia solani.*The effect of temperature on the antagonistic potential was also studied. The optimum temperature for the antimicrobial potential is 35 ◦C. This study reiterates that seaweed associated bacteria have the ability to produce bioactive compounds which may be useful in biomedical applications. Further studies are required for the purification and chemical characterization of the bioactive products.

Keywords: Bacterial epibionts, Antimicrobial potential, Secondary metabolites, *Pseudomonas Stutzeri, Rhizoctonia solani.*

INTRODUCTION

Marine bacteria are a profound resource on the development of natural product chemistry and the medical sciences. The improvement of natural products-based screening, rather than relying on synthetic sources, has been the aim of current pharmaceutical research and development. Alternative strategies under consideration include the identification of potential new antibiotics from commercial crude bacterial fermentations. Recent research reported that many bioactive natural products from marine invertebrates have striking similarities to metabolites of their associated microorganisms including bacteria. Compared with terrestrial organisms, the secondary metabolites produced by marine organisms have more novel and unique structures owing to the complex living circumstance and diversity of species, and the bioactivities are much stronger (Burgess J.G *et al*, 1999). The study of marine bacteria and their potential role in the production of metabolites is becoming a new topic for research. Several investigations have supplied an increasing number of biologically active and structurally unique compounds. Bacteria and other micro-organisms are ubiquitous in the marine environment. They are taxonomically diverse, biologically active, and colonize all marine habitats, from the deep oceans to the shallowest estuaries. It has been estimated that the majority of bacteria in natural aquatic ecosystems are organized in biofilms (Lemos *et al*, 1985).

The oceans bear most of the biodiversity on earth, the greatest part of which is still unknown. In addition, marine samples reveal a much higher hit rate for antitumor and antibiotic activities. These are very good reasons to intensively study and explore marine biodiversity for new drug candidates. It is now well recognized that the diversity of chemical structures from marine and microbial sources is the greatest, since all forms of life are subject to perpetual competition, it is not surprising that the organisms that live in the sea produce an enormous range of biological activity. Besides the compounds that repel predators by their toxicity, there are those which are attractive to make reproduction more probable. These compounds may be derived from primary or rather secondary metabolism of these organisms (Bérdy, 2005).

R. solani is a very common soil borne pathogen with a great diversity of host plants. *R. solani* primarily attacks below ground plant parts such as the seeds, hypocotyls, and roots, but is also capable of infecting above ground plant parts (e.g. pods, fruits, leaves and stems). The most common symptom of Rhizoctonia disease is referred to as "damping-off" characterized by non germination of severely infected seed whereas infected seedlings can be killed either before or after they emerge from the soil. Infected seedlings not killed by the fungus often have cankers, which are reddish-brown lesions on stems and roots. In addition to attacking below ground plant parts, the fungus will occasionally infect fruit and leaf tissue located near or on the soil surface. This type of disease often occurs because the mycelium and sclerotia of the fungus are close to or splashed on the plant tissue. The accumulation of several bodies of evidences that the associated microorganism might be the real source of some of the previously ascribed metabolites to marine macroorganism promoted us to isolate strains from marine macroalgae in a try to isolate substances that may have similar structures to known marine metabolites from marine animals. Therefore, this work was initiated to culture marine derived bacteria optimize their growth and production of antimicrobial metabolites and screen their crude extracts using different biological test systems.

In this study the seaweed samples were collected from Varkala,Thiruvananthapuram, Kerala (8.73 ^{0}N 76.71 ^{0}E).The study focuses on the epibiont bacteria of seaweed which is antagonistic to *R solani.*

MATERIALS AND METHODS

Seaweed samples were collected from the study sites, the samples were hand picked and immediately washed with seawater to remove the foreign particles, sand particles and macro epiphytes. The samples were transported to the laboratory on ice. The surface of sample was washed with fresh water. A bacterial sample was taken from the surface with a sterile cotton swab. The serial diluted samples were spread on Zobell marine agar plates. The plates were incubated for 24-48 h at 37°C. The pure bacterial cultures obtained were maintained on Nutrient Agar slants. Overnight bacterial culture (100 ml) in marine broth was centrifuged at 4° C at 7000rpm for 20min. The supernatant was collected and extracted with ethyl acetate. Organic layer was concentrated with a vacuum rotator.The concentrated solutions were used for bioassay against *R solani*. Mueller Hinton agar plates were prepared and uniformly swabbed with pathogen. Thereafter, it was punched with 5mm diameter wells and filled with 30µl of the concentrated bacterial extracts. The petri-dishes were incubated at 37°C for 24h. After incubation, plates were

examined for inhibition zones. The inhibitory activity was measured by calculating the area of clear zone.The culture of *R solani* was collected from National Chemical Laboratory ,Pune.The bacteria were separated based on colony morphology, cell morphology and Gram staining(Gram C,1884). The pure bacterial colonies were obtained by streaking and identified upto species level using 16s rRNA profiling.A loopful of 24h old culture of the isolate was added to the medium and the temperature was adjusted. The experiment was carried out in different temperature ranging from 15^0C 25^0C, 35^0C, 45^0C and 55^0C out in culture experiments.

RESULTS AND DISCUSSION

Seaweed samples of *Sargassum wightii* were collected from the study site. Five different isolates were obtained, out of which one isolate with high antimicrobial potential against *R solani* was selected and identified as *P stutzeri*. The isolate antagonistic to *R solani* is *P stutzeri*.The crude extract of *P stutzeri* was used for agar well diffusion against the culture of *R solani*. The inhibitory zone is given in plate 1.

Plate 1: Inhibitory zone of *P stutzeri* against *R solani*

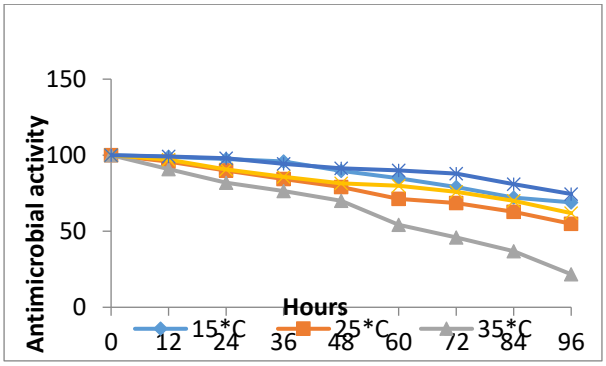

Fig 1 : Effect of temperature on the antagonistic activity of *P stutzeri.*

To determine the effect of temperature on antimicrobial potential the experiments were carried out at different temperatures. The temperature increases the rate of activity decreased because the catalytic activity of the enzymes starts to decrease beyond that temperature. Data shows that there was maximum antagonistic activity at room temperature of 35^0C .The effect of temperature on the antagonistic activity is shown in Fig 1.

P stutzeri is a nonfluorescent denitrifying bacterium widely distributed in the environment. The species has received much attention because of its particular metabolic properties.It has been proposed as a model organism for denitrification studies; many strains have natural transformation properties, making it relevant for study of the transfer of genes in the environment; several strains are able to fix dinitrogen; and others participate in the degradation of pollutants or interact with toxic metals. Phylogenetic studies of *P. stutzeri* strains' 16S rRNA sequences and other phylogenetic markers demonstrate that they belong to the same branch, together with related species within the genus, such as *P. mendocina*, *P. alcaligenes*, *P. pseudoalcaligenes*, and *P. balearica*. Typically, cells are rod shaped, 1 to 3 μm in length and 0.5 μm in width, and have a single polar flagellum. Phenotypic

traits of the genus include a negative Gram stain, positive catalase and oxidase tests, and a strictly respiratory metabolism. In addition, *P. stutzeri* strains are defined as denitrifiers. They can grow on starch and maltose and have a negative reaction in arginine dihydrolase and glycogen hydrolysis tests. *Pseudomonas stutzeri* is a ubiquitous bacterium with a high degree of physiological and genetic adaptability(Jorge Lalucat *et al* 2006).

In this study, there are five isolated strains, these bacteria could acquire the necessary nutrition such as vitamin, polysaccharide and fatty acid from their animal or plant hosts; while on the other, they could excrete products such as amino acid, antibiotic and toxin propitious for the development and metabolism of the hosts, or to improve the chemical defense capability of the hosts (Armstrong E,2001) .Marine microorganisms as model systems offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites and are currently being applied to the development of new drugs(Gibbons S and Gray A.I. 1998). In order to find more novel structures, new ways of screening of these compounds should be applied. Ecologically, the secondary metabolites produced by the different marine organisms may have a role in fish growth and protection from diseases. Development of economically feasible standard operating procedures for the production of extracts in large scale with reproducible antibacterial efficiency is necessary. The bioactive potential of epiphytic bacteria could be a potential source of marine bioprospecting in future. In the present study, *P stutzeri* isolated from *Sargassum* can inhibit *R solani*. The antagonistic activity was maximum at room temperature of 35^0C. Need of new alternatives is due to the decreased efficacy and resistance of pathogens to antibiotics. Further studies are required for the documentation of different bioactive compounds from seaweed associated bacteria.

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GOLD NANOPARTICLES SYNTHESIS IN EXTRACT OF *CURCUMA LONGA*, EVALUATION OF ITS TOTAL PHENOLIC CONTENT

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ABSTRACT

In this study, biosynthesis of gold nanoparticles (AuNPs) using the extract of Curcuma longa was undertaken, and antioxidant capacity of the synthesized nanoparticles was evaluated. Ultraviolet-visible spectrophotometer analysis confirmed the production of AuNPs at 500-600 nm where the color change in the solution from light yellow to pink indicated the formation of AuNPs. Plant extracts are known to contain polyphenols, flavonoids, tannins, which are known to be antioxidants. Therefore, an attempt was made to see if the biosynthesized AuNPs had better antioxidant activity compared to the extract by checking its antioxidant activities such as total phenolic content assay.

Keywords: *antioxidant, nanoparticles*

INTRODUCTION

The field of nanotechnology has recently witnessed spectacular advances in the methodology of nanomaterial's fabrication and utilization of their exotic physicochemical and Optoelectronic properties. Green nanotechnology offers the opportunity to stop the adverse effects of the use of chemical methods. Nowadays phytochemical mediated Metal nanoparticles have been employed and it has special chemical and physical properties (Mukherjee *etal*; 2001).

Plants are vital source of antioxidants in nature; they contain chemical compounds like flavonoids, phenols, and other compounds which show high antioxidant activity. Researchers are being carried out to find natural antioxidants from plants. Plants are safe and effective natural antioxidants, especially spices and herbs Polyphenols from food is important to prevent the oxidative stress due to over production of ROS (Sies, 1993).

In the present study the phenolic content of synthesized gold nanoparticle of *Curcuma longa* was evaluated.

MATERIALS & METHODS

Preparation of *Curcuma longa* aqueous extract

The rhizome, of *Curcuma longa* was washed thoroughly with tap water to remove debris. About 1 g of *C. longa* was mixed with 10 mL of distilled water and crushed in a mortar pestle. The aqueous extract of *C .longa* was filtered with Whatman No. 4 filter paper. The filtered extract was centrifuged at 1000 r/min for 10 min. The supernatant was collected and was kept at room temperature.

Green synthesis of GNPs:

Preparation of gold nanoparticle was done according to the method described by Sree lekshmi *et al*; 2013 with slight modifications. Biogenic gold nanoparticle was synthesized using Tetra Chloro auric acid solution ($HAuCl_4.3H_2O$ and *C. longa* extract. In a conical flask, 10 ml of *C. longa* extract was added to10µl of aqueous solution of 0.3M Chloroauric acid solution ($HAuCl_4.3H_2O$) at room temperature under static conditions. The reduction of Au NPs was clearly observed within the next 5-30 min. The solution has been modified from pale yellow to pink colour, which indicates the formation of biogenic (Au/*C. longa)* NPs.

Characterization of Au- NPs.

The reduction of Au-NPs was confirmed by using UV-vis spectroscopy in the range of 500 to 600nm (Shimadzu, UV-1601 UV-VIS Spectrometer).

Determination of Total Phenolic Content (TPC)

Total phenolic contents (TPC) determined by following the Folin- Ciocalteu method of slight modifications(Singleton *et al.,*1999) About One ml of plant extract and gold NP-s extracts added separately to a 25 ml volumetric flask filled with 9 ml distilled water. Folin Ciocalteu phenol reagent (0.5 mL) added to the mixture and shaken vigorously. After 5 min, 5 ml of Na CO solution was mixed up. The solution was instantly diluted to 4 ml with distilled water and mixed thoroughly. Thereafter it is then allowed to stand for 60 min before measurement .The absorbance measured at 750 nm versus the prepared blank. The total phenolics content (TPC) was revealed in terms of Gallic acid equivalent (GAE) in mg/g sample.

RESULTS AND DISCUSSION

In the present study gold nanoparticles were synthesised by using aqueous extract of *Curcuma longa* within 10 t0 20 minutes of incubation period pink colour was developed rapidly by the addition of $HAucl_4$ solution to the extract. The signatory pink colour was obtained which resulted due to the excitation of the surface Plasmon resonance vibrations of the gold nanoparticles formed.. The aqueous gold ions when exposed to herbal extracts were reduced in solutions thereby leading to the formation of gold hydrosol. The bio molecules found in the plants induce the reduction of Au^+ ions from auric chloride to gold nanoparticles (GNPs).Plants produce large number of H^+ ions during glycolysis along with NAD which act as redoxing agents this seems to be responsible for the formation of GNPs. Bio molecules like protein phenols and flavonoids not only play to reducing the ions to nanosize but also play an important role in capping of the nanoparticles (Jagadeesh et al; 2004). The synthesis of gold nanoparticles had been confirmed by measuring the UV –spectrum of the reaction medium. The UV spectrum of colloidal solution of GNPs synthesised from *Curcuma longa* has characteristic absorbance peak ranging from 500–600 nm(fig.1).The broadening of peak indicated that the particle are poly dispersed. An absorption band at 520nm is attributed to the aromatic amino acids of proteins. It is well known that the absorption band at 520 nm arise due to electronic excitation in tryptophan and tyrosine residues in the proteins. This observation indicates the release of protein into the solution by *Curcuma longa* and suggests a possible mechanism for the reduction of metal ions present in the solution.

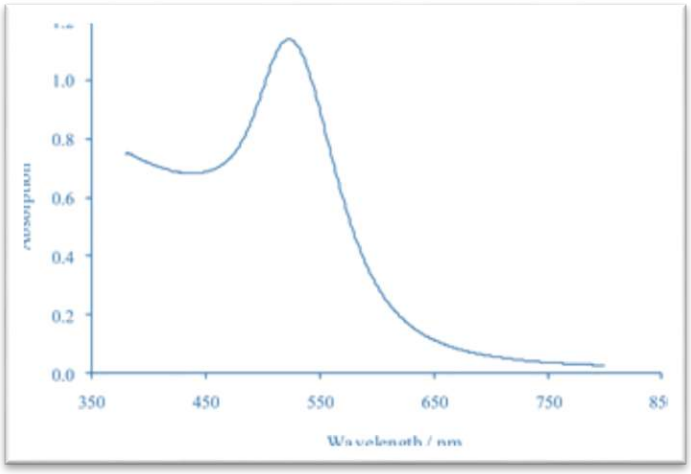

Fig 1: UV visible spectral analysis of biotransformed gold nanoparticles

Total phenolic assay

Phenolic compounds are responsible for the antioxidative action. polyphenolic compounds inhibits on mutagenesis and carcinogenesis the diet contains approximately 1 gm of phenolic substance rich in fruits and vegetable. Moreover it plays a vital role in stabilization of lipid peroxidation.The hydroxyl group present in the phenolic compounds are responsible for the radical scavenging activity.In the present study the total phenol present in C.longa also indicate a positive relationship exhibiting antioxidant activity. It was observed that the *C.longa* gold nano particles showed the higher amount equivalent of 84.07mg gallic acid than the control plant extract 77.12mg. Thus the *Curcuma longa* AuNps have the significant percentage of phenolic contents**.**

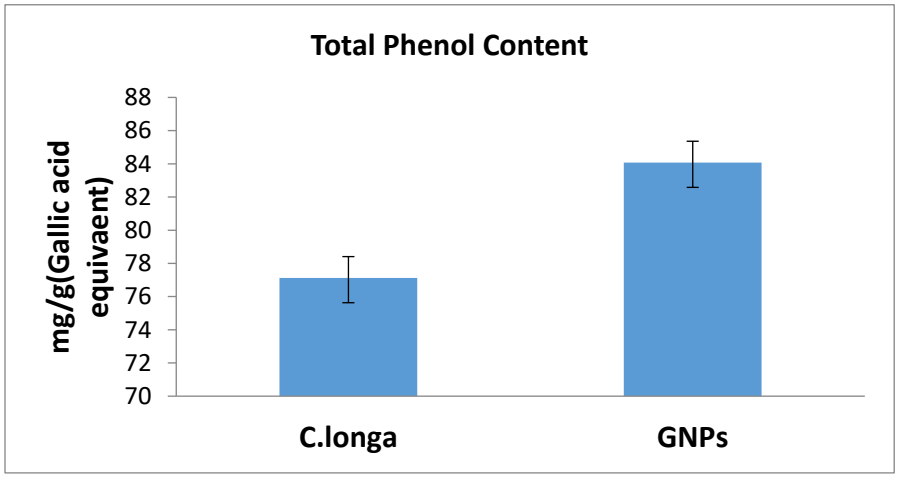

Fig 2: A comparative bar diagram showing TPC of *Curcuma longa* plant extract, AuNPs

CONCLUSION

In the present investigation, gold nanoparticles were synthesized from C.longa and the antioxidant properties of *C.longa* were studied. The total amount of phenol which is present in the plant gold nano particles was significantly high. The results in the present report suggest that the gold nanoparticle of the plant extracts of *C.longa* exhibited potent antioxidant activity and it can be further` subjected to evaluation of their bio-efficacies, active constituents, and molecular and biological mechanisms in vitro as well as in vivo on anti-oxidation or cancer chemoprevention effects

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PHYTOPLANKTON DIVERSITY AND WATER QUALITY ASSESSMENT OF VATTAKAYAL LAKE IN KOLLAM DISTRICT, KERALA, INDIA

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ABSTRACT :

The present work deals with the phytoplankton diversity and water quality assessment of Vattakayal Lake in Kollam district, Kerala. To fulfill the aim, phytoplankton and water samples were collected on monthly basis from October 2012 to September 2013. Phytoplankton samples were collected using 64 mm mesh size plankton net and preserved simultaneously Various water quality parameters like P^H, Temperature, Dissolved oxygen, Nitrate, Nitrite, Phosphate were analyzed into the laboratory using standard methods. Quantitative estimation was done by using standard method and identification of phytoplankton was carried out by using standard references. During the study period monthly variation in water quality parameters were recorded. In the present study 48 genera of phytoplankton belonging to four families were obtained from the Vattakayal Lake. Chlorophyceae (20 genera), Cyanophyceae (9 genera) Bacillariophyceae (15 genera) and Dinophyceae (4 genera). Among all chlorophyceae comprised highest generic diversity compared to others.

Key words : Phytoplankton, water quality, diversity, Vattakayal Lake

INTRODUCTION

The primary producers of organic matter in an aquatic ecosystem, which constitutes, the primary position in the tropic level in a food web and also forms the provides of food and oxygen to other organisms-plankton gather a wide range of importance. Phytoplankton in the autotropic component of the plankton community. Phytopkankton form the prime source of energy as primary producers and serve as a direct source of food to the other aquatic plants and animals (Saha *etal* 2000). The quality of phytoplankton is a good biopurifier and bioindicators of the aquatic systems. Phytoplankton ecology have focused on primary production and play a key role in maintaining equilibrium between components of aquatic ecosystem. (Pandey *etal* 2004) Physico chemical parameters, nutrient factors, biological interactions and carbon exchange significantly influenced the diversity and population of phytoplankton(Rajagopal *etal*.2010). Alteration in water quality ultimately affecting the phytoplankton community in terms of their diversity and abundance. In the point of view, the importance of phytoplankton in aquatic ecosystem present work was attempted to study the phytoplankton diversity and water quality in vattakayal lake in Kollam district, Kerala

MATERIALS AND METHODS

The present study was carried out monthly basis from October 2012 to September 2013 as per standard method (APHA 1998) . For the study Vattakayal Lake was selected as site. Vattakayal lake is situated in Kollam District. (8 º 56' N and 8º53 'N latitude and 76 º 32'E and $76^0$34'5 longitude). This lake is facing the bane of encroachment and varying degree of environmental degradation.

Measured quality of water (100 litre) from the surface was collected using plastic bucket, filtered through a plankton net made of boltng silk (standard grade No.25, mesh size = 64 mm) residue retained in the plankton net was transferred into plastic bottle containing 4 % formalin for further sorting and identification. The plankton were identified, using standard identification keys (Needham and Needham,1962, Palmer,1982, Krishna Pillai 1986, Edmendson 1992, Ward and Whipple 1992, APHA.1998) water quality parameters were analyzed once a month. Water samples were collected in selected sites.Temperature and were recorded immediately at the sites. Other physico chemical parameters viz. Dissolved oxygen, Nitrate, Nitrite and phosphate were analysed into the laboratory by using standard methods (APHA, 2005)

RESULT AND DISCUSSION

Monthly and seasonal variations in the density of phytoplankton at Vattakayal Lake in Kollam district during the period of October 2012 to September 2013 .Phytoplankton mainly represented by four taxonomic groups. Chlorophyceae, Cyanophyceae, Bacillariophyceae and Dinophyceae. Phytoplankton abundance was varied between 1100.01 to 4033.34 No.ml^{-1} . In chlorophyceae was varied from 400 to 1566.65 No.ml^{-1} , Cyanophyceae was varied from 100 to 966.67 No.ml^{-1}, Bacillariophyceae was varied from 366.67 to 1466.67 No.ml^{-1} and dinophyceae was 0 to 233.34 No. ml^{-1}. During the study period, temperature was varied between 24^0C to 32^0C. P^H of Vattakayal lake fluctuated between 6.1 to 7.8. Dissolved oxygen of Vattakayal lake varied from 1.6 to 7.8 $mg.^{1-1}$ Nutrients like Nitrate, Nitrite and phosphate were found as 28.8 to 42.8 μ g/l^{-1}, 0.21 to 0.93 kg/l and 13.1 to 52.6 μ g/l^{-1} respectively.

Healthy environment is very necessary for the growth and development of phytoplankton. Water temperature plays an important role in influencing the periodicity , occurance and abundance in the phytoplankton (Tripathi and Pandey, 1995). P^H is a major environmental factor of aquatic ecosystems, it supports the abundance and composition of phytoplankton. Dissolved oxygen is essential to the aerobic metabolism of all aquatic organisms. Nutrients availability in water is important for the phytoplankton growth. Nutrient level in water alters the abundance and composition of phytoplankton in water body. In the present study 48 genera of phytoplankton belonging to four taxonomic groups were obtained from the Vattakayal Lake. Chlorophyceae (20 genera), Bacillariophyceae (15 genera), Cyanophyceae (9 genera) and Dinophyceae (4 genera). Chlorophyceae was the most dominant group at all stations and Dinophyceae was generally the least represented. Dissolved oxygen support the growth of

chlorophyceae.Nirmal Kumar etal . 2011 reported that alkaline P^H supporting good population of Bacillariophyceae. The distribution of cyanophyceae depends upon availability of nutrients like nitrates and phosphates (Smith 1983)

CONCLUSION

In the present investigation attempts have been made to the phytoplankton diversity and variation in water quality parameter of Vattakayal Lake in Kollam district,Kerala. Phytoplankton dominated by chlorophyceae followed by bacillariophyceae, cyanophyceae and Dinophyceae. Monthly variations in water quality were recorded the study reveals that phytoplankton diversity and abundance depended on the water quality.

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A STUDY ON ARANEAN BIODIVERSITY OF KSM DB COLLEGE CAMPUS NEAR SASTHAMCOTTA LAKE

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INTRODUCTION

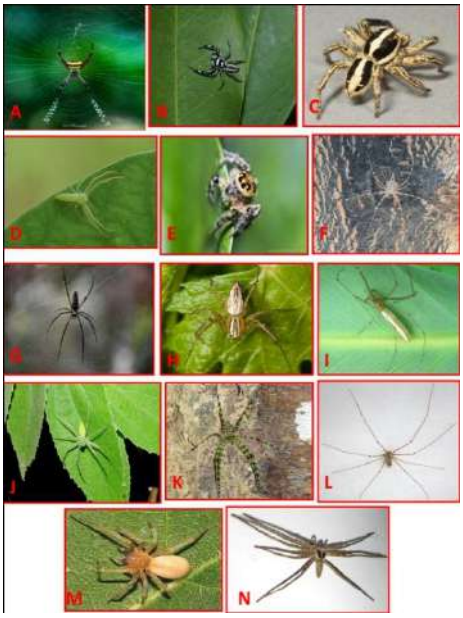
The Order Araneae include spiders, constituted a diverse invertebrate group of arachnids come under the phylum Arthropoda. They are the natural controllers of pest population. Spiders are present in everywhere except in Antarctica. There are more than 46,700 species found in all types of habitats. In India, 1,442 species in 59 families were reported. The class Arachnida is characterised by having two body regions a cephalothorax and an unsegmented abdomen, join at a narrow waist, called pedicel. The order Araneae comprises air - breathing arthropods that have 8 legs and chelicerae, with fangs that inject venom. The abdomen has no appendages except from one to four modified pairs of movable telescoping organs called spinnerets which produce silk. The aim of the present study was to investigate the biodiversity of spider in the campus of K.S.M.D.B. College Sasthamcotta, Kunnathur Taluk, Kollam (Dist).

METHODOLOGY

The study was conducted during the period from August 2018 to February 2019. In order to investigate the spider biodiversity in the KSM DB college campus, the spiders were searched on the ground, litter, undergrowth, bushes, tree trunks, foliage, and water bodies. When a spider was located, it was photographed using a digital camera (Nikon COOLPIX P900,83x optical zoom,166x dynamic fine zoom) and the identification of the spiders was done with the help of Tikader (1987) and Sebastian & Peter (2009), using internet search engines.

OBSERVATIONS AND RESULTS

The results revealed 14 spider species belonging to 9 families. They were *Argiope anasuja* (Araneidae), *Phintella versicolor* (Salticidae), *Oxytate virens* (Thomisidae), *Hyllus semicupreus* (salticidae), *Hersilia sp*.(Hersilidae), *Nephila pilipes* (Araneidae), *Oxyopus saiticus* (Oxyopidae), *Tetragnatha maxillosa* (Tetragnathidae), *Peucetia viridana* ((Oxyopidae), *Heteropoda Boiei* (Sparassidae), *Heteropoda venatoria* (Sparassidae), *Pholcus phalangiodes* (Pholcidae), and *Cheiracanthium* sp. (Miturgidae). The most observed spiders during the period of the study were *Hersilia* and *Hyllus* species.



A. ARGIOPE ANASUJA, B. PHINTELLA VERSICOLOR C. PLEXIPPUS PAYKULLI, D. OXYTATE VIRENS E. HYLLUS SEMICUPREUS F. HERSILIA SP., G. NEPHILA PILIPES H. OXYOPES SAITICUS I. TETRAGNATHA MAXILLOSA J. PEUCETIA VIRIDANA K. HETEROPODA BOIEI L. PHOLCUS PHALANGIOIDES; M. CHEIRACANTHIUM SP. N. HETEROPODA VENATORIA

DISCUSSION

About 40 families of spiders are recorded from Kerala. These belong to two major sub order called Araneomorph spider and Mygalomorph spiders. The first suborder included most of the commonly found spiders in and around our houses, while second sub order comprises of comparatively larger spiders, which live in the forest.

In the present study, 14 different spider species were around and near Sasthamcotta College.

CONCLUSION

The results revealed 14 spider species belonging to 9 families.Since spiders play crucial roles in the ecosystem, it is essential to know about their types, habit and habitat in order to keep the ecological balance and thereby the very existence of life on earth.

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CYTOMORPHOLOGY OF BLOOD CELLS IS THE MAJOR IMMUNE ORGANS OF *PRIACANTHUS HAMRUR*

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ABSTRACT

The present study was aimed to investigate the cytomorphology of blood cells in the major immune organs of *Priacanthus hamrur*. The moon tail bull's eye is an uncommon species found in outer reef slopes and deep lagoon pinnacles from 8 to least 80m. They are generally marketed fresh or may be salted or dried. The spleen is considered a secondary lymphoid organ that filters the blood and allows the B-cells that percolate through the white pulp to interact and respond to blood- borne antigen.In the *Priacanthus hamrur* in liver imprints only the mature lymphocytes and erythrocytes were seen. The population was dominated by lymphocytes. The head kidney or the pronephros has an important role in teleosts as the major blood producing organ involved in the production of erythroid, lymphoid and myeloid cells (Topf, 1953;smith.etal;1970). The present result fully supported the haemato poietic role of hard kidney, as in *Priacanthus hamrur*, head kidney imprints contained a respository of developmental stages of blood cells including haemocytoblasts. In *Priacanthus hamrur*, the thrombocytes are smaller than lymphocytes.

Key word : *Priacanthus hamrur*, lagoon, haematopoietic

INTRODUCTION

The immunology of teleosts has been the most intensity studied of all the fish groups owing to the great economical importance of several species as food source. In India Prianthrids are widely distributed all along southwest and east coast in the 50- 400 m depth zone with a peak concentration in the 100-200 m zone. Fish have a excellent immunological memory and so can be vaccinated against disease (Evans, 1998). In common with most other, vertebraten, the teleosts have spleen and thymus lymphoid organs. However, they do not have bone marrow or lymphnodes. The function of these tissues appears to be accomplished by two very important and unique lymphoid organs in the teleosts; the pronephros (head kidney) and the opisthonephros (trunk kidney). Several studies have shown that the pronophros ie, the major site of B-cell proliferation and antibody secretion (Anderson,2002). The spleen is considered to be erythropoietic (Mc Knight,1996; Haider;1963 b). The spleen is not a major site for antibody synthesis. Spleen is not a major site for antibody synthesis. Spleen functions as a lymphomyeloid organ in the fish. In the spleen imprints of *Priacanthus hamrur* only matured blood cells were present. Liver has a hematopoietic role as well as , particularly in young life stages of man and other vertebrates (Tizzard, 2004). In the liver imprints of *Priacanthus hamrur* only mature cells were seen.

Our current understanding of fish immunology is far behind that of mammals,even though many of the techniques and concepts workout on the latter are now being applied to fish.

MATERIALS AND METHODS

Specimens of these species were collected from the fish landing centre at Neendakara, Kollam . As soon as collected, the fishes were dissected out and the spleen liver and kidney of the *Priacanthus hamrur* were carefully exised and the imprints of each organ were prepared. The imprints were fixed in absolute methanol and stained with. Giema's stain properly stained imprints were made into temporary, xylene mounts and examined under oil immersion objective of a research microscope. Cell types present in each organ were studied for their shape and staining properly.

RESULTS AND DISCUSSION

In the present study, the imprints of the spleen of *Priacanthus hamrur* showed the presence of erythrocytes and lymphocytes. Presence of large number of lymphocytes attests the lymphopoietic role of spleen in *Priacanthus hamrur* however, whether this organ is involved in the genesis of erythrocyte is doubtful, as developmental stages in this lineage were not net with in the imprints. The majority of mature erythrocytes in the imprints were in a lysed state. During the present study, the spleen of *Priacanthus hamrur* seem to be more involved in destruction of erythrocytes than their production.

In the *Priacanthus hamrur*, in liver imprints only the mature lymphocytes and erythrocytes were seen. The population was dominated by lymphocytes.The present results fully supported the major haematopoietic role of head kidney, as in *Priacanthus hamrur* head kidney imprints continued a repository of developmental stages of blood including haemocytoblasts. Barring the presence of erythrocytes, the immune organs/sites examined of the teleost. *Priacanthus hamrur* -revealed the presence of several cell types, which in fishes are known to play crucial role in immunology such as lymphocytes, neutrophils and monocytes in the former and lymphocytes, neutrophils, eosinophils and monocytes after in the latter. Anderson (2002) reported that large number of blast cells or undifferentiated cells is seen in imprints of the head kidney of teleosts. Imprints of head kidney contained both mature and immature stages of erythrocytic and leucocytic series. The erythrocytes are sub spherical or oval in shape and their nuclei stain purple and cytoplasm bluish. Lymphocytes are round irregular cells. Nucleus stains pink and cytoplasm light blue. Platelets are small cells and they stain deep purple. The cytoplasm is indistinct. Immature cells present in the major lymphoid organs of the *Priacanthus hamrur* were the haemocytoblast, erythroblast, basophilic erythrocyte,lymphoblast and promonocyte. The haemocytoblasts are irregular cells with dark blue cytosome. Nucleus is darker than cytoplasm. Erythroblasts are rounded cells with centrally placed nuclei. Their cytoplasm stains bluish pink and neuclei purple. Basophilic erythrocytes are spherical cells. The nucleus is round and stains bluish pink and cytoplasm, light blue. Lymphoblsts are irregular cells with deep purple nuclei and pale blue cytoplasm. Promonocytes are rounded or irregular cells, their in nuclei are deep pink and cytoplasm, deep blue. In the present study promonocytes not abundant

there by attesting a good health status of the individuals of *Priacanthus hamrur* (head kidney and spleen) examined during the study.

CONCLUSION

In the liver imprints of *Priacanthus hamrur*, the mature cells were dominated by lymphocytes and a lesser number of erythrocytes. Hepatic cell nuclei were distinctively visible. In the spleen imprints, the mature RBCs are present, but majority of them were in the lysed state. In the head kidney imprints, both mature and immature stages of both erythrocytic and leucocytic series were met with. Haemocytoblasts were quite numerous.

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EVALUATION OF ANTIOXIDANT STATUS AND GLUCOSE LEVEL OF AIR BREATHING CATFISH, *CLARIAS BATRACHUS* (LINNAEUS, 1758) EXPOSED TO STREPTOZOTOCIN AND INSULIN

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Abstract

Oxidative stress plays pivotal role in progression and development of diabetes and its complications. The present study investigated the antioxidant potential and glucose level of streptozotocin (STZ)-induced diabetic fishes. Fish were induced diabetic by a single intraperitoneal injection of STZ (200 µg/kg) and the effect of insulin (40 IU/kg body weight) on air breathing Cat fish, *C. batrachus* were studied. In the present study, the activities of antioxidant enzymes like Catalase, SOD and GSH decreased while MDA level significantly increased after STZ treatment. Hyperglycaemia was evident in the blood of STZ exposed fish and insulin treatment lowered the condition. Hence, STZ induced diabetes model appears to be the most reliable and easily reproducible method of inducing diabetes mellitus in experimental animals like fishes.

Introduction

Oxidative Stress (OS) occurs when there is an increased generation of reactive oxygen species (ROS), decrease in antioxidant defenses, or a combination of both (Evelson *et al*., 2005). One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Streptozotocin (STZ). STZ was found to generate reactive species (ROS) leading to oxidative stress in the biological system (Szkudelski, 2001). The relationship between insulin and STZ has been poorly studied in fish. Hence, the aim of the present study was to examine the antioxidant status and glucose level of air breathing Cat fish, *C. batrachus* exposed to STZ and insulin.

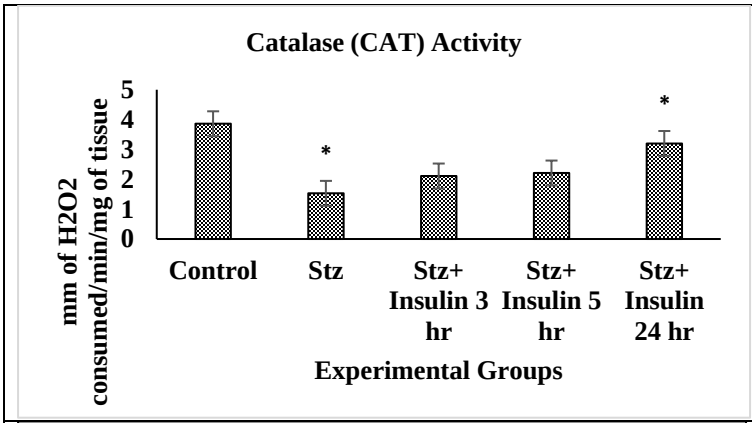
Materials and Methods

The healthy and adult fish, *Clarias batrachus* of approximate 45 ± 5g of body mass were collected from a local supplier near the Shasthamkotta lake, Kollam and acclimated in 20 L glass tanks with tap water at 28 ± 3°C (*p*H 7.2) under natural photoperiod (12L/12D) for three weeks prior to experiment. Experimental set up consists of thirty fishes which were divided into five groups of six each and placed in separate glass aquaria. Group I constituted 0.1 ml of saline injected controls for 24 hrs and Group II animals (Diabetic Group) were treated with 0.1 ml of 200µg/gm body weight STZ dissolved in saline. The third group of animal received STZ at a dosage of 0.1 ml of 200µg/gm b.wt and after 24 hrs were injected with 0.1 ml of 40 IU/mL Human insulin (Huminsulin, Recombinant DNA origin, Eli Lilly and company Pvt Ltd, India) and sacrificed after 3 hrs, 5 hrs and 24 hrs respectively. All injections were given intraperitoneally at the same time of the day (10 AM).

Results and Discussion

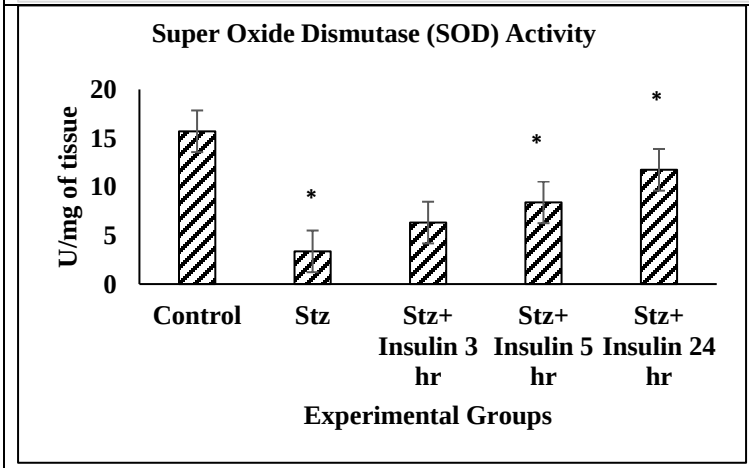
The CAT, SOD and Glutathione activities were significantly decreased ($P < 0.05$) after STZ treatment for 24 hr and reversal was observed after insulin administration at 3 hr, 5 hr and 24 hr respectively compared to the control group

(Fig. 1, 2 & 3). A significant elevation ($P < 0.05$) in MDA level was recorded in the liver tissue of fish in response to Streptozotocin (diabetic fish). But co-treatment with STZ and insulin lowered the MDA activity at 3 hr, 5 hr and 24 hr respectively (Fig.4). Blood glucose level was significantly increased ($P < 0.05$), Hyperglycaemia was evident on exposure to STZ treatment (Fig. 5) and significantly lowered ($P < 0.01$) the blood glucose level after insulin administration for 3 hr, 5 hr and 24hr respectively compared to the control.



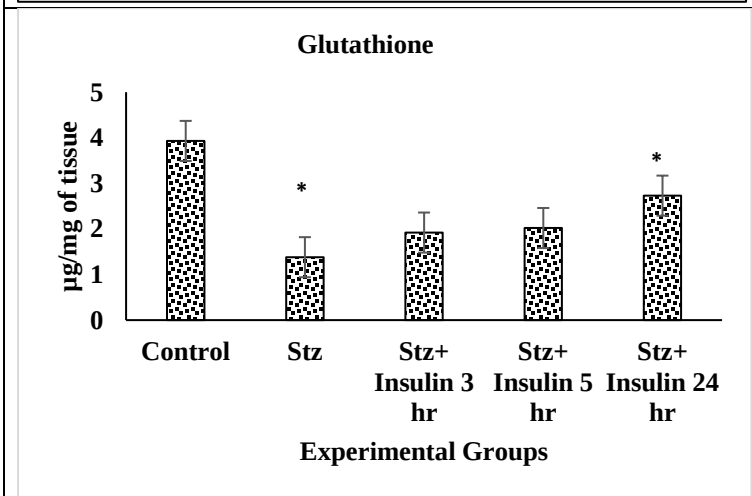
Data are represented as means ± SEM (n = 6).An asterisk (*) indicates significant difference ($P < 0.05$).

Fig. 1 Catalase activity in *C. batrachus* exposed to 24 hr Streptozotocin and insulin treatment



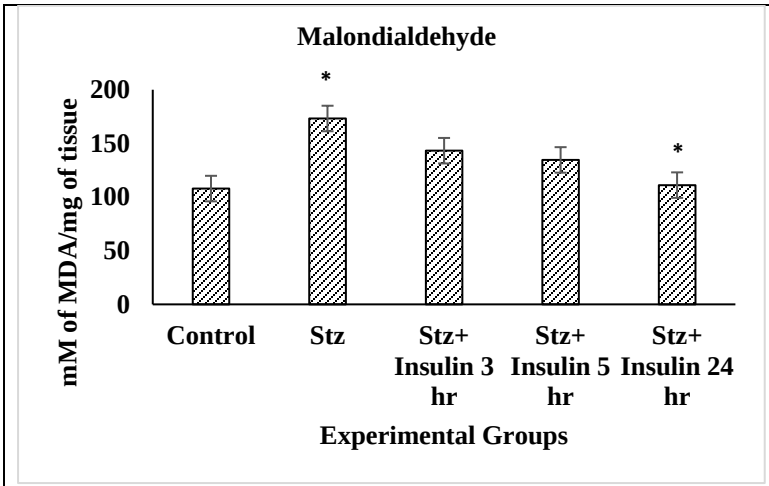
Data are represented as means ± SEM (n = 6). An asterisk (*) indicates significant difference ($P < 0.05$).

Fig. 2 Activity of SOD in *C. batrachus* exposed to 24 hr Streptozotocin and insulin treatment

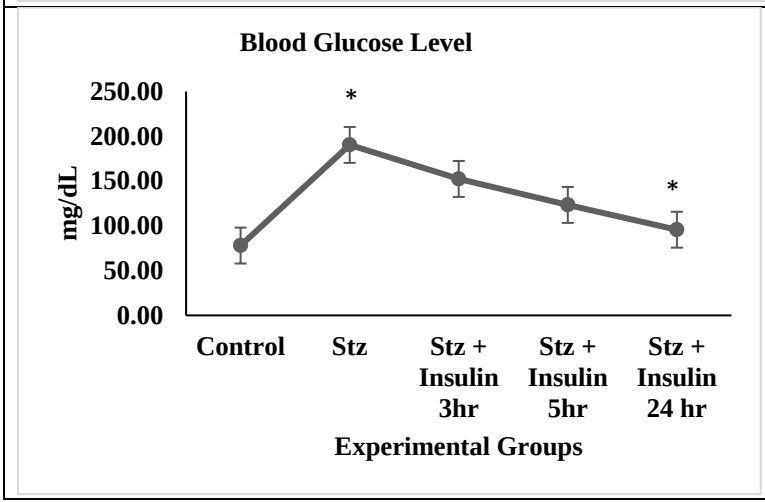


Data are represented as means ± SEM (n = 6). An asterisk (*) indicates significant difference ($P < 0.05$).

Fig. 3 Activity of GSH in *C. batrachus* exposed to 24 hr Streptozotocin and insulin treatment



Data are represented as means ± SEM (n = 6). An asterisk (*) indicates significant difference ($P < 0.05$).
Fig. 4 MDA level in *C. batrachus* exposed to 24 hr Streptozotocin and insulin treatment



Data are represented as means ± SEM (n = 6). An asterisk (*) indicates significant difference ($P < 0.05$).
Fig. 5 Blood glucose level in *C. batrachus* exposed to 24 hr Streptozotocin and insulin administration (3 hr, 5 hr and 24 hr)

Superoxide dismutase is one of the most important antioxidant enzymes that convert superoxide radicals into the hydrogen peroxide. However, CAT and GPx enzymes neutralize the hydrogen peroxide into the water in peroxisomes and cytoplasm, respectively. GSH is known to protect the cellular system against the toxic effects of lipid peroxidation. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of the enzyme, which have been reported to occur in diabetes (Sozmen *et al*., 2001). MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress. Hyperglycaemia was evident in the blood of STZ exposed fish which may help fish to meet critical needs of energy. Such elevation may also be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands.

Conclusion

The toxic actions of Streptozotocin on pancreatic B cells are generation of free radicals and disturbances in intracellular calcium homeostasis. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism, increased generation of reactive oxygen species, and decreased level of antioxidant defences such as GSH and SOD.The present study clearly demonstrated that the short term effect of Streptozotocin to Catfish, *C.batrachus* caused significant variation in antioxidant and glucose level in

fish. Hence, Streptozotocin induced diabetes model appears to be the most reliable and easily reproducible method of inducing diabetes mellitus in experimental animals.

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PHYTOCHEMICAL SCREENING AND COLOUR ENHANCING ABILITY OF *AQUILARIA MALACCENSIS* INCORPORATED FEED IN *BARBONYMUS SCHWANENFELDII* (BLEEKER, 1853)

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ABSTRACT

In view of the deteriorating effects on the environment due to use of synthetic pigments, the present work aims to emphasize the need for natural pigment enhancers and growth promotors sources as an alternative to synthetic chemicals. The work conducted by focusing on the objectives like accessing phytochemicals qualitatively with quantification of carotenoids.The present study attempts to investigate the efficacy of *Aquilaria malaccensis* supplemented feed and commercial feed to *Barbonymus schwanenfeldii* (Bleeker, 1853) for an experimental period of two month from 1-2-2018 to 30-3-2018. Fishes were fed twice a day, in the morning and the evening, kept in glass aquaria, denoted as Test tank and Control tank. After an acclimatization period of one week by feeding with commercial feed, fishes were randomly arranged with ten fishes in a tank. Thus each tank occupied ten fishes. The survival remained undeviated throughout the period. The carotenoid content evaluated in test fishes were 17.44 µg/g wet weight than the control fishes with 13.64 µg/g wet weight. Hence arrived at the conclusion that feed supplemented with *A. malaccensis* enhanced growth and colour hue due to the associated phytochemicals.

Keywords: *Bacopa monnieri*, *Barbonymus schwanenfeldii*, phytochemicals, carotenoids.

INTRODUCTION

Ornamental fish farming is the culture of attractive, colourful fishes of various characteristics which are reared in a confined aquatic system. In the recent decades ornamental fisheries sector has emerged as a globally growing million-dollar industry comprising cultivation of various fresh water fishes. The domestic trade with exotic species is far better with 20% growth annually due to its aesthetics with colour pattern. The attributes governing their market value are colour pattern, growth and disease resistance. Fishes evolved around 400 million years are excellent diversified vertebrates, refresh the viewers' sight being utilised by the Psychiatrist to treat psychic disorders. Hobbyists prefer rearing fishes as a part time activity (Divya and Sreeja, 2018) as the production cost is cheaper with huge returns within a short interval of time.

Phytochemicals literally mean plant chemicals are the naturally occurring chemicals in plants provides them colour, odour and flavour. Plant ingredients which could enhance growth along with pigmentation need to supplement while preparing aqua feed, promote survival in ornamental fishes proved by Divya and Sreeja, 2018. Ashley, 2007, suggested

that such alarming situation can be tackled by everlasting eco-friendly application of natural products in its crude or extract form also proved by Divya and Sreeja, 2018. Biodegradable plant products elevate antistress improved growth with anti-pathogen property not only in edible but also in ornamental fishes (Divya and Sreeja, 2016). The entire plant or its ingredients analysed for phytochemicals having the potential to enhance growth along with pigmentation need to supplement while preparing aqua feed to promote survival need to be successfully applied in ornamental fishes with available knowledge in edible forms proved by Divya and Sreeja(2018). Generally, harmless plant materials which are less toxic, cheaper with little side effects with a calibre to induce growth, colour and disease resistance attenuates the economic profile of ornamental fish culture.

REVIEW OF LITERATURE

In India, "ornamental aquaculture" demands true innovations and implementation of advanced technologies to promote itself to the next level to compete in the international market. Colour is one of major factor which determines the price of aquarium fish in the world market. Ornamental fish trade is very much dependant on the vibrant colour of the fish. Fish are coloured in nature often show faded colouration under intensive culture conditions. Fish like other animals do not synthesise carotenoids and depend on dietary carotenoid content for the colouration. Dietary factors-nutrients and chemical compounds that the fish eats which directly or indirectly influence colour (Sony Deposition, 2013). In view of the deteriorating effects on the environment due to use of synthetic pigments, the present work aims to emphasise the need for natural pigment colouring sources which will act as an alternative to synthetic chemicals. As the aqua feed industry seeks a natural, environment friendly source of pigment to improve colouration and to enhance commercial acceptability, there is a great potential for use of natural plant base carotenoids for pigmentation in aqua culture.

METHODOLOGY

Phytochemical screening

Aquilaria malaccensis leaves collected from the residence of Associate professor Sreeja J, HOD, Department of Zoology, Sree Narayana College for Women, Kollam, was thoroughly dried in the shade, mechanically grinded to fine powder and stored in air tight bottle for analysis. The extractive value or extractive yield calculated by the formula. Extractive yield % = $(W_1/W_2) \times 100$ where, W_1 = net weight of powder in grams after extraction and, W_2 = total weight of powder in grams taken for extraction. 1000mg of extract was dissolved in 100ml of its own mother solvent subjected to preliminary qualitative phytochemical screening tests outlined by Harborne, (1973).

Feed preparation and estimation of Carotenoids

The commercial feed was taken as the control diet while the experimental diet prepared with the commonly available ingredients like rice bran, ground nut oilcake, wheat, prawn and medicinal plant. After an experimental period of thirty

days the total carotenoid concentration in the fish muscle was analysed by using pigment extraction method as described by Briston (1995).

RESULTS AND DISCUSSION

The extractive yield% obtained for *Aquilaria malaccensis* in aqueous extract was 8.60. Phytochemical screening test performed revealed the presence of phytochemicals enlisted below accounted for their therapeutic values. The carotenoid content was higher for plant material incorporated feed (17.44 µg/g wet weight) compared to control fish with 13.64 µg/g wet weight.The qualitative phytochemical screening tests performed revealed the presence of polyphenols, recognised as the natural source of antioxidants stated by Giovanelli and Buratti, 2009.The Alkaloids in them well known for antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory, and pharmacological effects reported by Turan, 2006. But Yiadom, 1979 proved their host-mediated responses. Alkaloids are associated with nitrogenous compounds used in cancer treatment to interfere cell division.

Bohm, 1997 detailed about it as a source of cardiac glycoside. Terpenoids and tannins possess analgesic and anti-inflammatory activities heals wounds and inflamed mucous membrane fastly (Bruneton, 1999). Flavonoids detected are powerful antioxidants that neutralise the harmful free radicals, anti-ageing factor (Alcaraz *et.al*., 2000). Saponins perform cardio depressant properties (Olaleye, 2007). Thus we can infer that the secondary metabolites (phytochemicals) accounted for the medicinal value of *A.malaccensis* . Research works already proved efficacy of medicinal herbs as additives and growth stimulator, (Abdelwahab,2012; Malar and Charles, 2013). The active principles in the test diet chosen enhanced the colour of experimental fishes (Divya and Sreeja, 2018).

Total carotenoid concentration in the body of fishes fed with control and experimental diet revealed better result for plant material incorporated concerned to the control feed hence approved and support the work done in Koi carps with 250mg of astaxanthin (Liang *et al*., 2012) with a saturation point in the accumulation of carotenoids in dwarf cichlids (Harpaz, 2007). The conducted work thus suggests that even though fishes are unable to synthesise carotenoids the supplementation of carotenoid enhancers in feed augment their colour hue by Divya and Sreeja (2018) though its effectiveness remained species specific (Ha *et al*.,1993).Similar results were obtained for gold fish by feeding with spirulina (Kiriratnikom *et al*., 2005).

CONCLUSION

In the present study, it was found that most of the biologically active phytochemicals were present in the aqueous extracts with which they provide the defence to plants. The generated data thereby not only provided the basis for its wide uses as a therapeutic both in traditional medicine but also opened a gateway of sustainable ornamental fish production.

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METAZOAN PARASITES IN TWO EDIBLE FISH SPECIES

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ABSTRACT

Fishes present in polluted water are more prone to infection with metazoan parasites than those found in non-polluted water.In the polluted water fishes are infested parasites than other metazoan groups more with copepod. Copepods infesting the fishes of polluted waters comprise mainly Ergasilus and Dermoergasilus species.Nematodes, cestodes and termatodes are seen rarely infesting the fishes of polluted waters.Percentage of incidence of infestation of parasites in most of the fish species is more in females than in males and in immature ones. The highest infestation was observed in the gills and lowest infestation in the intestine. Gills are the favourite site for the attachment of many fish parasites.

INTRODUCTION

Fish and fisheries products are important sources of protein and contribute a great deal to available food resources worldwide. Over-fishing and environmental degradation are already threatening most of the larger fish stocks, and a further increase in fisheries production seems to be dependent on the cultivation of aquatic organisms within semi-extensive and intensive mariculture. An intensive culture leads to an increasing risk of infection by disease causing agents, such as fungi, viruses, bacteria, and parasites (Palm,2007). Marine parasitology is an important field in aquatic science. Because of its close linkage to other fields in marine sciences such as fisheries, mariculture, fish ecology and environmental monitoring, marine parasitology should be seen in the context of other marine science disciplines.

Present study presents the comparison between the metazoan parasite faunas of E. *suratensis* and *R. kanagurta* in their natural environment. In the present work, an attempt has been made to assess the presence of parasites, prevalence, intensity, and percentage of infestation in these fish species along the region of Neendakara and Kureepuzha region of Kollam, Kerala, India. The study conjointly provides an insight into the rising pollution status along the aquatic systems of Kerala.

Fishes present in polluted water are more prone to infection with metazoan parasites than those found in non-polluted water. In the polluted water fishes are infested parasites than other metazoan groups more with copepod. Copepods infesting the fishes of polluted waters comprise mainly *Ergasilus* and *Dermoergasilus* species. *Nematodes, cestodes* and *termatodes* are seen rarely infesting the fishes of polluted waters. Percentage of incidence of infestation of parasites in most of the fish species is more in females than in males and in immature ones. The highest infestation was observed in the gills and lowest infestation in the intestine. Gills are the favourite site for the attachment of many fish parasites. The intensity of infestation of parasites in most of the fish species is greater in females than in males and immature ones. The study conjointly provides an insight into the rising pollution status along the aquatic systems of Kerala.

METHODOLOGY: Fishes were collected from Neendakara and Kureepuzha a part of Ashtamudi Lakes during February 2019. Two sampling sites were selected representing differing water quality and parasitic occurrence, that are likely to affect fish infestation. The fishes and water samples for the present study were collected from the two sampling sites.

Site 1 – Neendakara (8º 56' N latitude & 76 º 32' E longitude) and **site 2** – Kureepuzha: A part of Ashtamudi lake (9°54' and 9°55'N L and 76°46' and 76°48' E L): characterized by organic pollution of sewage and litters. For the detailed study of nature infections, two species of fishes from the two different habitats were selected. Collection of fishes were carried out from Neendakara harbour and Kureepuzha between 6.00 am to 9.30 am in the morning. Fishes were brought to the laboratory in fresh condition and maximum number of them were examined for the presence of parasites based on their sex.

PARASITOLOGICAL STUDIES: Relevant techniques for parasitological examination were adopted from Fernando et al.,(1972). The body surface and fins were examined using a magnifying glass or whenever necessary by using Stereo dissection microscope (Maximum magnification 40 X). The parasites removed were recorded. The alimentary canal was cut open and thoroughly examined under SDM. The parasites and their numbers were recorded.The data regarding standard length, weight and sex of the host fishes were recorded.

THE HOST FISHES

The host fishes, collected from the two sites, examined for parasites are the following

Etroplus surratensis (Bloch, 1790) & ***Rastrelliger kanagurta (Cuvier, 1816)***

Difference in Parasite Faunas

The metazoan parasite faunas of the two fishes differed; *R. Kanagurta* was comprised only 12 species representing five taxa whereas in *E. suratensis* it was represented by 08 species belonging to six taxa (Table 1).

SITE I

Out of the twenty-three fishes examined from Neendakara nine fishes were infested with parasites. A total of 62 parasites were collected. Maximum number of parasites infested in a fish was 06 *i.e*., in a male fish, 05 in a female fish and 03 in an immature fish. The parasites collected were copepods belonging to Ergasilidae family. They were *Dermoergasilus hoi, Eragiasilus sp, Pseudohaliotrema sp, Centrocestus formosanus, Eyelevera sp, Prodistomum orientalis (Layman,1930), Lecithocladium angustiovum, Orbitocolax aculeatus Pillai, 1967, Nothobomolochus sp, Caligus sp, Lernanthropus sp* and *Peniculus sp*. The percentage of incidence of infestation is 75% in male fishes, 60 % in immature fishes and 42 % in females. The intensity of infestation in immature fishes is 7, in males it is 2.16 and in females it is 5.60.

Table II Parasites present in *Rastrelliger kanagurta*

Rastrelliger kanagurta	Immature	Male	Female	Total
Number of fishes examined	05	08	12	23
Number of fishes infested with parasites	03	06	05	14
Number of parasites collected	21	13	28	62
Percentage of Incidence of Infestation (%)	60	75	42	
Intensity of Infestation	7	2.16	5.60	

SITE II

Out of the twenty seven fishes examined for parasites only one fish was infested with parasites (Table II). The parasite infested in a female host was Monogenea belonging to *Pseudohaliotrema sp, Cleidodiscus sp and Enterogyrus globodiscus; Monogenea belonging to Ergasilus parvitergum, Saccocoelioides sp, Trematoda* belonging to *Opegaster ditrematis and copepod belonging to Dermoergasilus hoi*. Total number of parasites collected were 3 and the maximum number of parasites infested in a fish was 3 *i.e.*, in a female. ln males and immature fishes no parasites were seen. The percentage of incidence of infestation was zero in immature ones and males whereas in females 82%. The intensity of infestation was recorded only in females it is 5.

Table III Parasites present in *Etroplus suratensis*

Etroplus suratensis	Immature	Male	Female	Total
Number of fishes examined	08	08	11	27
Number of fishes infested with parasites	03	05	09	17
Number of parasites collected	0	0	05	05
Maximum number of parasites infected in a fish	0	0	22	22
Percentage of Incidence of Infestation (%)	0	0	82	
Intensity of Infestation	0	0	05	

DISCUSSION

The parasitological terms like prevalence and mean abundance were calculated to determine the abundance of parasitic species. At site I, out of the twenty-three fishes examined , nine fishes were infested with parasites. A total of 62 parasites were collected. Maximum number of parasites infested in a fish was 06 i.e., in a male fish, 05 in a female fish and 03 in an immature fish. The parasites collected were copepods belonging to *Ergasilidae family. They were*

Dermoergasilus hoi, Eragiasilus sp, Pseudohaliotrema sp, Centrocestus formosanus, Eyelevera sp, Prodistomum orientalis (Layman,1930), Lecithocladium angustiovum, Orbitocolax aculeatus Pillai, 1967, Nothobomolochus sp, Caligus sp, Lernanthropus sp and *Peniculus sp*.

At site II, among the twenty seven fishes examined for parasites, only five female fish was infested with parasites. The parasite infested in a female host was Monogenea belonging to Pseudohaliotrema sp, Cleidodiscus sp and Enterogyrus globodiscus; Monogenea belonging to Ergasilus parvitergum, Saccocoelioides sp, Trematoda belonging to Opegaster ditrematis and copepod belonging to Dermoergasilus hoi. Total number of parasites collected were 3 and the maximum number of parasites infested in a fish was 3 i.e., in a female. ln males and immature fishes no parasites were seen.

The present study investigates that there is the presence of *Ergasilus* sp in the host fish Kanagurta may be no way fatal, high levels of infestation can cause serious damage to individual fish and consequently to fish population as a whole (Radhakrishnan, 1979 and Jayarajan, 1986). The highest infestation was observed in the gills and lowest infestation in the intestine. Gills are the favorite site for the attachment of many fish parasites. It is clear that in the Kureepuzha, the host fishes are minimal parasitic infection except R.kanagurta of Neendakara region. The most abundant species of parasite infested was the *Dermoergasi hoi* present in all host species of fishes. So it can be concluded that D. hoi is a resistant sp of copepod that can tolerate pollution to a great extent and thus the parasite is not host specific. Pollution decreases the immunity of fishes and this in turn makes the fish more susceptible to infection. Dissolved oxygen content is less due to pollution and this might both in reason fishes in the polluted water are more infested.

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A STUDY ON THE CHROMATOPHORE DISTRIBUTION OF TWO CEPHALOPODS FROM KOLLAM COAST

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ABSTRACT

Cephalopods have been a source of mystery and wonder for generations ,and the subject of interest to researchers for quite some time .What is the most striking is its ability to produce as well as change its colour quickly. Many reasons have been attributed to this phenomenon –camouflage, attraction of the opposite sex for mating, warning, hunting etc.

A study of this kind has not been reported from the cephalopods of kollam coast and hence the objective of our study The tables 1-3 shows the distritribution of chromatophores in Cistopus indicus in different rejoins and tables 4-6 shows the distribution of chromatophores in Loligo duvuacelli. It was found that the octopus species had a varied distribution of chromatophores like erythrophores, xanthophores, melanophores, iridophores,etc. While the Loligo species did not appear to be very colourful with just xanthophores and melanophores on their skin.. One of the reasons for this difference in distribution could be because of the difference in the habitat. The Cistopus species being more of the a bottom dweller needs to complete with the larger predators for its existence while the Loligo species , a mid dweller has better availability of food.

Extensive research need to be undertaken to understand the implications of this differential distribution of chromatophores in the two different cephalopod species. This study serves to throw some light in this direction.

KEYWORDS: *Cistopus indicus, Loligo duvuacelli, chromatophore index, colour,distribution*

INTRODUCTION

cephalopods A Cephalopod is any member of the molluscan Class Cephalopoda (head-feet) such as squid, octopus or nautilus. Cephalopods became dominant during the Ordovician period, represented by primitive nautiloids. The class now contains two, only distantly related, extant subclasses: Coleoidea, which includes octopuses, squid, and cuttlefish; and Nautiloidea, represented by Nautilus and Allonautilus. About 800 living species of cephalopods have been identified. Two important extinct taxa are Ammonoidea (ammonites) and Belemnoidea (belemnites). An estimated 11,000 extinct have been described, although the soft-bodied nature of cephalopods means they are not easily fossilized (Wilbur et al., 1985).Brief squid found in Chesapeake Bay is the only species which can tolerate brackish water (Bartol et al., 2002).Cephalopod diversity is greatest near the equator (~5 species captured at 60^0N) (Nixon et al., 2003).surprisingly, given their ability to change colour, all octopods(Boyle and Rodhouse,2004) and most cephalopods (Messenger et al., 1998) are considered to be colour blind. Chromatophores are coloured pigment cells that expand and contract in accordance to produce colour and pattern which they can use in a startling array of fashions (Kingston et al., 2015).the bioluminescence is produced by bacterial symbionts; the host cephalopod is able to detect the light produced by these organisms (Tong, D.et al., 2009).Cistopus is a genus of octopus in the family Octopodidae from the Indo- Pacific region, colloquially known as "old lady" octopuses. *Cistopus chinensis, C.indicus, C.platinoidus, C.taiwanicus species* are classified in genus

cistopus. Uroteuthis is a genus of 14 species of common inshore squids in the family Loliginidae from Indo-West Pacific region. Which is divided into 3 subgenera; Subgenus Uroteuthis, Subgenus Aesturiolus, Subgenus Photololigo and Subgenus Incertae (i.e. uncertain).However *Loligo duvuacelli or Uroteuthis duvuacelli* comes under the genus Photololigo. Tremendous research has been carried out on cephalopods worldwide but scientific research on cephalopods from Indian waters is noted to be meager. The present study on the two commonly available *Cistopus indicus and Loligo duvuacelli* was undertaken to fill this lacuna of research on cephalopods from Indian waters.

MATERIALS AND METHOD

As a reference we used a work on the seasonal variations in chromatophore index in *Punitius sophore* which is a fish species from Jammu water bodies done by Krishan Raj Kant, Kadambri gupta, Seema Langer , published in the International Journal of Fisheries and Aquatic studies 2016; 4(4): 425-430 . The octopus and squid specimens were collected from Neendakara harbor and brought to the lab.

With the help of a needle and scissors, a fine peel of octopus skin was taken. It was neatly placed on the slide. 2-3 drops of 0.5% KCl was added and a coverslip was placed over it without making any air bubbles. After observing the preparation under the microscope, the chromatophore index was calculated after taking the count in three different positions. The dorsal, ventral and tentacular regions were observed. The same process is alsodone in loligo.

The chromatophore index is calculated by the method;

the chromatophore index in the region = Average of chromatophores in the position taken in a region.

RESULT

The skin of two cephalopods namely octopus , *Cistopus indicus* and squid , *Loligo duvuacelli* were observed to study the types and distribution of chromatophores on its skin.The chromatophores observed were xanthophores, cyanophores,melanophores,erythrophores and iridophores.In octopus, more of cyanophores were seen on all the region viz;dorsal, ventral, tentacular skin while 19% of cyanophore was seen in the dorsal region (plate II,III &IV), 24% was seen on the ventral region(Fig:2), while 16% (Fig 13) was observed in the tentacular region.

This was followed by the presence of xanthophores more on the ventral rejoin (Fig 11&12) and tentacular region when compared to the dorsal region.Presence of iridophores was observed on the tentacular region (Table IV, Fig.13) of *Cistopus* species.The chromatophores observed in *Loligo* were xanthophores and cyanophores only. The distribution of xanthophores was high on the ventral region (20%) (plate VI,Fig.18) and tentacular region (17%) and least on the dorsal region(12%) (table V,Fig.15) was observed on the dorsal

regoin followed by 15% on the tentacular region and (13%) on the ventral region (13%) (plate VI&VII) of *Loligo species.*

DISCUSSION & CONCLUSION

The present study was just an attempt to understand the distribution of chromatophores present in the two cephalopods obtained from the Arabian sea and how it could be related to its habitat. It was seen that *Cistopus spp*. , being a bottom dweller, had a wider and colourful distribution of different chromatophores when compared to its counterpart the *Loligo* species, a mid-dweller, which seemed to have just xanthophores and melanophores on them. The reason for this difference could be because of the greater exposure of Octopus to larger predators that live at the bottom of the oceans and seas. Cephalopods have been a source of mystery and wonder for generations, and researchers continue to discover new and even more fascinating aspects of these bizarre creatures. Suffice to say when you are able to see them outside of their camouflage these creatures are definitely more than meets the eye.

Cephalopods have been a source of mystery and wonder for generations, and the subject of interest to researchers for quite sometime. What is most striking is its ability to produce as well as change its colour quickly. Many reasons have been attributed to this phenomenon- camouflage, attraction of the opposite sex for mating, warning, hunting, etc. A study of this kind has not been reported from the cephalopods of Kollam coast and hence the objective of our study. It was found that the octopus species had a varied distribution of chromatophores like erythrophores, xanthophores, melanophores, iridophores, etc. while the Loligo species did’nt appear to be very colourful with just xanthophores and melanophores on their skin. One of the reasons for this difference in distribution could be because of the difference in its habitat. The Cistopus spp. being more of a bottom dweller needs to compete with the larger predators for its existence while the Loligo spp., a mid-dweller has better availability of food. Extensive research needs to be undertaken to understand the implications of this differential distribution of chromatophores in the two different cephalopods species. This study just serves to throw some light in this direction.

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A STUDY OF VECTOR INDICES OF AEDES AEGYPTI IN MUNDAKKAL AREA, KOLLAM

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ABSTRACT

In Kerala, Kollam district has been reporting high Dengue cases as per disease surveillance and newspaper reports. So a study was carried out to calculate vector indices around Mundakkal area, Kollam corporation in Kerala State. A cross sectional study with entomological survey was conducted in houses in the above area. A total of 289 houses were visited by students and entomologists from District Medical Office, Kollam. House index, container index and Breteau index werecalculated after the survey. The prevalence of fever was less at this time. Breteau index was higher in many areas under study. Conclusion of the study was a possible outbreak of epidemics in the rainy season. All the households were cleared of sources and awareness could be given to the population in the survey area. It is recommended that public should be more vigilant in source reduction and they should cooperate with health department.

INTRODUCTION

In epidemiology, a disease vector is any agent who carries and transmits an infectious pathogen into another living organism. Arthropods form a major group of pathogen vectors with mosquitoes, flies, sand flies, lice, fleas, ticks, and mites transmitting a huge number of pathogens.

In Kollam district, Kerala there has been an increase in number of fever cases as reported from Official disease notification system integrated disease surveillance project (IDSP) and several newspapers and media. Similarly a few studies have been made by Chadee et al.,1990, Anderson et al., 1991, Kumar eta al.,2012 etc. The data from the district shows that dengue cases were increasing since 2009 (Gopakumar et al 2018). The objective of the present study is larval survey of Mosquitoes so that Dengue larvae prevalent in that particular area, sources and indices can be understood and awareness may be given to public.

MATERIALS AND METHODS

The survey was conducted in Mundakkal area of Kollam corporation on 21/11/2018 by students of BSc Zoology in S N College for Women, Kollam along with entomology team from District hospital, Kollam. 11 batches of students with 6 members each inspected the area. The indices that are commonly used to monitor Aedes aegypti infection levels are

i) House index (Hl): percentage of houses infected with larvae and/or pupae

HI = Number of Houses infected Number of Houses inspected X100

ii) Container Index (Cl): percentage of water holding containers infected with larvae or pupae.

Cl = Number of positive containers Number of containers inspected X100

iii) Breteau Index (Bl): number of positive containers per 100 houses inspected Bl: Number of positive containers Number of houses inspected X100

Larvae are collected with a dipper, a plastic cup attached to the end of a thin pole. Larval breeding areas are sampled by dipping the cup into water and inspecting it for larvae. Inspections are performed on several types of sources such as tanks, basins, coconut shells, plastic containers, covers, ponds, tubs and so on. The data was tabulated in a Data sheet. A final consolidated statement was also prepared.

RESULTS

The results are given in tables 1-3 and Figure 1.

The survey team visited 289 households as 11 teams. A total of 836 subjects were surveyed who resided in that area. The number of fever cases reported were 20. However 58 houses were positive for Aedes breeding. 325 containers were examined for mosquitos and found that 92 containers were positive for Aedes breeding.

The indexes were calculated for each team

Team 1(26 houses)	**House index was 26.92**	**Container index was 25.71**	**Breteau index was 34.61**
Team 2(25 houses)	House index was 48	Container index was 51.4	Breteau index was 76
Team 3(29 houses)	House index was 17.24	Container index was 18.18	Breteau index was 27.58
Team 4(20 houses)	House index was 45	Container index was 37.5	Breteau index was 60
Team 5(25 houses)	House index was 24	Container index was 17.3	Breteau index was 32
Team 6 (30 houses)	House index was 16.66	Container index was 100	Breteau index was 16.6
Team 7 (25 houses)	House index was 20	Container index was 59.25	Breteau index was 64
Team 8 (35 houses)	House index was 8.57	Container index was 20.68	Breteau index was 17.14
Team 9 (35 houses)	House index was 2.85	Container index was 10	Breteau index was 5.71
Team 10 (22 houses)	House index was 4.55	Container index was 13.33	Breteau index was 18.18
Team 11 (17 houses)	House index was 23.53	Container index was 20	Breteau index was 23.53

Table 1 showing types of various indices

Team	House index	Container index	Breteau index
1	26.92	25.71	34.61
2	48	51.4	76
3	17.24	18.18	27.58
4	45	37.5	60
5	24	17.3	32
6	16.66	100	16.6
7	20	59.25	64
8	8.57	20.68	17.14

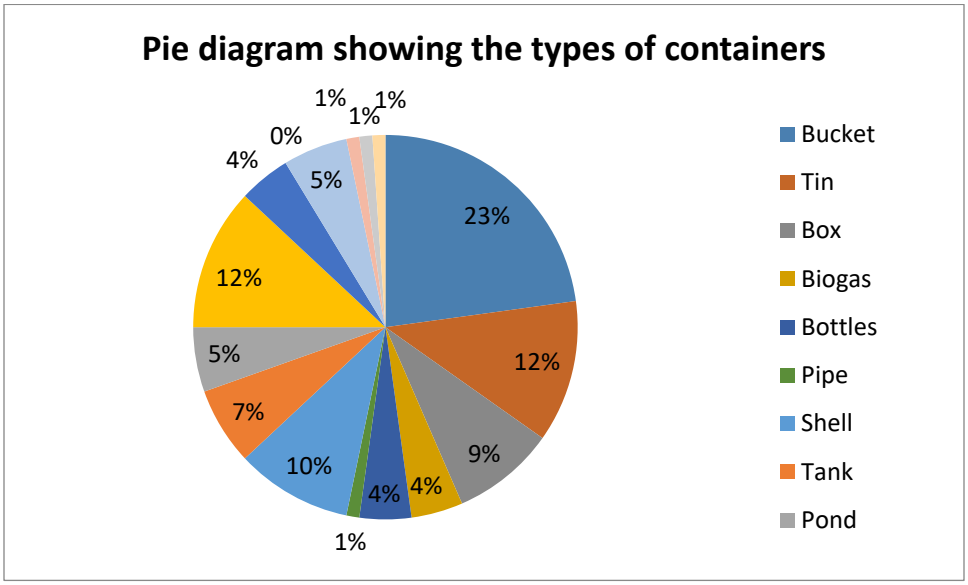
9	2.85	10	5.71
10	4.55	13.33	18.18
11	23.53	20	23.53

Table 2 showing population covered, fever cases, positive containers etc

Total teams	Total houses inspected	Population covered	Number of fever cases	Number of houses + for Aedes breeding	Number of containers checked	Positive containers
11	289	836	20	58	325	92

Table 3 showing types of Containers where positive cases were reported

Sl. No	Type of containers	No of positive cases
1	Bucket	21
2	Tin	11
3	Plastic box/cup	8
4	Biogas	4
5	Bottles	4
6	Pipe	1
7	Coconut shell	9
8	Tank	6
9	Pond/pool of water	5
10	Flower pot	11
11	Steel vessels	4
12	Fridge	0
13	Tyre	5
14	Well	1
15	Sheet	1
16	Grinder	1
	Total	92



DISCUSSION

The proportion of dengue fever was less in our study (6.92%). 14.8% and 20% were the prevalence obtained in the previous studies of Kumar et al.,2012 and Serological study 2018. However, in the study by Gopakumar et al., 2018 a high value of 50% was reported. Breteau index could be used as a predictor of dengue transmission which was high (For teams 2, 4 and 7) in the present study. Four areas in our study showed slightly higher container index than the 32.2% in Tiruchirapalli district of Tamil Nadu (2012 - 13). Storage of water containers such as buckets for use at home was 21. There was no collection of water under refrigerator. Tin containers were 11. Plastic cups were 8 and bottles and pipes were 4 each. Coconut shells were also breeding sites (9). Flower pots were more prone to larval breeding (11). Both House Index and Breteau Index which are considered better predictors than container index are well above acceptable limits in many areas and hence the area studied is at high risk for epidemics.

The conclusion from the present study that although Breteau index was higher and fever cases were low suggests climatic factors are also responsible. With the onset of monsoon all the sources can be reduced and thereby prevent dengue in that area. More awareness needs to be generated among the public on the importance of identifying and destroying the vector breeding sites or sources around households.

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ASSESSMENT OF PRIMARY PRODUCTIVITY OF ACHANKOVIL RIVER BASIN WITH SPECIFIC REFERENCE TO SOUTH WEST MONSOON

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ABSTRACT

Productive nature enhances the floral faunal growth and overall characteristics of a water body. Productivity widely varies in rivers and assessment of individual rivers is necessary to evaluate the productive nature of the same. The present work analyses the productivity and relative physicochemical parameters of Achankovil river basin at 8 selected locations in its full length during southwest monsoon 2018. Achankovil river is an important river that originates from Southern western ghats and travels through Kollam, Pathanamthitta, and Alappuzha districts to join Pampa river. Gross primary productivity ranged between 1.87mgC/l/hr to 2.9mgC/l/hr and Net Primary Productivity ranges from .65mgC/l/hr to 1.52mgC/l/hr. Water temperature, pH, Dissolved Oxygen, BOD, nitrate nitrogen, phosphate were also estimated as per standard methods. The study showed that values of gross and net primary productivity of Achankovil river basin at its various locations is comparable to the values obtained from other rivers of the region and are influenced by the prevalent physicochemical characteristics of the river water.

INTRODUCTION

The basic life dependency, the universal solvent "water", is now in an exhausting stage due to rapid anthropogenic activity. Pollution of water greatly affects the basic characteristics of water, hence affecting the productivity of the water body. The basis of ecosystem functioning is the biological production of autotrophs which is manipulated by primary productivity of water body (Odum et al, 1971). Major factors controlling productivity are nutrients light, season, and climate (Deka, 2017). Estimation of primary productivity reflects the health of an ecosystem which is essential to understand the food chain and food web (Sontakke and Mokashe, 2014). The present study aims to evaluate productivity and certain physico chemical parametrs of Achankovil River basin. Thousands of people depend for their fresh water requirement on Achankovil river which flows 128km through the districts of Kollam, Pathanamthitta, Alapuzha. Many sacred temples are located on its bank and hence the river is considered to be a sacred river. Eight stations along the full length of 128 km are selected for the present study.

MATERIALS AND METHODS

The stations selected are Achankovil, Konni, Omalloor, Konathumoola, Kaipuzha, Attuva, Pallipad and Veeyapuram and monthly collections are made from June 2018 to September 2018. Impact of heavy rainfall that caused the

devastating flood in August 2018 on the productivity and physico chemical parameters are also examined in the study. Water temperature, Ph were noted in situ. DO, BOD, Nitrate, phosphate, GPP, NPP, were analysed using standard procedures (APHA, 2012). Clean sterilized bottles are taken for the collection and all the parameters were analyzed within short period.

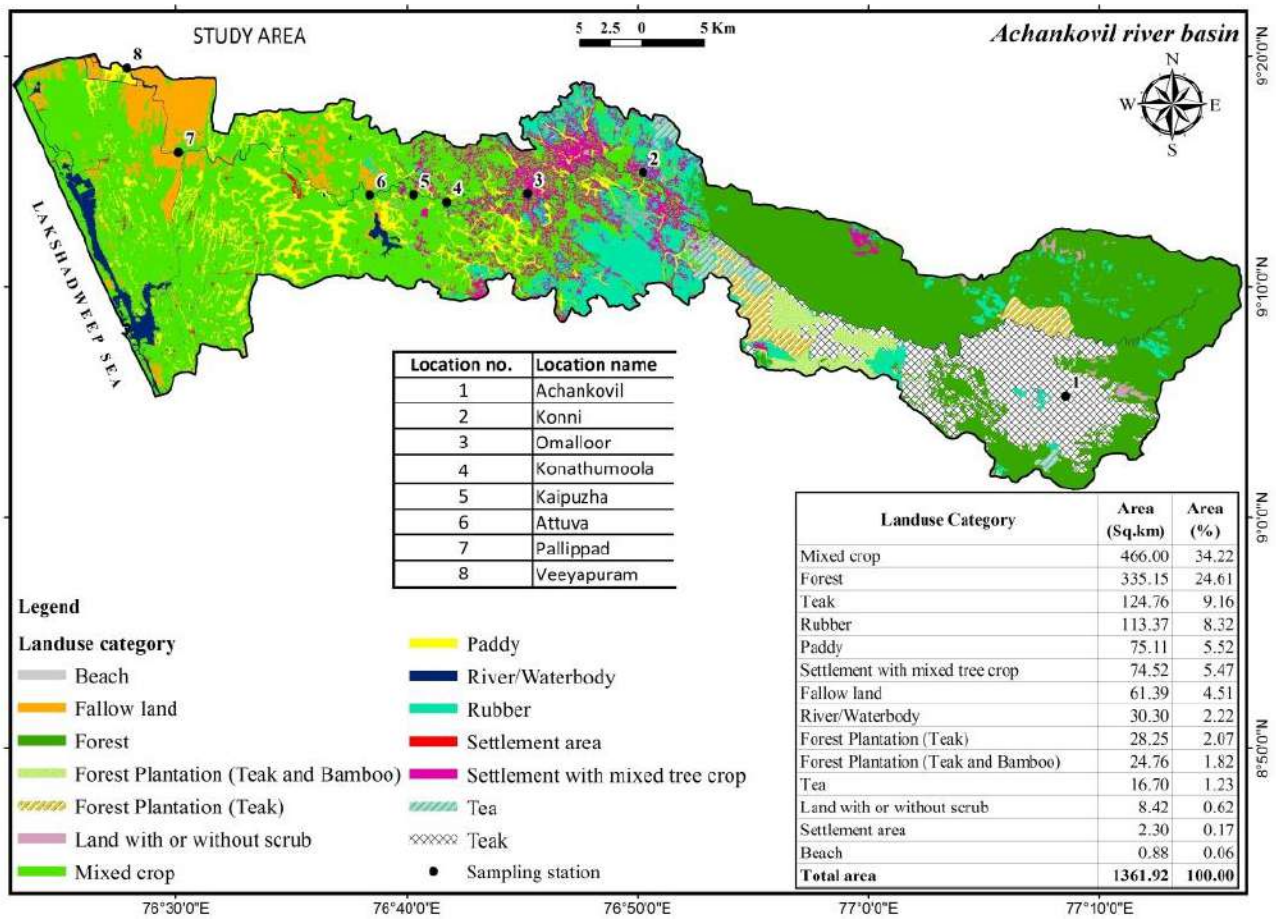
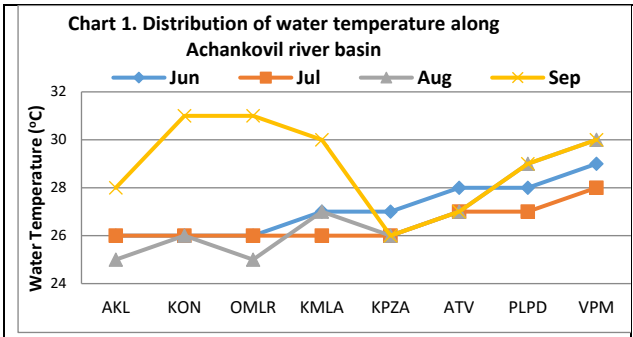
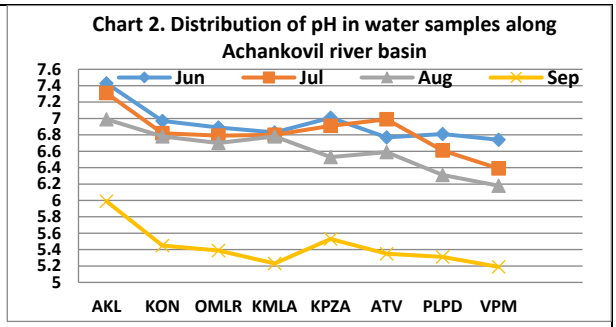
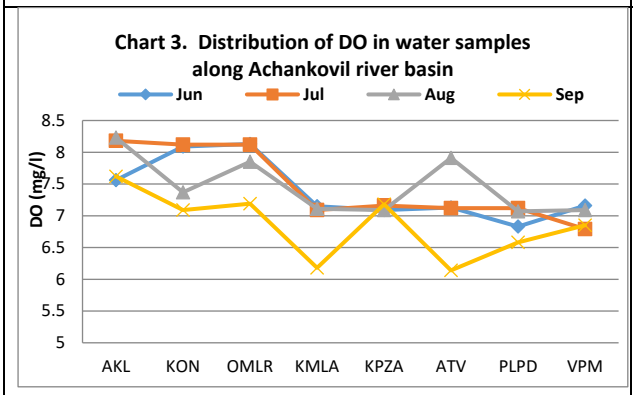


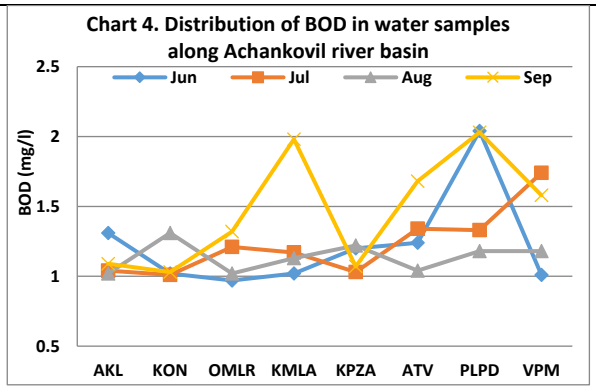
Fig. 1. Location Map of the study area

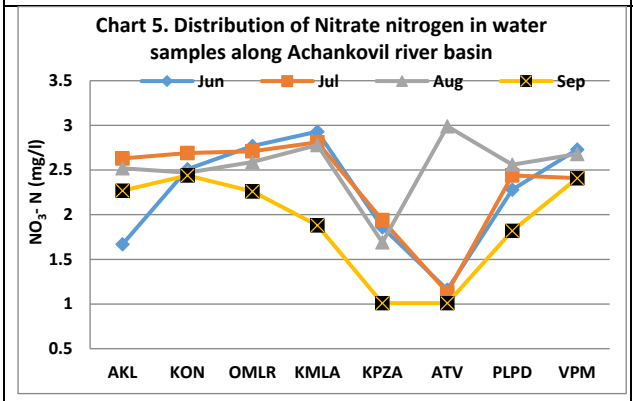
RESULTS AND DISCUSSION

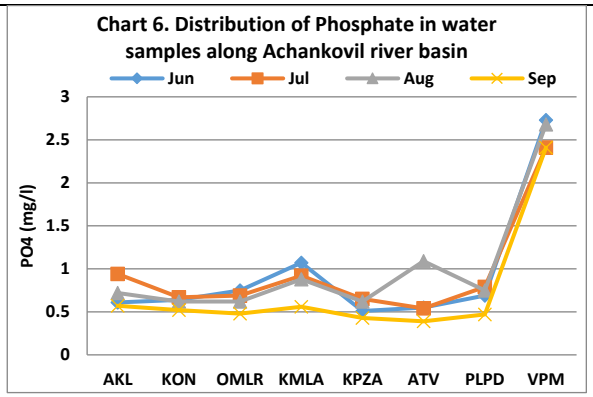


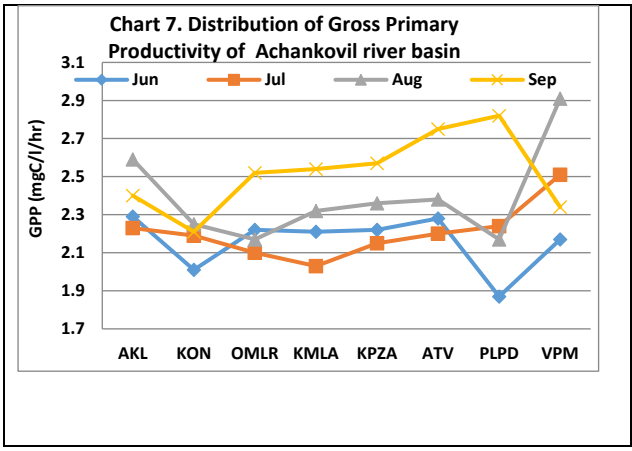


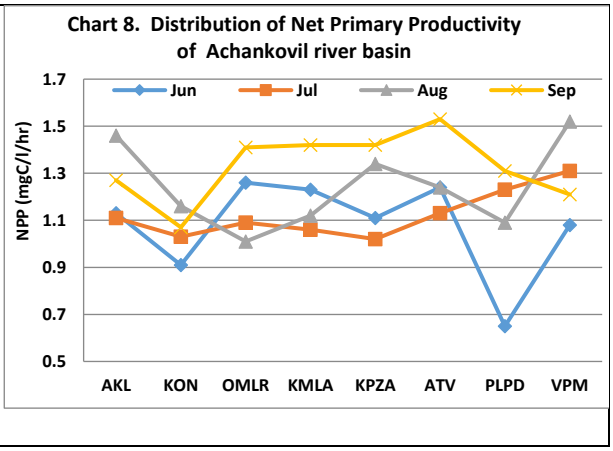












The maximum water temperature was recorded from Konni and Omallor (31±0.5ºC) in September while the minimum temperature was from Achankovil and Omalloor (25±1.0ºC) in August. Since monsoon climate prevailed water temperature was generally low at all stations. Temperature affects chemical reactions and reaction rates within the water, thereby influence the oxygen dissolution and favours the biodiversity and suitability of water use (Metcalf and Eddy, 2003). Lowest pH was noted at Veeyapuram (5.19±0.15) in September where highest value was recorded from Achankovil (7.43±0.1) in June. Acidic pH noted in September may be due to effect of intensive leaching of clay during flood. In Pampa and Manimala rivers also similar reduction in water pH was reported immediately after the flood (Joshy, 2018). The monthly pH variation in the range of 7.12 to 8.12 was reported from Kallayi river by Akshad et al (2017). Attuva station showed the lowest DO concentration (6.14±0.06mg/l) in September while Achankovil station showed the highest value (8.23±0.13mg/l) during August. In general, moderate to high DO was recorded in all station possibly due to heavy rain in the monsoon 2018. DO seemed to be high on upstream portion which indicated no pollution especially in first station. Dissolved oxygen is an important limnological parameter indicating level of water quality and organic pollution in the water body (Kataria *et al*; 1996). The water showing more dissolved oxygen during the rainy season was also reported by Akshad et al (2017) from Kallayi river in Kerala. BOD was comparatively low (0.97±0.01mg/l) at Omalloor and high at Pallipad (2.04 ±0.11), both in the month of June. BOD was found to be increased towards lower stream which showed the impact of disposal of wastes including food waste, plastic, toilet waste etc directly into water body from the midstream onwards. Nitrate nitrogen concentration was found to be in an increasing manner from station 6 which may be attributed to the high anthropogenic activities prevalent in the downstream region especially from Kaippuzha onwards. Water sample at Attuva showed the highest nitrate nitrogen concentration (2.99±0.22mg/l) in August which dropped to 1.01±0.05mg/l in September, after the flood in the same station. The lowest phosphate concentration was recorded from Attuva in September (.39± 0.03mg/l) where the highest value was noted from Veeyapuram (2.73± .070) during June. High concentration in the confluence region might be because of the accumulation and suspension of sewage and solid waste drawn to the station. Washing and bathing was also prevalent in the location which might also have contributed to phosphates in water.

GPP ranged between 1.87±0.08mgC/l/hr at Pallipad in June and 2.91± 0.13mgC/l/hr at Veeyapuram in August. The lowest NPP was recorded from Pallipad in June (0.65± 0.02mgC/l/hr) and the highest at Attuva in September (1.53 ± 0.06mgC/l/hr). In an earlier study, GPP ranging from 0.13mgC/l/hr to .478mgC/l/hr and NPP varying from 0.025mgC/l/hr to 0.158mgC/l/hr was reported from Achankovil river in monsoon season (Rajan and Samuel, 2016). They observed that during monsoon period, gross productivity and net productivity become comparatively low and started increasing at the initiation of the post monsoon season. The GPP values in the range of 0.90 – 3.95 mg C/l/day and NPP values in the range of 0.13 to 0.84 mg C/l/day was reported in a study at Chaliyar river by Dhanalakshmi and Priyatharsini (2017). The values recorded in the present study in Achankovil are much higher than the values reported from Chaliyar. However, the primary productivity of the river Kharashrota (Orissa) analysed by Supriya et al (2011) both spatially and seasonally showed an annual average GPP ranging 0.075±0.009gC/m^3/h to 0.938±0.103gC/m^3/h and NPP varying from 0.012±0.001gC/m^3/h to 0.832±0.081 gC/m^3/h.

CONCLUSION

The present study indicates an overall healthy ecosystem in the river basin of Achankovil as of now. As per the data obtained in the present study, the water quality of the river at its upstream are uncontaminated but with deteriorated downstream. Downstream stations exhibited pollution possibly attributed to domestic and municipal waste disposal. Overall trend in values of GPP reflected a healthy ecosystem in the upstream stations. The factor that props up the growth of primary producers and the improved primary production seems to be the water temperature followed by DO and nutrients, though nutrients (N and P) and DO recorded comparatively lower values during the month of September but temperature was high . Flood occurred during August 2018 seems to have negligible impact in overall water quality and primary production except a decrease in pH immediately after the flood.

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QUALITY ASSESSMENT OF *SARDINELLA LONGICEPS* COLLECTED FROM THE LOCAL FISH MARKET

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ABSTRACT

Fish is an important food stuff and source of protein all over the world. In india especially kerala, fisheries sector contributes a lot in case of earning foreign currency and meeting domestic need of animal protein. To meet the domestic need, fish and fish products are procured from the neighboring states. But it is evident from several studies that raw fish items sold in kerala are heavily contaminated with pathogenic bacterial and toxic chemicals like formalin, ammonia etc which are highly hazardous and carcinogenic. Contamination may be caused by food-borne pathogens which are naturally present in aquatic environments, such as *Vibrio spp*. *E.coli*etc, or derived from sewage contaminated water such as *Salmonella spp*. Consumption of these contaminated fish may cause infection or intoxication to the consumers. Water and ice quality is also an important factor for good quality fish, because water and ice used for fish processing may contaminate the quality.

Therefore, the present study was carried out to investigate the quality of the edible fish, collected from the local market, for raising food safety concern and awareness in the public.

Key words: Fish quality, Microbial analysis, *Vibrio spp*. Carcinogenic, *Salmonella spp*

INTRODUCTION:

Fish contributes about 60% of the world supply of protein, and 60% of the developing world derives more than 30% of their animal protein from fish (Emikpe *et al.*, 2011, Sohana Al Sanjee and Md. EkramulKarim 2016). Fish allows for protein improved nutrition in that it has a high biological value in term of high protein retention in the body, low cholesterollevel and presence of essential amino acids (Emikpe*et al.*, 2011). Fishes are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues (WHO, 2007). Microbial contamination on environmental surfaces may be transferred to the food products directly through surface contact or by vectors such as personnel, pests, air movements or cleaning regimes Pal, 2010, Abdullah Diler& İsmail YükselGenç 2018. Microorganisms exist on the skin/slime, gills and the gut of live and newly caught fish.

In India , more people have to be fed each year and the most reliable source of protein for many is fish. Although there is an argument regarding the concept of "fish for all" in India initiated by the World Fish Centre, since

30 to 50% of the Indian population is predominantly vegetarian, there is no dispute that fish is very popular diet among the people of the states of West Bengal, Goa and Kerala (Sakthivel, 2003). Bacteria present on the fish are normally associated with those found in their natural environment and influenced by the season and the harvesting conditions. . To assess the quality and microbiological assessment of oil sardine collected from the local market of cherthala. To assess the quality of ice used in fishes.

MATERIALS AND METHODS:

The study was carried out in the local markets of cherthala. During the study period, the presence of pathogenic organisms namely, *Salmonella spp*., *Staphylococcus aureus, E.coli* and *Vibrio cholerae*, of public health significance from the Indian Oil Sardine –*Sardinella longiceps* and total coliforms and*E.coli* in ice (storage temperature −20∘C) which were used during the processing of samples were investigated. All the frozen fishes were gutted and organoleptically good enough to carry out further bacteriological analysis.

Sample Collection: For microbiological analysis, raw fish samples were collected from the cherthala market during 10:00 to 11:00 AM in the morning and transferred to the laboratory in a using a sterile aseptic polythene bags together with ice .The ice was collected in 1-liter sterilized container from different location. The collected samples were preserved in the refrigerator (4∘C), when analysis was delayed for more than 3 hr. Isolation and Identification of pathogens of public health interest like. *Staphylococcus aureus ,E.coli , Salmonella spp., Vibrio cholera* in raw fish as well as Total Coliforms and *Vibrio cholarae* in Ice were enumerated using standard microbiological manuel. (Kannan *et al* 2002)

RESULTS

The source and QIM score of *Sardinella longiceps is* recorded in the present experiment . The sensory quality parameters like general appearance, colour of the eyes and smell of the fish scored the lowest QIM scores (0) which emphasize the freshness of the fish (Table-1). But the Total Coliform bacterial count was higher in ice used to preserve *Sardinella longiceps*(Table-2).

Table- 1: Quality Index Method (QIM) as applied to assess quality of*Sardinella longiceps*

Quality parameter	Character	Observation	Score
General appearance	Skin	bright	0
	Blood spot	None on gill cover	0
	Stiffness	Stiff, in rigor morti	0
	Smell	Fresh	0
Eyes	Clarity	Clear	0
Gills	Colour	Red	0
	Smell	Fresh	0

Tables- 2. List of Bacterial species isolated from ice associated with – *Sardinella longiceps.*

SL.NO	PARAMETER TESTED FOR	TEST RESULT	MAXIMUM LIMIT	TEST METHOD
1	Total coliforms	7.8MPN/GM	<1.8MPN/GM	USFDA(8AM) Chapter 4 ,2010
2	*Vibrio cholerae*	Absent	Absent	USFDA(8AM) CHAPTER 9 ,2004

Tables 3. List of Bacterial species isolated from raw fish – *Sardinella longiceps*

SL.NO	PARAMETER TESTED FOR	TEST RESULT	MAXIMUM LIMIT	TEST METHOD
1	*Staphylococcus aureus*	<10 CFU/GM	100 CFU/GM	USFDA(8AM)8Edn Chapter 12,2001
2	*E.coli*	<10 CFU/GM	20 CFU/GM	USFDA(8AM)8Edn Chapter 4 ,2013
3	*Salmonella.spp*	Absent	Absent	USFDA(8AM)Edn Chapter 5,2014
4	*Vibrio cholerae*	Absent	Absent	USFDA(8AM)Edn Chapter 9 ,2004

Of the four species analysis *Staphylococcus aureus and E.coli* were the prevalent species present in the raw fish , but in both case it was present within the minimum limit ie., <10 CFU/GM . Both *Vibrio cholerae*, *Salmonalla* spp were completely absent in the*Sardinella longiceps*collected from the local market(Table-3)..

DISCUSSIONS

In samples collected from the local market of Cherthala, the predominant pathogenic bacteria include *Staphylococcus aureus and E.coli* which indicated poor quality .The poor quality may be due to poor handling, improper storage system and sanitary condition at all the steps in the fish processing and selling.

Staphylococcus aureus are not part of the normal fish micro biota (Huss, 1988; Van den Broek *et al*, 1984). They can cause food poisoning and may occasionally cause infections in patients whose immune system is compromised (Karl, 1975; Wesley, 1975,Sohana Al Sanjee and Md. Ekramul Karim2016).

Hence, the presence of *Staphylococcus aureus* in seafood samples indicates the post-harvest contamination due to poor personnel hygiene or due to the disease in fish (Austin and Austin, 2007; Huss, 1988, Abdullah Diler& İsmail YükselGenç 2018).

The present study revealed that the microbial quality of seafood samples from cherthala fish market was not good due to the presence of *Vibrio cholerae* in the samples. Our study revealed that the raw sea foods sold at cherthala fish market could be a source of food borne bacterial pathogens. . The levels of Total Coliforms Count in ice indicate the urgent need require improving the Quality control and Quality assurance systems of Cherthala Fish Market.

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DISTRIBUTION OF ANTIBIOTIC RESISTANCE AMONG BACTERIAL ISOLATES FROM DISEASED *GYMNOCORYM BUSTERNETZI*

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ABSTRACT

The ever-increasing demand for aquarium fishes gradually paved the way towards global trade of ornamental fishes. Diseases in aquaculture systems are recognized as an important limiting factor to production and trade. In the present study six antibiotics were selected and all isolates were sensitive to chloramphenicol and gentamycin. One isolate showed multiple drug resistances, with resistance ampicillin, nalidixic acid and intermediate sensitivity to ciprofloxacin. Among the antimicrobial agents commonly used in aquaculture, several are classified by the WHO as critically important for use in humans.

INTRODUCTION

The majority of bacterial fish pathogens are natural inhabitants of the aquatic environment (Rainer and, Daniel, 2017). The widespread distribution of these bacteria in the aquatic environment and the stress induced by intensive culture practices predisposes fish to infections.The disease problems are treated with antibiotics, the indiscriminate use of which can result in the rapid spread of multi-drug resistant pathogens across the system (Akinbowale *et al* ,2006). Misuse of any antibiotic can lead to the creation of resistant bacteria in a facility. So there is a need to identify the bacteria by running culture and sensitivity tests, and thereby avoid

MATERIALS AND METHODS

Live diseased black tetra , *Gymnocorym busternetzi* were collected and pure cultures of bacteria were isolated.The medium prepared for bacterial culture is nutrient broth. Muller Hinton agar plates, nutrient agar plate and slants were used. Bacteria were isolated from different parts of fish like, body surface, fin, gill and intestine. Using sterile scissors, small piece of dorsal fin, skin, gill and intestine was cut and extracted separately with 0.9 % sterile NaCl solution (saline). The extracts were serially diluted using sterile saline. The dilutions used were 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Using a sterile pipette, 0.1 ml of each sample was placed on nutrient agar plates and plated by spread plate method. The plates were incubated at room temperature for 18-24 hours. Morphologically different colonies were then selected and purified by repeated streaking on nutrient agar plates. The purified bacterial culture were then inoculated nutrient agar slants and stored at 4^0C.The isolates were named as GT1, GT2, GT3, GT4, GT5, GT6, GT7, GT8 and GT9.The 6 antibiotics and **disc** content (mcg) used in the study are Ampicillin (10 mcg) Chloramphenicol (30 mcg) Ciprofloxacin (5 mcg) Gentamycin (10 mcg) Nalidixic acid (30 mcg) Vancomycin (30 mcg).

RESULTS AND DISCUSSION

Bacterial colonies formed on nutrient agar plates. Morphologically different colonies were streak plated on nutrient agar plates for isolation of pure colonies. Pure colonies are then transferred to nutrient agar slants and maintained as stock culture.Figure 6 and 7 shows the result of Antibiotic susceptibility test of isolate GT1 and GT3 respectively. Zone of diameter in mm of each isolate was tabulated in Table 2. Among the nine isolates tested GT1 showed resistance against vancomycin. GT3 isolate was resistant to ampicillin and nalidixic acid. GT3 isolate exhibited intermediate sensitivity to ciprofloxacin. The GT9 isolate showed intermediate sensitivity to nalidixic acid. GT2, GT4, GT5, GT6, GT7 and GT8 isolates exhibited sensitivity to all the antibiotics tested.GT3 isolate was found to be multidrug resistant with resistance against ampicillin and nalidixic acid. This isolate showed only intermediate sensitivity to ciprofloxacin.

Use of antimicrobial agents in aquaculture has resulted in the emergence of reservoirs of antimicrobial-resistant bacteria in fish and other aquatic animals, as well as in the aquatic environment (Aoki 1998). In the present study all isolates were sensitive to chloramphenicol and gentamycin. One isolate showed multiple drug resistances, with resistance against ampicillin, nalidixic acid and intermediate sensitivity to ciprofloxacin.

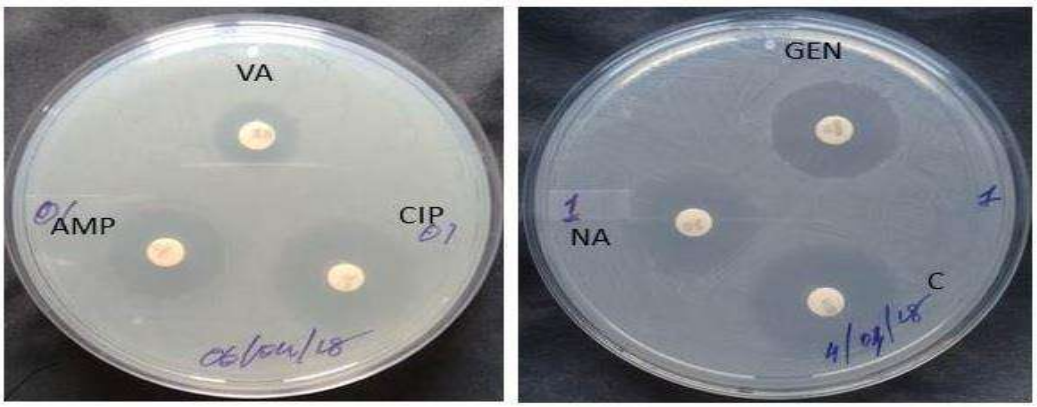

Figure 1. Antibiotic sensitivity test of GT1 isolate showing resistance against vancomycin. VA-Vancomycin, AMP-Ampicillin, CIP-Ciprofloxacin, GEN- Gentamycin, C- Chloramphenicol.

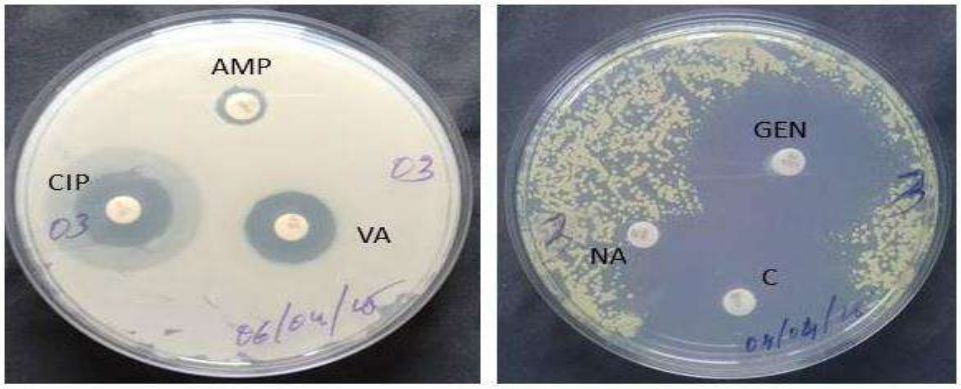

Figure 2. Antibiotic sensitivity test of GT3 isolate showing resistance against ampicillin and nalidixic acid. VA- Vancomycin, AMP- Ampicillin, CIP-Ciprofloxacin, GEN- Gentamycin, C- Chloramphenicol.

Sl. No.	Antibiotics	Disc content (mcg)	Bacterial isolates – Zone diameter (mm)								
			GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8	GT9
1	Ampicillin	10 mcg	21 (S)	45 (S)	10 (R)	24 (S)	26 (S)	48 (S)	26 (S)	30 (S)	30 (S)
2	Chloramphenicol	30 mcg	26 (S)	34 (S)	35 (S)	32 (S)	27 (S)	35 (S)	29 (S)	37 (S)	18 (S)
3	Ciprofloxacin	5 mcg	25 (S)	42 (S)	18 (I)	40 (S)	28 (S)	47 (S)	27 (S)	40 (S)	26 (S)
4	Gentamycin	10 mcg	22 (S)	31 (S)	31 (S)	28 (S)	24 (S)	25 (S)	23 (S)	35 (S)	22 (S)
5	Nalidixic acid	30 mcg	20 (S)	24 (S)	11 (R)	36 (S)	20 (S)	39 (S)	20 (S)	27 (S)	17 (I)
6	Vancomycin	30 mcg	14 (R)	30 (S)	17 (S)	22 (S)	21 (S)	33 (S)	20 (S)	29 (S)	18 (S)

Table 2 .Zone diameter and antibiotic susceptibility of bacterial isolates.

CONCLUSION

From the aquatic reservoir some drug-resistant pathogenic bacteria can be transferred to humans directly and more importantly, resistance genes may be disseminated by horizontal gene transfer and reach human pathogens (Acar and Moulin ,2012). Occurrence of resistance to antimicrobial agents in human pathogens severely limits the therapeutic options in human infections (David *et al*, 2009) .

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MOLECULAR PHYLOGENY OF SELECTED EDIBLE FRESH WATER FISHES OF VELLAYANI LAKE

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Abstract

Present study was carried out on Molecular phylogeny of selected edible fresh water fishes from Vellayani Lake. 16s Genes of mitochondrial DNA were analyzed. 3 sequences of samples were compared with the sequence retrieved from NCBI. Phylogenetic tree constructed using ML method on Kimura 2 parameter model using MEGA 7.

Introduction

Mitochondrial DNA has widely used in phylogenetic studies because it evolves much more rapidly than nuclear DNA, resulting in the accumulation of differences between closely related species. (Timm *et al.*,2008). In the past two decades, highly conserved mitochondrial 16SrRNA gene has been used to explore the phylogenetic relationships of fishes. (Richily *et al.*, 2004).

METHODOLOGY

SAMPLE COLLECTION

Three samples were collected from landing site at Vellayani Lake, Trivandrum, Kerala

DNA isolation

Whole genomic DNA was isolated using "Phenol chloroform method" of Sambrook et al(1989). 16SrRNA were used for the amplification of genomic DNA. The amplification reaction were conducted.

The PCR product was analyses on a 1.8% agarose gel. The amplified product was commercially sequenced at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

RESULTS AND DISCUSION

The sequences were analysed. Similar sequences from NCBI were compared with the samples. (Acession number:DQ42666.1, GU566027, JQ699194.1), all sequences were aligned using Clustal W.

Phylogenetic Analysis:

In this study, we examined phylogenetic of Fishes from Vellayani Lake, Trivandrum using 16S rRNA mitochondrial gene to elucidate species relationships. A frog species clusters far away from the fishes were used as the out group.. The tree also indicates that *O.mossambicus* and *E.suratensis* are closely related species when compared to other species and *C.dussumieri* are highly distant to each other

Finally, it is concluded that the three taxonomically identified fish species used in the study belongs to *O.mossambicus*, *E.suratensis* and *C.dussumieri* respectively, partial sequence information of 16S rRNA gene can be used as a diagnostic molecular marker in identification and resolution of taxonomic ambiguity of fishes.

CONCLUSION

The present study aims to provide a phylogeny and of selected fishes found in Vellayani lake. Since this study is a preliminary and pioneering molecular characterization of sfishes in Vellayani, it is suggested that more comprehensive studies of fishes in Vellayani, especially on characterization and population genetic structure be carried out.

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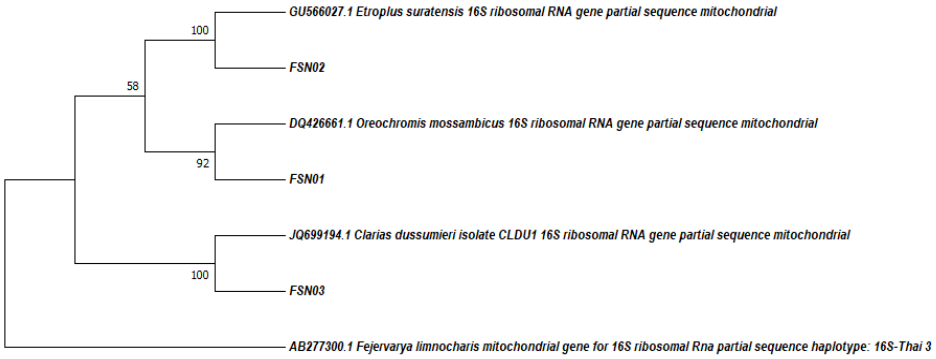
Sl. NO	Scientific name	ID	Date	Place	Habitat
1	*Oreochromis mossambica*	FSN01	4/4/2019	Vellayani	Fresh water
2	*Etroplus suratensis*	FSN02	4/4/2019	Vellayani	Fresh water
3	*Clarias dussumieri*	FSN03	4/4/2019	Vellayani	Freshwater



ICTHYODIVERSITY OF KADAMAKUDY ISLANDS, KERALA, INDIA

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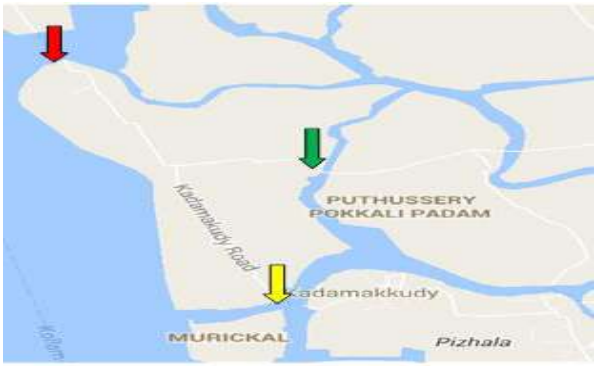
Abstract

Survey and sampling carried out in three different sites of Periyar River revealing the occurrence of 30 species. The survey was undertaken during the period of February to December 2017 in the Kadamkudy Island of Ernakulum to find out the biodiversity status of fishes of selected sites of Periyar River system. This is a pioneer study on the fish diversity of Periyar, Kadamakudy Island that is a part of the Vembanad estuary.

Introduction

Faunal diversity of Periyar river is threatended by human activities and exotic species. Thus, only by the study of diversity and distribution of fishes, one could design scheming and implementing conservational strategies (Dahanukar, *et.al.*, 2004). The Present paper aimed studying of the Icthyodiversity of Kadamakudy Island.

Materials and methods



Site of Investigation

Kadamakudy islands are a cluster of fourteen islands belonging to the Varapuzha panchayath.

Figure 1.Sampling sites

Sample collection

Sampling was carried out in three selected sites (Fig 1.) in Periyar River in three seasons (pre monsoon, monsoon, post monsoon) throughout 2017. Samples were collected from local fishermen and using various gears and samples are preserved in 10% Formaldehyde solution. Fishes were identified up to species level using Keys of Jayaram, (1999), and Munro, (2000) and FAO sheets.

Analysis

Shannon index (Shannon, C. E.*et.al.*,1949), and Simpson's dominance index (D) (Simpson, 1949) were used.

	A	B	C	Average
Taxa_S	20	25	18	21
Individuals	92	99	89	93.33333
Dominance-D	0.1073	0.08581	0.08623	0.093113
Simpson-1-D	0.8927	0.9142	0.9138	0.9069
Shannon-H	2.576	2.776	2.618	2.656667

Result and discussion

In the present work, 30 species belonging to 27 families and 12 orders were recorded.

Fish species diversity, abundance and distribution

Among the three sampling locations, high species diversity was observed in site 2 and low diversity was observed in site 1.

Table 1 Statistical analysis
Figure 1. Threat status

In the present study, 30 species were collected. During monsoon, salinity is very less. Intensity of rainfall is a factor contributes more freshwater species. Many marine species are migrated through marine water inflow. Temperature, PH, climate changes and activities like land

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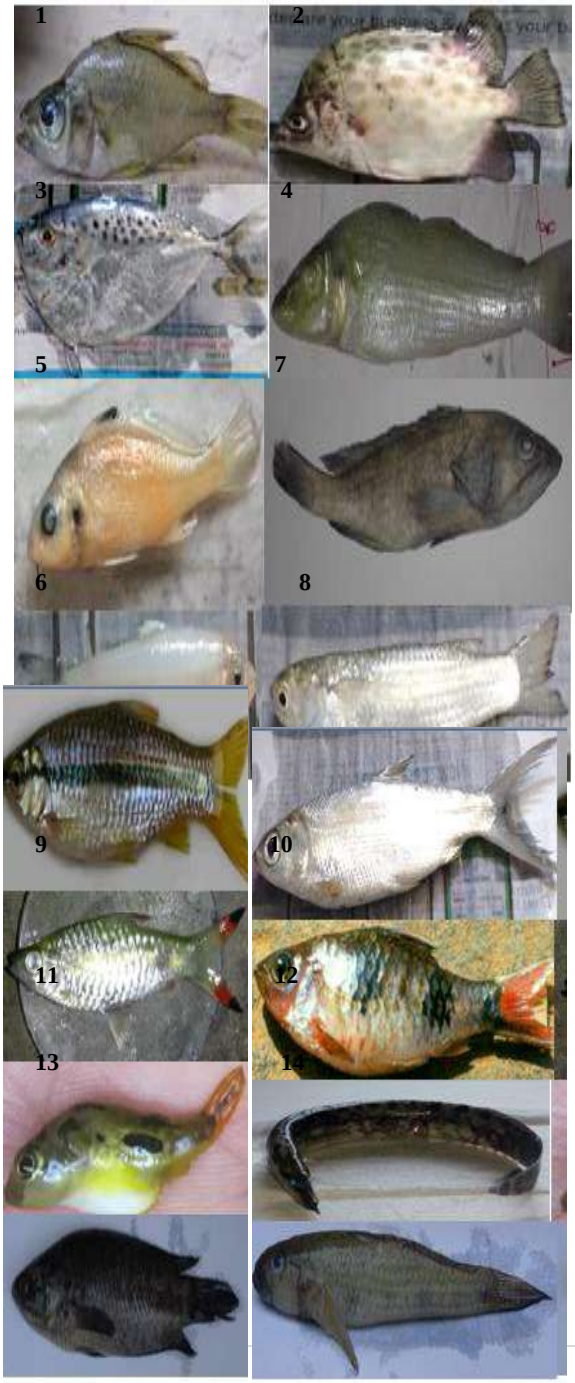
filling, pollution also affects the abundance of fishes. The difference in salinity and other factors affect diversity. The comprehensive listings, distribution as well as the continuous monitoring are the critical requirements in the protection of fish fauna.

Conclusion

The present study illustrates the biodiversity status of Kadamakudy Island. The presence of endemic and threatened fish species necessitates, proper conservation and management strategies, which have to be developed and implemented in the Periyar River.

Plate



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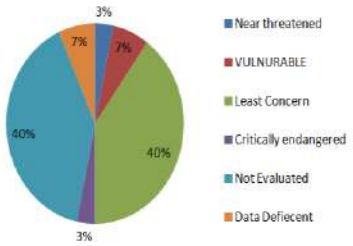

No.	**Scientific name**
1.	Parambasis thomsonni
2.	Scatophagus argus
3.	Mene maculate
4.	Sillago indica
5.	Argyrosomus amoyensis
6.	Stolephorus commersonni
7.	Lates calcarifer
8.	Mugil cephalus
9.	Rasbora daniconians
10.	Chanos chano
11.	Puntius filamentosa
12.	Puntius aurilus
13.	Carinoteradon travencoricus
14.	Mastacembalus armatus

15.	Anabas tesidineus
16.	Glossogobius giuris
17.	Etroplus suratensis
18.	Platycephlus indicus
19.	Gerres subfasciatus
20.	Apolocheilus panchax
21.	Carangoides malabaricus
22.	Psttodes erumei
23.	Triacanthus biaculeatus
24.	Arius maculates
25.	Hyporhamphus limbatus
26.	Xenatodon cancila
27.	Collectichthys dussumerri
28.	Cynoglossus macrostomi
29.	Etroplus maculates
30.	Oreochomis mossambicus

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PESTS AND PREDATORS OF *P. FUCATA* IN VIZHINJAM WATERS ALONG THE SOUTHWEST COAST OF INDIA

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Abstract

A field experiment was conducted in Vizhinjam Bay of Southwest coast of India on the occurrence of pest and predators of Akoya pearl oyster, *P. fucata*. Adults of *P. fucata* with an average size of 60 mm (DVM), 56 mm (HL), 28 mm (THK) and 27g (WGT) were selected for the present study. Monthly three plastic baskets were introduced with 15 oysters and fouling data was recorded. Baskets were hung from the raft system at an average depth of 5 - 6 meters which was moored in the Vizhinjam bay. The oysters were selected from the hatchery bred batch and prior to study they were gently cleaned off the fouling species. Study was continued for a period of two years (January 2016 - January 2018). Results showed that although biofoulers are present all throughout the year around in Vizhinjam waters, seasonal variation was found among species and encrusting sponges *viz*; *Callyspongia* sp., *Clathria* sp, *Aaptos* sp, *Paratimea constellata*, *Dysidea etheria*, green and white encrusting sponges, barnacles of the species *B. amphitrite* and predator crabs including *Thalamita* sp., *Charybdis* sp, box crabs are the major pests identified which leads to oyster mortality.

Introduction

In India, pearl production has been successful for nearly three decades and *P. fucata*, the Akoya pearl oyster is the major pearl oyster resource. Biofouling on pearl oysters especially over the periostracum of the oyster and cages has been considered as a serious problem. It leads to mortality of the farmed stock or reduces the growth rate of oysters affecting the quality of pearls (Nishii 1961; Miyauti 1968; Shirai 1970; Wada 1973; Mohammad 1976; Alagarswami 1991; Doroudi 1996; Taylor *et al.* 1997; Pit and Southgate 2003). Too much fouling is detrimental to the farmed stock and removal or control of fouling is crucial for the effectual management of pearl farms. Pearls produced by heavily infested oysters are also of poor quality. Extensive research has been carried on biofouling of pearl oysters but detailed studies on the pearl oysters of Vizhinjam coast is lacking. Present study mainly focuses on the major pest and predators of *P. fucata* in Vizhinjam waters along the South west coat of India.

Materials and methods

Samples for the present study was obtained from the hatchery bred stock in the Vizhinjam bay. Adult oysters of *P. fucata* with an average size of 60 mm (DVM), 56 mm (HL), 28 mm (THK) and 27 g (WGT) were selected for the current study. Prior to study, the oyster shells were cleaned off the biofoulers and monthly three plastic baskets were introduced with 15 oysters inside. The plastic baskets used had a diameter of 24 cm, height of 13.5 cm and a total volume of 6104.16

cm^3 and the total available opening of a plastic basket was 700 cm^2. It has rows of rectangular openings of size 1 cm X 1 cm on the body and 1cm X 0.5 cm openings on the lid. Each month, plastic baskets were taken, pests and predators were recorded with an introduction of new three baskets. The study was continued for a period of two years.

Results

In Vizhinjam waters, foulers and borers were present all around the year and during the present study; community development of biofoulers was common and is present over the growth process, periostracum and hinge region of the oyster shells. Monthly variation was observed on the attached and non attached fauna during the two year period and barnacles are the major foulers noticed. Barnacle of the species *B. amphitrite* was found settled on oysters and the intensity varied throughout the year between 5 - 10 numbers. But a maximum of 20 - 60% was observed during September - October - November - December period. Rather than barnacles, the encrustation of various sponges was a severe problem along with the juvenile predator crabs.

Biofoulers *viz;* encrusting sponges including *Callyspongia* sp., *Clathria* sp, *Aaptos* sp, *Paratimea constellata*, *Dysidea etheria*, green and white encrusting sponges and predator crabs including *Thalamita* sp., *Charybdis* sp, box crabs were noticed. Occurrence of the boring polychaete, *Polydora* sp. over the oyster shells was noticed throughout the course of study. No attack of predator fish was noticed. Other pests observed were nudibranchs, brittle star, ascidian sp. *Phallusia nigra*, *Ophiothrix* sp., and cowries (Family: Cypraeidae).

During the present study, sponges including white encrusting sponge and *Clathria* sp. were found encrusted over the plastic baskets. Other foulers like *P. nigra* and *B. amphitrite* was also found as settled over the lid and corners of plastic baskets blocking the openings of baskets thereby restricting the free flow of water and nutrients for the oysters. Results also showed heavy encrustation and settlement during the months of September - November results in mortality of oysters.

Discussion

Present study results give an account on the pest and predators of Akoya pearl oyster *P. fucata* in Vizhinjam waters. In Vizhinjam waters, biofoulers were present all throughout the year and seasonal variation in occurrence of different species was noted. Encrusting sponges and barnacle, *B. amphitrite* were the major foulers and crabs were the predators. Encrustation of sponges over the periostracum which prevents opening and closing of shell valves is a grave problem in Vizhinjam waters which bring mortality of oysters. Fouling has been found to affect several physiological activities of pearl oysters causing oyster mortality (Miyauti 1967, 1968). Present results also elucidated the colonial behaviour of *B. amphitrite* over the periostracum of oysters which reduces the growth of oysters. Mohamed 1976 previously reported on the stunted growth exhibited by pearl oysters due to the barnacle fouling.

Pit and Southgate 2003, previously reported on the requirement of regular cleaning of fouling organisms which settled on the periostracum which is a significant fact for the commercial production of pearl oysters. It is observed that in

Vizhinjam waters, the fouling of *P. fucata* is a serious problem if not cleaned periodically. During the present study the entry of small predatory crabs seems to be a menace which causes mortality of pearl oysters. Fortnightly inspection on crabs and monthly cleaning of oysters to get rid of the fouling species according to the level of fouling is a requirement. Kripa *et al.* 2012 also opined on the occurrence of low diversity indices of fouling communities in Southeastern Arabian Sea, is related to the accumulation of silt over the periostracum of oysters. Present study gives an idea about the seasonal and group wise occurrence of pest and predators in the Vizhinjam waters which has commercial significance in the farming of pearl oyster *P. fucata* and pearl production.

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ROLE OF INSULIN ON ALLOXAN - INDUCED DIABETES: BIOCHEMICAL STUDIES OF THE INDIAN FRESHWATER TELEOST, *OREOCHROMIS MOSSAMBICUS* (PETERS, 1852)

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Abstract

Alloxan-induced diabetes is one of the widely used model to induce Type I diabetes mellitus in the experimental animals. The current study sought to investigate single intraperitoneal injection of alloxan induced diabetes (24 hr) and the effect of mammalian insulin (40 IU/kg body weight) on alloxan pretreated fresh water teleost, *O. mossambicus* after 4hr, 8 hr and 24 hr respectively. The present study clearly demonstrated that the short term effect of alloxan to Tilapia, *O. mossambicus* caused significant variation in the biochemical studies in the fish. Biochemical indices like SGOT, SGPT, Blood glucose and Cholesterol level increased after alloxan treatment. Hyperglycaemia was evident in the blood of alloxan exposed fish and insulin treatment lowered the condition. Therefore, Tilapia proved to be a promising experimental model for studies and advances in research involving diabetes mellitus.

Introduction

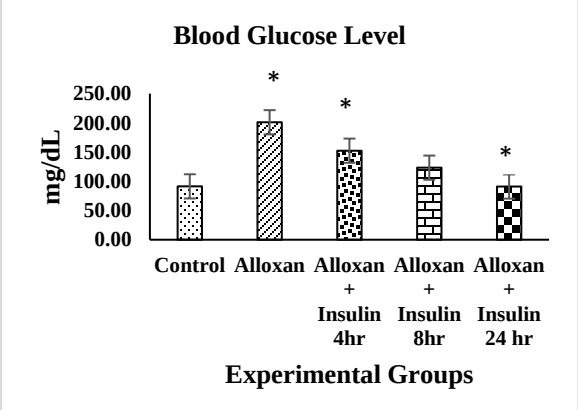
Diabetes Mellitus (DM) is a commonly occurring metabolic disorder characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. DM occurs due to absolute or relative deficiency of insulin or insulin resistance (Joo and Hoober, 2012). One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan. Administration of the drug alloxan provide a quick and convenient method for producing experimental diabetes in a variety of vertebrates (Turner and Bagnara, 1976). Alloxan, the β-cytotoxic drug, causes loss of membrane potential swelling of mitochondria and alter O_2 consumption by inhibiting the active respiration; the overall effects may be due to mitochondrial damage (Boquist, 1984). Our knowledge of the role of alloxan in impairing the blood glucose homeostasis in lower vertebrates, including fish is scanty and undeducible. Hence, the aim of the study was to examine the short term effect (24 hr) of alloxan induced diabetes and insulin (40 IU/kg body weight) on biochemical studies on the fresh water teleost, *O. mossambicus*.

Materials and Methods

The adult fish, *O.mossambicus* of 30 ± 5g of body mass were collected from a local supplier near the Sasthamkotta lake, and treated with 0.05% KMnO4 to remove any parasitic infections, and they were transferred to large glass aquaria and acclimatized for 20 days. The average values for water characteristics data holding in aquaria were temperature 25±3ºC and pH 6.8. Experimental set up consists of thirty fishes which were divided into five groups of six each and placed in separate glass aquaria. Group I constituted 0.1 ml of saline injected controls for 24 hrs and were sacrificed on the next day. Group II animals (Diabetic Group) were treated with 0.1 ml of 0.2 mg /gm body weight alloxan monohydrate (2, 4, 5, 6-Tetraoxypyrimidine, 5, 6 Dioxyuracil, SRL Mumbai) dissolved in saline and sacrificed after 24 hrs. The third group of animal received alloxan at a dosage of 0.1 ml of 0.2 mg /gm b.wt for 24 hrs and were injected with 0.1 ml of 40 IU/mL Human insulin (Huminsulin, Recombinant DNA origin, Eli Lilly and company Pvt Ltd, India) and sacrificed after 4 hrs, 8 hr and 24 hrs respectively. All injections were given intraperitoneally at the same time of the day (9 AM).

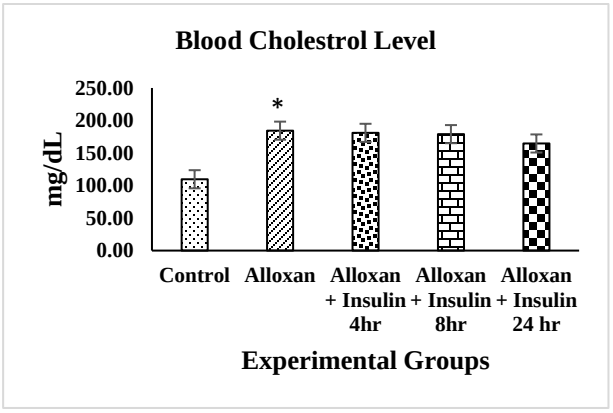
Results and Discussion

Blood glucose level was significantly increased ($P < 0.05$), Hyperglycaemia was evident on exposure to alloxan treatment (Fig. 1) and significantly lowered ($P < 0.01$) the blood glucose level after insulin administration for 4 hr, 8 hr and 24hr respectively compared to the control. There was a significant elevation ($P < 0.05$) of Cholesterol level in the blood after alloxan and insulin administration (Fig. 2). Aspartate aminotransferase (Serum glutamic oxaloacetic transaminase (*SGOT/AST*) and Serum glutamic-pyruvic transaminase (SGPT), called alanine aminotransferase (ALT) significantly elevated ($P < 0.05$) in alloxan treatment but the simultaneous administrations of alloxan and insulin resulted in the inhibition of AST, ALT activity (Fig. 3).



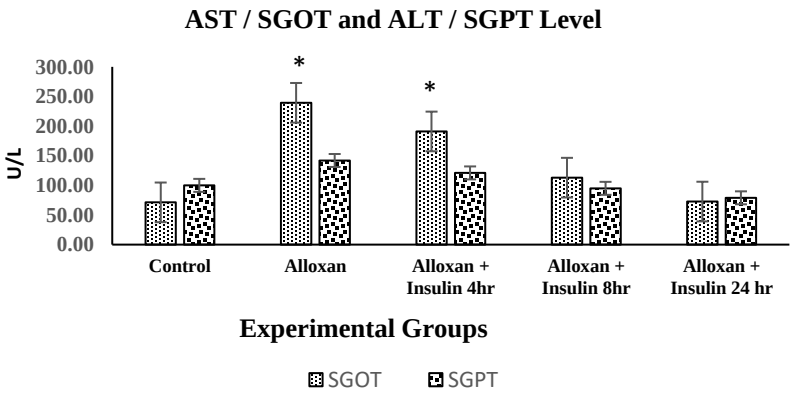
Data are represented as means ± SEM (n = 6). An asterisk (*) indicates significant difference ($P < 0.05$).

Fig. 1 Blood glucose level in *O. mossambicus* exposed to 24 hr alloxan and insulin administration (4 hr, 8 hr and 24 hr)



Data are represented as means ± SEM (n = 6). An asterisk (*) indicates significant difference ($P < 0.05$).

Fig. 2 Cholesterol level in *O. mossambicus* exposed to 24 hr alloxan and insulin administration



Data are represented as means ± SEM (n = 6). An asterisk (*) indicates significant difference ($P < 0.05$).

Fig. 3 AST and ALT level in *O. mossambicus* exposed to 24 hr alloxan and insulin administration (4 hr, 8 hr and 24 hr)

Hyperglycaemia was evident in the blood of exposed fish which may help fish to meet critical needs of energy. Such elevation may also be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands. Hypercholesterolemia which occurred in diabetic induced fishes may be caused by the increased cholesterol synthesis and the decrease in synthesis of bile salts from cholesterol due to decreased hepatic phenol-2

monooxygenase enzyme activity which responsible for formation of bile salts from cholesterol. AST and ALT are sensitive indicators to monitor the liver function under drugs treatment or with acute viral hepatitis. The elevated AST and ALT values in the blood sample indicate liver damage or injury. Hence present study clearly revealed the pathological conditions of fish after alloxan exposure. The increased level of ROS (Oxidative stress) can produce hepatic damage, and as AST and ALT are present in large amount in hepatocytes, they leak from hepatocytes upon its destruction and as the pressure of damaged hepatocytes on bile canaliculi causing bile stasis, so the activities of AST and ALT were increased in diabetic -induced fishes compared to control healthy animals group.

Conclusion

This study attempts to establish Tilapia as a promising experimental model for studies and advances in research for the study of targets applicable to diabetes. The present investigation on the fresh water fish, *O. mossambicus* treated with alloxan and insulin reveals the susceptibility of the fish to the toxic stress. From the obtained data, it can be concluded that treatment with alloxan and insulin has a potent effect against biochemical alterations caused by diabetes in fish. Such results associated with the success of insulin therapy in tilapias demonstrated the potential of experimental model in studies and advances in researches involving diabetes mellitus.

Acknowledgement

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A STUDY ON THE EFFECT OF *EUPHORBHIA MILII* ON HELA CELL LINE

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ABSTRACT

The present study focused on elucidating the chemo preventive and therapeutic action of *Euphorbia milii* and deciphering its molecular targets on human cervical carcinoma cells. In order to determine the differential cytotoxicity towards cancer cells, the effect of varying concentrations of the extract of *E. milii* was evaluated on *HeLa* cells. *E. milii* was used to estimate the extent of toxicity of plant extract, cellular changes induced by it, to determine the percentage of cell viability and to determine the action of cytotoxicity of *E. milii* in *HeLa* cell line. The results showed that *E. milii* extract could inhibit the viability of cancer cells in a dose and time dependant manner. The study also showed the inhibition of the proliferation and increased necrosis in response to *E. milii* extract in high concentration on cervical cancer line. The results showed cytotoxicity of plant extract increased in relation to increasing concentrations and incubation times. The percentage of viable cells was highly reduced as the concentration and time increases. The decrease of viability means the inhibition of cell proliferation is high that depends on concentrations and increase of duration of time.

Key Words: *Euphorbia milii, HeLa cell line*, cytotoxicity

ASSESSMENT OF BIOCHEMICAL CONSTITUENTS AND HEAVY METAL ACCUMULATION IN *RASTRELLIGER KANAGURTA* AND *METAPENEUS DOBSONI* OF KOLLAM COAST, SOUTH INDIA

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ABSTRACT

The present study was conducted to assess the biochemical constituents and heavy metal accumulation in the edible fish species, *Rastrelliger Kanagurta* and *Metapeneus Dobsoni* seen in the Arabian Sea coast of Neendakara, Kollam, South India, during the period February to May 2019. The fishing activities in Neendakara harbour and industrial effluent discharge from factories are considered as the main sources of pollution in Kollam coast including tons of sewage from fish market and oil pollutants from motor boats. The important physical parameters and heavy metals in the seawater samples were analyzed following the procedures by Grasshoff (2009). The biochemical and heavy metal analyses in fish species were done following the procedures by Jayaraman (1981) and Topping (1973). The sea surface water analyses revealed that it is alkaline in nature and showed high values of electrical conductivity indicating water is enriched with dissolved ions. In different months (February and May) of the study period the finfish (*Rastrelliger kanagurta*) and shellfish (*Metapenaeus* dobsoni) tissues showed changes in biochemical constituents. The results showed a depletion in total proteins, carbohydrates and glycogen content during the month of February which indicate that the fish species were exposed to stressed condition in the sea and it affected its metabolic processes. The concentration of heavy metals in the tissues of finfish and shellfish were estimated and it varied in the months of February and May. In both the fish species studied lead and cadmium showed concentration above the permissible limit set by FAO/WHO, 2003. The rate of heavy metal accumulation determined as BAF was found high in the liver tissue of finfishes. The bioaccumulation factor of lead in fish tissues was found high compared to that of other heavy metals. The daily consumption of these edible fishes by humans may result in bioaccumulation of toxic heavy metals in their organs and cause different diseases.

Keywords: *Biochemical constituents, Rastrelliger kanagurta, Metapenaeus dobsoni Heavy metal, Bioaccumulation Factor*

ECOLOGICAL AND BIOCHEMICAL ASPECTS OF EDIBLE BIVALVES OF ASHTAMUDI LAKE, KERALA.

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ABSTRACT

Oysters, clams and mussels are the most exploited bivalve resources in India, with an annual production of 84,483 tonnes (CMFRI, 2017). A major portion of this is resourced from the Ashtamudi Lake, Kerala, one of the Ramsar wetland of International importance. Considering the unique nature of fishing methods, Marine Stewardship Council (MSC) certification was granted to the Ashtamudi Lake on November, 2014. The Lake is characterised by its unique biodiversity and provide diverse kinds of habitat for different bivalve species. The dominant edible species include *Marcia recens*, *Villorita cyprinoides*, *Perna viridis* and *Crassostrea madrasensis*. Since the demand of proteinaceous food is increasing especially in developing countries, the knowledge of the biochemical composition of edible organisms along with the environmental parameters is extremely important to develop suitable management strategies for their rational exploitation and also to develop sustainable aquaculture practises. The environmental factors such as pH, temperature, salinity, Dissolved oxygen, carbon dioxide, hardness, substrate quality, TDS etc. was compared between the two ecologically different sites of Ashtamudi Lake. The ecological differences of the studied sites resulted in the spatial variation in the availability of the bivalves (*Marcia recens*, *Villorita cyprinoides*, *Perna viridis* and *Crassostrea madrasensis)* as well as the collected specimens from the two sites showed differences in the biometric (total weight, meat weight, shell weight, meat content, length, height, width and shell volume) and biochemical parameters (Ash, Moisture, Protein, Carbohydrate and Lipid). This study envinced that the nutritional qualities of these edible bivalves are highly influenced by the changes in the hydrological indices and suitable measures should be taken against the increasing pollution load in order to ensure the sustainable aquaculture practises in the Lake.

Keywords: Ashtamudi Lake, Bivalves, Hydrological indices, Biochemical parameters.

OPTIMIZATION OF *DROSOPHILA MELANOGASTER* CULTURE MEDIA TO STUDY THE DEVELOPMENTAL STAGES

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ABSTRACT

Drosophila melanogaster, also known as the "fruit fly," is a small insect that is commonly found near ripening fruit. *Drosophila* is used for scientific research and the study of this organism has provided insight into eukaryotic genetics and human disease. The genus *Drosophila* consists of various species of fruit flies which are widely used as model organisms as *Drosophila* has well- defined phylogeny. Besides, the short generation time of Drosophila aided the studies on genetics especially studies of the laws of heredity (Markow & O'Grady,2006). This species of fruit flies not only possesses of well-defined genetics information, they also have short generation time in which one pair of parent flies is able to produce several hundred offspring which ease the process of genetics (Demerec & Kaufmann, 1996). Thomas Hunt Morgan discovered many details of the chromosomal basis of heredity of *Drosophila*, during the first three decades of the century.

Drosophila in biological research began in the early years of the 20th century. One of the many reasons that make Drosophila an extremely valuable organism is that the molecular, cellular, and genetic foundations of development are highly conserved between flies and higher eukaryotes such as humans. *Drosophila* progress through several developmental stages in a process known as the life cycle and each stage provides a unique platform for developmental research *Drosophila melanogaster* embryos and larvae are easy to manipulate and develop rapidly by mechanisms that are analogous to other organisms, including mammals. Prepare culture medium using dissolve agar and add crushed banana. Mix it thoroughly. Add Brewer's yeast and boil the mixture for ten minutes with constant stirring. Remove from heat and add a drop of propionic acid. Pour this to the culture vials using a funnel. Tap the bottom of vial to exclude the air bubble and to maintain uniform surface. A sterilized strip of paper may be introduced into the vial to facilitate pupation. Plug the vial with sterilized cotton.
Observations from the present study revealed the occurrence of four main stages in *Drosophila* life cycle, which are egg, larva, pupa and adult stages. Drosophila embryos develop in the egg membrane. The egg hatches and produces a larva which feeds by burrowing through the medium. The larval period consists of three stages, or instars, the end of each stage marked by a molt. The first instar is the newly hatched larva; the third instar is the final larval stage, where the larva may attain a length of 4.5mm. Near the end of the larval period, the third instar larva will crawl up the sides of the culture vial, attach themselves to a dry surface (the jar, the filter paper, etc.) and form pupae. After a period of time the adults emerge. It takes one or two days for Drosophila eggs to hatch into larvae, four to five days for the larvae to enter pupae, and four days for the pupa stage. The following are the images taken during the study period.

The *Drosophila melanogaster* was selected for study nearly a hundred years ago, a great deal has been learned about its genome. The *D. melanogaster* is considered a model organism due to its small size, short life cycle, fast reproductive rate, low cost in maintenance. Four different stages of fruit fly were clearly observed from the present study in *D. Melanogaster*. By using *Drosophila*, we can understand Mendalian genetics and inheritance of traits, obtain an understanding of the life of *D. melanogaster* (an insect which exhibits complete metamorphosis), learn techniques to manipulate flies sex them and keep concise journal notes and learn culturing techniques to keep the flies health

IMPACT OF SELECTED HEAVY METALS ON THE CHROMATOPHORES OF *ETROPLUS SURATENSIS* FROM TWO DIFFERENT SITES OF ASHTAMUDI LAKE

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ABSTRACT

Chromatophores have been used extensively as a tool to study specific responses to a wide range of pharmacologic and bioactive agents and their analogues in a number of

different species. We are using chromatophores as biosensors to detect a broad range of bioactive compounds, without necessarily knowing the exact mechanisms inducing changes in the cells without necessarily knowing the exact mechanisms inducing changes in the cells.The present study was an attempt to study the effect of different heavy metals on the chromatophores of *Etroplus suratensis* from two different sites, Mukkad and Munrothuruthu. Chromatophores are pigment containing and light reflecting cells, or groups of cells, found in a wide range of animals including amphibians, fishes, reptiles, crustaceans and cephalopods.

Mature chromatophores are grouped into subclasses based on their colours. They include: xanthophores,erythrophores, iridiophores, leucophores, melanophores and cyanophores.

It was observed that there was a drastic change in the size, shape and number of chromatophores on exposure to various heavy metals such as cadmium chloride, mercuric chloride and lead nitrate when compared to the control.

Concentrated, punctate, stellate, dispersed and highly dispersed states of chromatophores were observed in the control. But it was observed that the size, shape and number of chromatophores gradually changes on exposure to heavy metals such as cadmium chloride, mercuric chloride and lead nitrate.

Inspite of altering the chromatophores, these heavy metals when injested by the fisht can also cause various health problems and diseases including hormonal imbalance, abnormal behavior, reduction in spawning, high death rate, larval deformation, colour change, kidney disfunctioning etc

Unfortunately these chemical contaminants are stored within the lipid components of the fish so they are well protected in entering the human body. But in the long run, consuming fish may cause various health hazards to human beings too, which include diarrhea, vomiting, failures in bone, damage to DNA, damage to nervous system etc.

The present study has shown significant changes in the chromatophores upon exposure to certain heavy metals. This may be synonymous to the polluted aquatic habitat of the lake, which is home to the candidate species i.e. *Etroplus suratensis*. Hence chromatophores may be considered as a “BIOSENSOR” in fishes.

COMPARISON OF SEDIMENTOLOGICAL PARAMETRS ALONG THE KOLLAM CANAL RELATION WITH MICROBIAL CONTENT

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Sediments are one of the access channels in aquatic surveilling. Apart from water, sediments are also accountable of nutrients and pollutant transport in the aquatic environment. Bottom sediments have an important function as an efficient natural trap for diverse substances (including contaminants) and also as a natural regulator of the processes that occur on the seafloor. They can store large amounts of organic matter and affect the oxygen content of bottom water. Bottom sediments also constitute a source of nutrients for the water column above them leading to benthic-pelagic coupling and influencing primary productivity (Jorgensen 1996). Kollam Canal or Quilon Canal is a 7.7 km long canal system passing through the city of Kollam, India. It is a part of National Waterway-3 and is 78 km long once under the Kollam-Trivandrum waterway project. The Kollam canal was built in the year 1880 and is a bustling part of 560 km long Thiruvananthapuram – Shoranur canal (TS Canal) waterwayproject, also used as a means for transport of both people and goods, as an avenue forleisure and the water even used for irrigation and drinking. The main objectives of the present study are to analyse the sediment of different polluted environment, to compare sedimentological parameters along the three sites, to analyse and compare the microbial content of the sediment samples, to compare the relationship between the microbial content and sediment pollution.

The study helped to compare the various sedimentological parameters of the three sites of Kollam canal and understand the pollution status, the study also knows about the microbial content. The data from the study gave the information in designing and developing the pollution prevention and management strategies. Wide occurrence of aquatic anthropogenic pollution is rising at an alarming rate. Bio monitoring offers an appealing tool for the assessment pollution in aquatic ecosystems. Environmental changes over longer time periods can be monitored using sediment quality, the data generated from the project will enable the officials concerned to formulate a rational scientific aquatic zone planning protocol. During the study period, correlation of sediment parameters with total microbial content was positively correlated with temperature, nutrients, and sediment texture. Whereas pH, phosphate and clay showed a negative correlation. During the present study, the sediments are collected from the study sites and data is analysed to describe the health condition of the canal. Sedimentological parameters showed spatial variations though, some of the parameters varied only within a narrow range. Municipal solid waste (solid as well as domestic) and sewage from local areas are one of the major sources for pollution as it uses the canal region as a ‘waste sink’ the other waste dumping sites for disposing untreated waste to the region. It also contains large quantities of non-degradable plastic bags and containers. There are no proper management practices for disposing the waste from slaughterhouse and hospital in the city. Therefore, the study will provide valuable information not only on the plankton diversity and total microbial content but also on its present ecological status. The biomonitoring network may pro-vide important information

on the aquatic pollution level and wide occurrence of aquatic anthropogenic pollution is rising at an alarming rate. Biomonitoring offers an appealing tool for the assessment pollution in aquatic ecosystems. Environmental changes over longer time periods can be monitored using plankton and microbes are as potential candidates. Studies based on the identification of pollution indicators may help in the better understanding of stress in the environment.

KERALA STATE COUNCIL FOR SCIENCE, TECHNOLOGY AND ENVIRONMENT

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KSCSTE an autonomous Institution of Govt. of Kerala is committed for the promotion of Science, Education, Research and Scientific temper in the State. KSCSTE prepares the road map for development of the State through advancements in scientific research and innovation in technologies. Achieving excellence in basic research, academia-industry interactions, strengthening indigenous technologies initiatives, and building strong infrastructure and developing a high quality science education system in the state are our targeted goals. There are Seven R&D centres under the umbrella of the Council to coordinate Research and Development activities in the specific mandated domains.

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PG & Research Department of Zoology

(DST-FIST Supported)

Fatima Mata National College (Autonomous), Kollam

HISTORY OF THE DEPARTMENT

Prof. K. T. Kurien (1951-1970) our first Head of the Zoology Department, is the main architect of the department, through whose dedicated and concerted efforts, the department rose to the status of a first grade College in 1953 with the introduction of B. Sc. Degree course followed by MSc. Course in 1964. Dr. E. I. Thomas who took over as the next HOD was an eminent academician and researcher with a D.Sc to his credit (The second D. Sc. from the University of Kerala).

Dr. V. R. Prakasam, one of our most enterprising faculty member, was the driving force in elevating the department as a recognized Research Centre (the first in the college) in 1988. To date 47 candidates have secured doctoral degree and currently 11 students have enrolled for their PhD programme.

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In this issue (40 articles)

State Level Seminar

NANOSCIENCE AND NANOTECHNOLOGY

Date: 16-01-2019

PROCEEDINGS

Organized by

Post Graduate & Research Department of Chemistry

Fatima Mata National College, Kollam

Sponsored by the

Kerala State Council for Science, Technology and Environment

In Association with

Academy of Chemistry Teachers (ACT)

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Preface

It is a matter of pride to organize the seminar on **Nanoscience and Nanotechnology** at Fatima Mata National College, Kollam, sponsored by the Kerala State Council for Science Technology and Environment (KSCSTE) on 16-01-2019.

Fatima Mata National College was founded in 1951 by His Excellency Rt. Rev. Dr. Jerome M Fernandez, with a view to providing facilities for higher education in the Diocese of Quilon. Department of Chemistry was established in the year 1952 with BSc Chemistry course. The Department now offers MSc Chemistry and BSc Polymer Chemistry. In 2013 the Department was elevated to the status of Research Centre.

The aim of the seminar is to disseminate knowledge on the recent advancements made in the area of Nanoscience and technology. Nanoscience is the branch of science that studies systems and manipulates matter on atomic, molecular and supramolecular scales. On such a length scale, quantum mechanical and surface boundary effects become relevant, conferring properties on materials that are not observable on larger, macroscopic length scales.

Nanoscience is the science of objects with typical sizes of 1-100 nm. If matter is divided into such small objects the mechanical, electric, optical, and magnetic properties can change. Interfaces rather than bulk properties dominate. Quantum effects due to the size limitation come into play. Nanoscience and Nanotechnology are interdisciplinary, crossing boundaries between physics, chemistry, chemical, electric and mechanical engineering. Nanoscience, that is the science of objects with typical sizes of 1-100 nm, is one of the most important developments in the last decades. Miniaturization of electronic devices to sizes of the elementary units below 1 μm has revolutionized our daily live. New technologies were required to enter the nanoscale because many of the traditional techniques do not work at the nanoscale. The relation between nanoscience and technology is like a symbiosis. Scientific discoveries lead to new technologies. The technology enables new fundamental insights.

Two new technologies which enabled the progress of nanoscience are scanning tunneling and scanning force microscopy. They allow to image and manipulate objects on surfaces with sufficient precision even in ambient conditions or in liquids. Most properties of solids are altered when their dimensions approach the nanoscale. As an example, consider a particle of 1x1x1 nm^3 . This contains roughly 43 to 64 atoms. Only 8 atoms of them are in the interior, while 87% of the atoms are at the surface. The electronic, magnetic, chemical, and mechanical properties of nanoparticles are therefore dominated by surface atoms. Simply by finely dispersing ordinary bulk materials new properties can be created: inert materials become catalysts, insulators become conductors, or stable materials become combustible. A rather inert material like Au may for example become an efficient and selective catalyst when of the size of a few nm. The characteristic length scale of a system can often be given intuitively. For example, for a spherical particle one would use the diameter, for a thin film the thickness. For more complex systems intuition can, however, lead to ambiguous results. Nanoscience is thus the science of systems with λ in the range of 1 to 100 nm. The most simple nanomaterials are particles dispersed in a medium. Although nanoparticles have been made since the time of Michael Faraday and colloid science has particularly flourished in the 1920s and 30s, making functional nanoparticles will remain a challenge.

With great pleasure I place on record my sincere thanks to Kerala State Council for Science Technology and Environment (KSCSTE) for the financial assistance. I would like to express my heartfelt gratitude to Rev Fr. Rolden Jacob, Manager, Fatima Mata National College, Kollam, Dr. Vincent B Netto, Principal, Dr. Apsara AP, Head, Department of Chemistry, and all my colleagues for their kind and support for the successful conduct of the Seminar.

Dr Manohar. D. Mullassery
(Organizing Secretary & Editor)

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Invited Talk I

Supramolecular Chemistry with DNA

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Introduction: Supramolecular chemistry refers to the domain of chemistry beyond that of molecules and focuses on the chemical systems made up of a discrete number of assembled molecular subunits or components. While traditional chemistry focuses on the covalent bond, supramolecular chemistry examines the weaker and reversible noncovalent interactions between molecules. These forces include hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions and electrostatic effects. Important concepts that have been demonstrated by supramolecular chemistry include molecular self-assembly, folding, molecular recognition, host-guest chemistry, mechanically-interlocked molecular architectures, and dynamic covalent chemistry. The study of non-covalent interactions is crucial to understanding many biological processes from cell structure to vision that rely on these forces for structure and function. Biological systems are often the inspiration for supramolecular research. In the presentation, individual supramolecular interactions with specific examples were discussed.[1-3]

Electrostatic interactions: Electrostatic interactions are between cations and anions. Electrostatic interactions can be either attractive or repulsive, depending on the signs of the charges. Favorable electrostatic interactions cause the vapor pressure of sodium chloride and other salts to be very low. The electrostatic interactions within a sodium chloride crystal are called ionic bonds. But when a single cation and anion are close together, say on the surface of a protein, or within a folded RNA, those are favorable non-covalent electrostatic interactions. Electrostatic interactions can be very strong, and fall off slowly with distance (1/r).

Dipole Interactions: In a molecule composed of atoms of various electronegativities, the atoms with lowest electronegativities hold partial positive charges and the atoms with the greatest electronegativities hold partial negative charges. In a methanol molecule (CH_3OH), the electronegative oxygen atom pulls electron density away from the carbon atom. In a water molecule, the electronegative oxygen atom pulls electron density away from the hydrogen atoms. The oxygen atom carries a partial negative charge. The hydrogen atoms carry partial positive charges. This phenomenon of charge separation is called polarity. Water is a polar molecule.

Dipole-dipole interactions: The strength of a dipole-dipole interaction depends on the size of each dipole and on their relative orientation. The interaction energy between two dipoles can be either positive or negative. Parallel end-to-end dipoles attract while antiparallel end to end dipoles repel.

Dipole-induced dipole interactions: A molecule with a permanent dipole moment will induce a dipole moment in a second molecule that is located nearby in space. This phenomenon is called polarization. The strength of a dipole-induced dipole interaction depends on the size of the dipole moment of the first molecule and on the polarizability of the second molecule. Polarizability is a measure of the ease with which electrons are shifted by an external electronic field. Molecules with π electrons, such as phenylalanine and tryptophan, are more polarizable than molecules such as isoleucine that lack π electrons. Dipole-induced dipole interactions are important even between molecules with permanent dipoles. A permanent dipole is altered/modulated by the dipole of an adjacent molecule. For example, the dipole of one water molecule will influence the electron distribution of an adjacent water molecule. The dipole of a water molecule will induce a change in the dipole of a nearby water molecule, compared to the permanent dipole of an isolated water molecule.

Dipole-charge interactions: A molecule with a permanent dipole can interact favorably with charged species. This type of interaction is why sodium chloride (cationic sodium ions and anionic chloride ions) and other salts tend to interact well with water (strong dipole).

Fluctuating dipoles (Dispersive interactions, London Forces): Molecules behave like oscillating dipoles. In molecules that are located nearby to each other the oscillators are coupled. The movements of the electrons in molecules are correlated. Electrons tend to run away from each because of electrostatic repulsion. Coupled fluctuating dipoles experience favorable electrostatic interaction known as dispersive interactions. The strength of the interaction is related to the polarizabilities of the two molecules (or atoms). Fluctuating dipole interactions fall off with $1/r^6$.

Hydrogen Bonding: An acceptor atom (A) with a basic lone pair of electrons (i.e., a Lewis Base) can interact favorably with an acidic proton bound to an electronegative atom (D). A strong hydrogen bond requires that both atoms A and D are electronegative atoms. The most common hydrogen bonds in biological systems involve oxygen and nitrogen atoms. Sulfur can also engage in hydrogen bonds. Hydrogen bonds where atom D is a carbon atom are observed although these are relatively weak interactions. Hydrogen bonds are essentially electrostatic in nature, although the energy can be decomposed into additional contributions from polarization, exchange repulsion, charge transfer, and mixing. In traversing the Period Table, increasing the electronegativity of atom D strips electron density from the proton, increasing its partial positive charge, and increasing the strength of the hydrogen bond. Hydrogen bond strengths form a continuum. Strong hydrogen bonds of 20-40 kcal/moll, generally formed between charged donors and acceptors, are nearly as strong as covalent bonds, Weak hydrogen bonds of 1-5 kcal/mol, sometimes formed with carbon as the proton donor, are no stronger than conventional dipole-dipole interactions. Moderate hydrogen bonds, which are the most common, are formed between neutral donors and acceptors are from 3-12 kcal/mol. All these different supramolecular interactions were discussed with appropriate examples. Apart from this general introduction, two very recent research papers were also discussed in the talk.[4,5]

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Invited Talk II

ANALYSIS OF NANO MATERIALS; UNRAVELING THE HIDDEN SECRETS

Dr Renjith S

Central Analytical Facility, SCTIMST

Abstract: Nano materials gather tremendous research attention in the recent times owing to their intriguing properties which makes them suitable candidates for a variety of applications in our daily life. As Richard Feynman rightly pointed out, there is plenty of room at the bottom to explore and many research groups are working ardently in this line. The characterization of nano materials is quintessential before deploying them for any applications since they can pose a threat to the very same application if present in undesirable chemical combination or morphology. It also helps to confirm the formation of targeted compound in the desired chemical combination, crystalline form, morphology and purity. This talk is intended mainly to give an overview of the basic characterization tools used for the analysis of nano materials.

Oral Presentation I

Ethylene diamine modified chitosan in heavy metal pollution mitigation: Removal of Pb(II) from aqueous medium

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Abstract
Environmental contamination is a major problem being faced by the society today. Due to the rapid growth in technology, industrial, agriculture, and domestic wastes are discharged in to several receivers. Heavy metal pollution is of significant environmental importance because of their interaction with solid phase materials of geological origin and because of their influence on biological process. The present work explores the potential use of Ethylene Diamine Modified Chitosan (EDMC) for the removal of Pb (II) from aqueous solution. Batch adsorption studies were performed to assess the influence of various parameters such as solution pH, contact time, initial Pb (II) concentration and temperature. Equilibrium, kinetic and thermodynamic studies were made for the adsorption of Pb (II) on to EDMC at pH 5. The equilibrium data has been analyzed using Langmuir and Freundlich isotherms. The suitability of the pseudo-first order and pseudo-second order equation was tested. The pseudo-second order equation is well fit with the kinetic data and it was confirmed by regression analysis.

Introduction

Water is a unique substance, because it can naturally renew and cleanse itself, by allowing pollutants to settle out or break down, or by diluting the pollutants. This natural process takes time, and is difficult when excessive quantities of harmful contaminants are added to the water. Water pollution includes all of the waste materials that cannot be naturally broken down by water. Anything that is added to the water, above and beyond its capacity to break it down, is pollution. Pollution, in certain circumstances, can be caused by nature itself, such as when water flows through soils with high acidities. But more often, human actions are responsible for the pollutants that enter the water. The term "heavy metals" refers to any metallic element that has relatively high density and is toxic or poisonous even at low concentration. "Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3 or 5 times or more. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and the platinum group elements. Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed.

The bio toxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. Although individual metals exhibit specific signs of their toxicity, the following have been reported as general signs associated with cadmium, lead, arsenic, mercury, zinc, copper and aluminium poisoning: gastrointestinal (GI) disorders, diarrhea, stomatitis, tremor, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia when volatile vapours and fumes are inhaled.

Adsorption is recognized as an effective and economic method for heavy metal wastewater treatment. The adsorption process offers flexibility in design and operation and in many cases will produce high-quality treated effluent. In addition, because adsorption is sometimes reversible, adsorbents can be regenerated by suitable desorption process. For many applications, this process has been proven to superior to other technique for a variety of reasons including the simplicity

of design, low cost, high removal efficiency, ease of operation, and availability. Activated carbon is considered as universal adsorbent for effluent treatment and is commonly used for the removal of various pollutants from water. Chitosan, a biocompatible polysaccharide obtained from deacetylation of chitin, is non-toxic, biodegradable and antibacterial compound which can be used for the removal of heavy metal ions. Meng et al (2010) studied chitosan-coated sand (CCS) can be utilized for the removal of copper (II) and lead (II) ions from water.

Materials and Experimental Methods

Materials

All chemicals used were of analytical grade. Distilled water was used throughout the study. The adsorbent used for this study is Ethylene diamine modified Chitosan. Chitosan, a biocompatible polysaccharide obtained from the deacetylation of chitin (Fig. 1). The stock solution (1000mg/L) was prepared by dissolving 1.5985g of lead nitrate in distilled water. The experimental solution was prepared by diluting the stock solution to desired concentrations using distilled water. HNO_3 and NaOH solutions were used for pH adjustments.

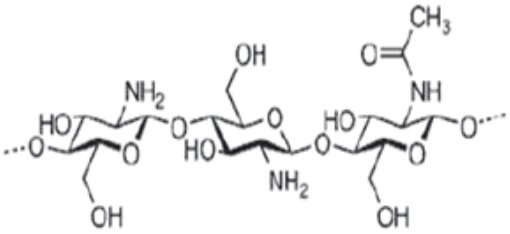

Fig. 1. Chitosan's structure

Adsorbent

The adsorbent used for this study is Ethylene diamine modified Chitosan. Chitosan, a biocompatible polysaccharide obtained from the deacetylation of chitin, is a non-toxic, hydrophilic, bio-compactable, biodegradable, and antibacterial compound (Fig. 2). It can be used to efficiently remove lead ions. Chitosan obtained was grounded and sieved to obtain 60 (250 microns) mesh size particles using standard test sieves.

Fig. 2. Chitosan powder

Analytical tools and methods

The estimation of lead was performed using Atomic Absorption Spectrometer (AAS).The pH measurements were made using a Cyber pH meter (Ph-14L) coupled with a combined electrode. Weight measurements were made on Shimadzu made electronic balance (Model: AUX 220). A temperature controlled shaker with digital display was used for adsorption experiments. Standard test sieves were used for sieving chitosan for getting particles of 60 mesh size. All the glass wares were of Borosil class-A type and thoroughly washed with tap water followed by distilled water and dried before each experiment.

Pb (II) Analysis- Experimental Procedure

The stock solution (1000mg/L) of Pb (II) is prepared by dissolving accurately 1.5984g lead nitrate in distilled water. Experimental solutions were prepared by diluting the stock solution with distilled water. Batch adsorption experiments were conducted by agitating 0.1g of adsorbent with 50ml of lead nitrate solution of different concentrations. The pH of the solution was adjusted using different concentration of HNO_3 and NaOH solutions. The solution was then subjected to constant shaking in a temperature controlled shaker. At predetermined time intervals, the supernatant solution was taken, diluted and analyzed for residual lead in the aqueous phase. The amount of lead was estimated using Atomic Absorption Spectrometer. The amount of lead adsorbed on the solid surface was calculated as

$$q = \frac{[(C_O - C_A)V]}{m}$$

Where 'q' is the amount of lead adsorbed on to unit amount of the adsorbent (mg/g); C_O ,the concentration of lead in the initial

solution(mg/L); C_A, the residual lead concentration in the aqueous phase after adsorption (mg/L); V, the volume of the aqueous phase (mL); and m, the weight of the adsorbent (g).

Effect of pH

The influence of pH on the adsorption of lead was studied by agitating different initial lead ion concentration of 5, 10, 25, and 50 mg/L with constant amount of adsorbent at 30°C. Solution of lead with different initial concentrations were shaken with 0.1g of adsorbent at different pH values (4-6) for 120 minutes by keeping a constant agitation speed.

Effect of contact time

About 0.1g of the adsorbent was agitated with 50ml of lead solution of desired concentrations at optimum pH and at 30°C with predetermined time intervals 1, 5, 15, 30, 60, 90, 120 minutes. The batch experiments were carried out for different initial lead concentrations of 5, 10, 25 and 50mg/L. The kinetic data obtained by the above experiments were modeled using pseudo-first order and pseudo-second order kinetic expression for finding out the rate of the adsorption reaction. Based on the coefficient of regression it possible to find out the best fit kinetic model.

Effect of temperature

The influence of temperature on the adsorption process was determined over temperatures 20, 30, 40, and 50°C for different initial lead concentrations of 5, 10, 25, 50, 75, 100 and 125 mg/L. The operational conditions such as solution pH, adsorbent dosage and contact time were kept constant.

Effect of initial concentration

The influence of initial lead concentration on the adsorption of lead on to solid surface was carried out for different initial concentrations 5, 10, 25, 50, 75, 100, and 125 mg/L by keeping the following operational conditions constant: contact time, adsorbent dose, temperature and pH. The equilibrium data obtained were modeled using Langmuir and Freundlich isotherm models and thereby it possible to find out the adsorption capacity and energy of adsorption.

Results and discussion

In the present study, the adsorptive removal of Pb (II) on to EDMC has been studied. The systematic characterization of investigated adsorbent material was carried out using standard method. Batch experiments were conducted to study the adsorption of Pb (II) on to EDMC. The factors affecting the extent of adsorption process such as solution pH, initial concentration, and contact time were determined. The kinetic and equilibrium data were modeled to find out the best fit kinetic and isotherm model to describe adsorption phenomenon. Batch adsorption studies were performed to assess the influence of various parameters such as solution pH, contact time, initial Pb(II) concentration and temperature. The adsorption of Pb (II) ions was markedly affected by the solution pH because it influences the metal chemistry in aqueous media as well as the surface chemistry of the adsorbent. The percentage of Pb (II) adsorption increased with increase in pH and reaches a maximum at pH 5 and then decreased. At an initial concentration of 10mg/L, maximum Pb (II) adsorption of 99.9% (4.995mg/g) on to chitosan was observed at pH 5.

Adsorption is an equilibrium process and reaction time is one of the important factors influencing the adsorption of metal at solid-liquid interface. An optimum contact time of 2hr was observed on the uptake of Pb (II) onto chitosan. As initial concentration increases, the amount of adsorption decreases. For initial concentration of 5mg/L, maximum Pb (II) adsorption of 99.9% on to chitosan was observed at pH 5 and at contact time of 2hr.

The adsorption of Pb (II) on to EDMC was investigated as a function of temperature and the maximum removal of Pb (II) ions was observed at 40°C. This may be due to the increased mobility of metal ions and an increase in number of active sites for adsorption with rise to temperature. The results indicates that adsorption of Pb(II) on to EDMC is an endothermic process. The various adsorption studies is presented in Fig. 3

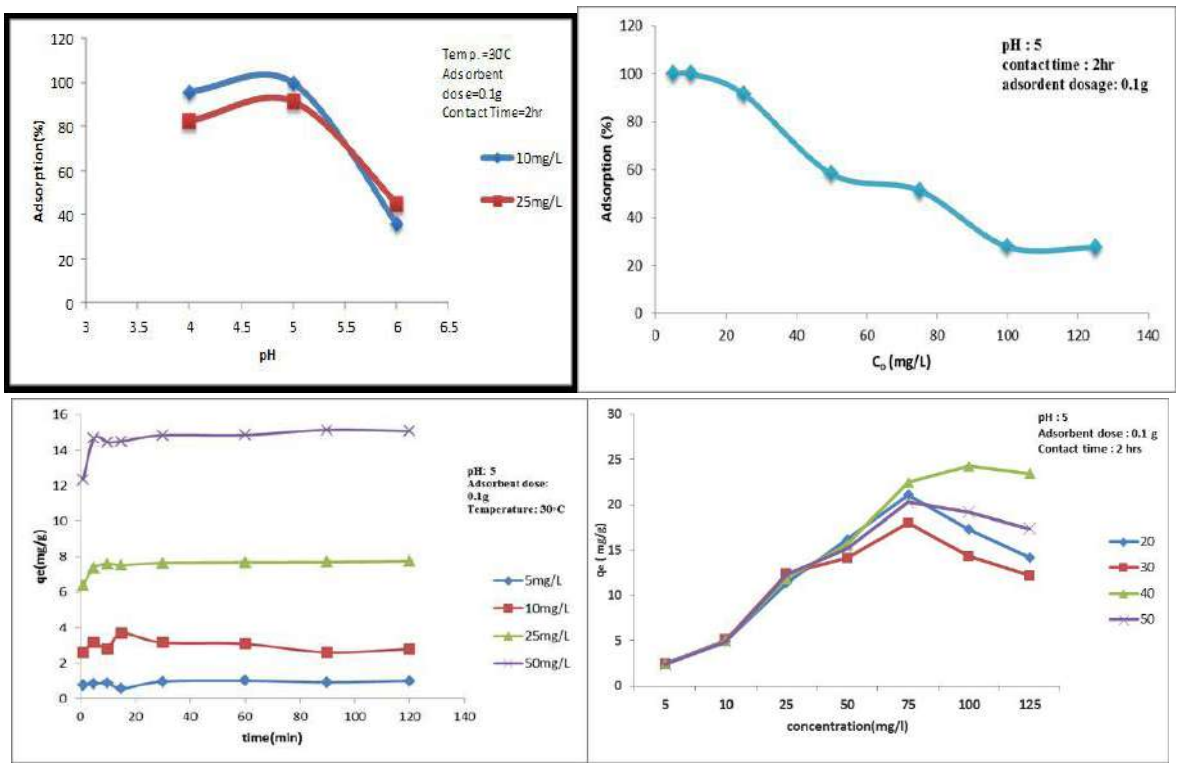

Fig. 3. Various adsorption studies for the removal of Pb(II) using chitosan

Adsorption kinetics

The kinetic parameters, which are helpful for the prediction of adsorption rate, give important information for designing and modeling the adsorption process. In order to investigate the mechanism of adsorption various kinetic models have been suggested. In this study, two of these models were investigated to find out the best fitted model for the experimental data obtained. The Lagergren pseudo-first order rate equation (Lagergren, 1898) and pseudo-second order equation (Ho and McKay, 1999) were used as kinetic models. The pseudo- first order kinetic model equation is

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303}$$

where q_e and q_t are the amount of Pb(II) adsorbed at equilibrium and at time 't' in mg/g and k_1 is the pseudo- first order rate constant (min-1) for the adsorption. The rate constant k_1 is obtained by plotting log (q_e-q_t) verses 't' and the results are given in the Table 1

The pseudo-second order kinetic model is expressed as follows.

$$\frac{t}{q_t} = \frac{1}{k_{2q_e}2} + \frac{t}{q_e}$$

where qe and qt are the amount of Pb (II) adsorbed at equilibrium and at time't' in mg/g and k_2 is the equilibrium rate constant of pseudo- second order adsorption (g/mg/min). Values of k_2 and qe were calculated from the plot of t/qt against t. The values of rate constant k_2 for adsorption of Pb (II) onto chitosan are given in the Table 1. The kinetic plots drawn using pseudo-first-order and pseudo-second-order kinetic expressions is presented in Fig. 4.

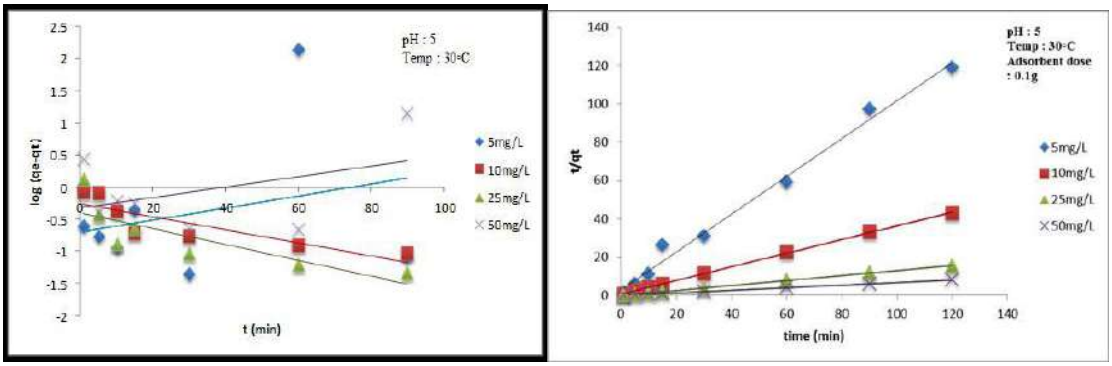

Fig. 4. Pseudo-first-order and pseudo-second-order kinetic plots

Models	Parameters	Initial Concentrations (Mg/L)			
		5	10	25	50
Pseudo- first Order	K_1 (min^{-1})	-0.021	0.0232	0.028	-0.019
	q_e (mg/g)	0.198	0.553	0.393	0.471
	r^2	0.068	0.736	0.648	0.174
Pseudo-second order	K_2 (min^{-1})	0.333	0.227	10.482	0.148
	q_e (mg/g)	1.011	2.8	7.722	15.128
	r^2	0.990	0.999	0.999	0.999

Table 1 Adsorption kinetic parameters

The values of k_1 for the adsorption of Pb (II) onto EDMC at initial concentration of 5, 10, 25, and 50mg/L were found to be -0.021, 0.0232, 0.028 and -0.019 min-1 respectively. In the case of pseudo-second order kinetic expression K_2 values were 0.333, 0.227, 10.482 and 0.148 g/mg/min for the adsorption of Pb (II) on to EDMC at initial concentrations of 5, 10, 25 and 50 mg/L respectively. The values of correlation coefficients for the adsorption of Pb (II) onto EDMC were found to be 0.068, 0.736, 0.648 and 0.174 for Lagergren first-order kinetic model and 0.990, 0.999, 0.999 and 0.99 for pseudo-second order kinetic model at initial concentrations of 5, 10, 25 and 50mg/L. The calculated qe values agree with the experimental values and also correlation coefficients for the pseudo-second order kinetic plots were very high. This result implies that the adsorption follows pseudo-second order kinetic model than the Lagergren first order kinetic model.

Conclusion

The adsorption of Pb (II) on to EDMC was investigated as a function of temperature and the maximum removal of Pb (II) ions was observed at 40°C. This may be due to the increased mobility of metal ions and an increase in number of active sites for adsorption with rise to temperature. The results indicates that adsorption of Pb (II) on to EDMC is an endothermic process. Equilibrium, kinetic and thermodynamic studies were made for the adsorption of Pb (II) on to EDMC at pH 5. The equilibrium data has been analyzed using Langmuir and Freundlich isotherms. The characteristic parameter for each isotherm and related correlation coefficients has been determined. The higher regression coefficient is observed for Langmuir isotherm indicating that Langmuir model is best to study the adsorption of Pb (II) on to EDMC. Kinetic studies were carried out for the adsorption of Pb (II) on to EDMC. The suitability of the pseudo-first order and pseudo-second order equation was tested. The pseudo-second order equation is well fit with the kinetic data and it was confirmed by regression analysis. The present study concludes that EDMC can be employed as an ecofriendly and efficient adsorbent for the removal of Pb (II) from water and waste water.

Acknowledgements

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Oral Presentation II

DFT investigations and molecular docking study of benzoxazole derivative

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Abstract
The synthesis, FT-IR, FT-Raman spectral analysis of an antimicrobial active benzoxazole derivative, 5-[(4-methylphenyl)acetamido]-2-(4-tert-butylphenyl) benzoxazole (MPATB) is reported. The localization of HOMO, LUMO plots in the title compound over the title molecule shows the charge transfer in the molecular system through the conjugated paths.The electrophilic and nucleophilic sites are revealed from the molecular electrostatic potential map. The first hyperpolarizability of the title compound is greater than that of the standard nonlinear optical material urea and the title compound and its derivatives are good objects for further research in nonlinear optical analysis. The docked title compound forms a stable complex with thymidylate synthase and got a binding affinity value of -8.5kcal/mol and the title compound can be a lead compound for developing new anti-cancerous drug.

Keywords: Benzoxazole; DFT; Molecular docking.

1. Introduction

Heterocyclic compounds such as benzoxazoles have attracted attention due to their diverse pharmacological and biological properties like antibacterial, antifungal, anti-tubercular,anti-tumorandantiviral[1,2].Studies of reactive properties of newly synthetized organic molecules with potential important biological activities are very important for the development and improvement of methods for water purification. Namely, molecules that are active components of pharmaceutical products are synthetized to be very stable, thus natural conditions and conventional purification methods are not enough effective for their degradation [3] .Unfortunately, due to various reasons drugs are entering the environment and are accumulating especially in the water resources, where they are toxic to aquatic organisms. So far these types of compounds have been detected in all types of waters [4].

2. Experimental

The chemicals and solvents were purchased from Sigma-Aldrich (Munich, Germany) and Fisher Scientific (Pittsburgh, PA, USA); they were used without purification. Silica gel HF_{254} chromatoplates (0.3 mm) were used for thin layer chromatography, and the mobile phase was chloroform/methanol (10:0.5). Melting point was recorded on a Stuart Scientific SMP1 instrument (Bibby Scientific Limited, Staffordshire, UK) and is uncorrected. NMR spectra were recorded on a Varian Mercury 400 MHz NMR spectrometer (Palo Alto, CA, USA); trimethylsilane (TMS) was used as an internal standard. The mass spectra was recorded on a Waters ZQ Micromass LC-MS spectrometer (Milford, MA, USA) using the ESI(+) method.
The FT-IR spectrum was recorded using KBr pellets on a DR/Jasco FT-IR 6300 spectrometer. The FT-Raman spectrum was obtained on a Bruker RFS 100/s, Germany. For excitation of the spectrum the emission of Nd:YAG laser was used, excitation wavelength 1064 nm, maximal power 150 mW, measurement on solid sample. The spectral resolution after apodization was 2 cm^{-1}.
Firstly, 5-amino-2-(4-tert-butylphenyl)-benzoxazole was synthesized by heating 0.02 mol 2,4-diaminophenol·2.HCl with 0.02mol 4-tert-butylbenzoic acid in 25 g polyphosphoric acid (PPA) and stirring for 3-4 h. At the end of the reaction period, the residue was poured into an ice/water mixture and the solution was neutralized with 10%
NaOH. The resulting precipitate was filtered, washed with distilled water, dissolved in boiling ethanol with 0.2 g charcoal, and filtered off. Then distilled water was added to the
filtrate slowly in order to stimulate crystallization. The crude compound was

obtained by filtering and drying the crystalline material. Then, 4-methylphenyl acetic acid (0.5 mmol) and thionylchloride (1.5 ml) were refluxed in benzene (5 ml) at 80°C for 3h. excess thionylchloride was removed *in vacuo*. The 4-methylphenyl acetic acid chloride was dissolved in ether (10 ml) and this solution added during 1 h to a stirred, ice-cold mixture of 5-amino-2-[4-tert-butylphenyl]benzoxazole (0.5 mmol), sodiumbicarbonate (0.5 mmol), diethylether (10 ml) and water (10 ml). The mixture was kept stirred overnight at room temperature and filtered. The precipitate was washed with water, 2M HCl and water, respectively and finally with ether to give compound. The product was re-crystallized from ethanol-water as needles which are dried *in vacuo*.

3. Computational details

The molecular geometry optimization, polarizabilities and natural bond orbital analysis for the title compound are calculated by density functional using B3LYP/6-311++G(d,p) [5] level of theory using Gaussian09 software [6]. The calculated caled wave numbers are scaled by using scaling factor as reported in literature [7]. With the help of potential energy distribution analysis [8] and Gaussview program [9] the vibrational assignments were carried out.

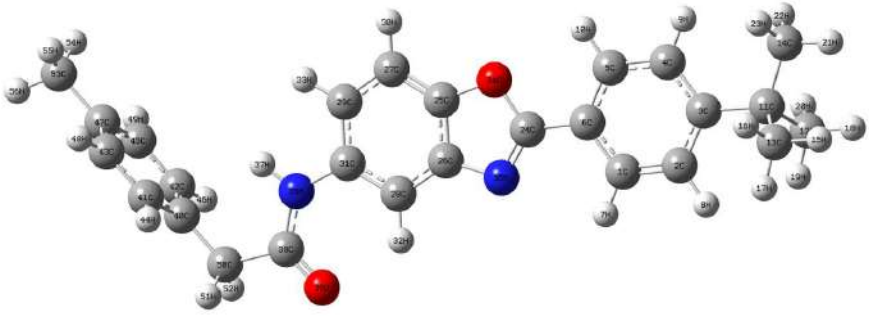
Jaguar 9.0 program [10], as implemented in Schrödinger Materials Science Suite 2015-4, was also used for the DFT calculations of ALIE, Fukui functions and BDEs with B3LYP exchange-correlation functional [11], together with 6-311++G(d,p), 6-31++G(d,p) and 6-311G(d,p) basis sets, respectively. For MD simulations Desmond program [12], also as implemented in Schrödinger Materials Science Suite 2015-4, was used with OPLS 2005 force field [13] within NPT ensemble class. Simulation time was set to 10 ns, while the whole system was modeled by placing one MPATB molecule in the cubic box with ~3000 water molecule. Other parameters include cut off radius set to 12 Å, temperature to 300 K and pressure to 1.0325 bar. Solvent was treated within simple point charge (SPC) model [14]. Noncovalent interactions were determined by using the method of Johnson [15], as implemented in Jaguar 9.0 program. In all cases when Jaguar and Desmond were used, input and output files were manipulated by Maestro graphical user interface application for Schrödinger Materials Science Suite 2015-4.

4. Results and discussion

In the following discussions, the rings, C_{41}-C_{43}-C_{47}-C_{45}-C_{42}-C_{40}, C_{29}-C_{27}-C_{25}-C_{26}-C_{28}-C_{31}, N_{35}-C_{24}-O_{34}-C_{25}-C_{26} and C_1-C_2-C_3-C_4-C_5-C_6 are designated as PhI, PhII, PhIII and PhIV, respectively.

4.1 IR and Raman spectra

According to literature [16], the NH vibrations are expected in the following regions: stretching mode: 3500-3300 cm^{-1}; deformation modes: around 1500, 1250 and 750-600 cm^{-1}. For the title compound, the NH stretching modes are assigned at 3440 cm^{-1} in the IR spectrum, 3449 cm^{-1} in the Raman spectrum and at 3453 cm^{-1} theoretically and the NH deformations are assigned at 1507, 1263, 646 cm^{-1} theoretically and experimentally bands are observed at 1498, 644 cm^{-1} in the Raman spectrum and 644 cm^{-1} in the IR spectrum. The NH stretching mode



has a PED of 100% with IR intensity 108.48 and Raman activity 440.07. The PED of the NH deformation modes are in between 40 and 50%. For the mode at 646 cm^{-1}, the IR intensity and Raman activity are very low, less than 10. The reported values of NH modes are at 3462 cm^{-1} in the IR spectrum, 3450 cm^{-1} in the Raman spectrum, 3400 cm^{-1} theoretically (stretching modes), 1508, 1219, 655 cm^{-1} (DFT) (deformation modes) [17] and 1587, 1250, 650 cm^{-1} (IR), 1580, 1227, 652 cm^{-1} (DFT) (deformation modes) [18]. The C=N stretching mode of the title compound is assigned at 1521 cm^{-1} in the IR spectrum, 1519 cm^{-1} in the Raman spectrum and at 1522 cm^{-1} theoretically. The C-N stretching modes of the title compound are observed at 1192, 1115 cm^{-1} in the IR spectrum, 1242, 1189 cm^{-1} in the Raman spectrum and at 1244, 1189, 1118 cm^{-1} theoretically which are expected in the range 1300-1100 cm^{-1}. All the CN stretching modes have PEDs from 36 to 42% and for the modes, 1522, 1244 cm^{-1} the Raman activity is very high and bands are seen the Raman spectrum. In the present case, the C=O stretching mode is observed at 1677 cm^{-1} in the IR spectrum, 1670 cm^{-1} in the Raman spectrum, 1674 cm^{-1} theoretically while the C-O stretching modes are assigned at 905 cm^{-1} in the Raman spectrum and at 1174, 903 cm^{-1} theoretically. For the modes, 1674 and 903 cm^{-1} the Raman activities are high, 64.43 and 181.17 and experimentally bands are observed in the Raman spectrum and the PEDs are 74 and 39%. Also the mode at 1674 cm^{-1} has a high IR intensity of 334.39 and a PED of 74%. According to literature, the C=O stretching modes are expected in the region 1850-1550 cm^{-1} [19] and the C-O-C stretching modes are in the region 1200-900 cm^{-1} [20].

The methyl stretching modes of the title compound are observed at 2960, 2903 cm^{-1} in the IR spectrum and at 2983, 2955, 2914 cm^{-1} in the Raman spectrum as expected [16]. The bending modes of the methyl groups are observed at 1427, 1361, 1342, 1018, 984 cm^{-1} in the IR spectrum and at 1461, 1433, 1359, 1338, 1002, 969, 905 cm^{-1} in the Raman spectrum. The DFT calculations give these modes in the ranges 2984- 2904 cm^{-1} (stretching) and 1463-901 cm^{-1} (deformation modes) [16]. The CH_2 modes of the title compound are observed at 1410, 1149 cm^{-1} in the IR spectrum, 1276, 1156 cm^{-1} in the Raman spectrum experimentally and the PED analysis gives these modes at 2968, 2933 cm^{-1} (stretching) and 1408, 1276, 1159, 893 cm^{-1} (deformation modes).

The CC_3 stretching modes are expected in the ranges 1295-1175 cm^{-1} and 890-710 cm^{-1} [21] and in the present case, these modes are assigned at 1235, 809 cm^{-1} in the IR spectrum, 812 cm^{-1} in the Raman spectrum and at 1234, 1176, 811 cm^{-1} theoretically with PEDs 47, 48 and 52%. For the mode 1234 cm^{-1} the IR intensity is 40.31 and for the other two modes the IR intensity is less than 10.00. The deformation modes of the CC_3 group are expected in the regions, 435± 85, 335 ± 80 and 300 ± 80 cm^{-1} (total five modes, two asymmetric, one symmetric and two rocking) and for the title compound, these deformation modes are assigned at 446, 384, 374, 342, 284 cm^{-1} theoretically with PEDs, 62, 35, 38, 43, 33% and the IR intensity and Raman activity values are less than 10% and the reported values are 496, 326, 313, 290, 219 cm^{-1} theoretically [22].

The phenyl CH stretching modes are observed at 3020 cm^{-1} in the Raman spectrum for PhI, 3078 cm^{-1} in the IR spectrum, 3121 cm^{-1} in the Raman spectrum for PhII and 3052 cm^{-1} in the IR spectrum, 3085, 3053 cm^{-1} in the Raman spectrum for PhIV [21]. Theoretically these CH stretching modes are assigned in the ranges, 3049-3031 cm^{-1} for PhI, 3124-3041 cm^{-1} for PhII and 3083-3052 cm^{-1} for PhIV as expected [21]. The phenyl ring stretching modes are assigned in the ranges 1589-1296 cm^{-1} for PhI, 1595-1320 cm^{-1} for PhII and 1586-1280 cm^{-1} for PhIV while experimentally bands are observed at 1578, 1545, 1521, 1279 cm^{-1} in the IR spectrum and at 1610, 1580, 1551, 1519, 1397, 1322 cm^{-1} in the Raman spectrum [21].

Trisubstituted phenyl rings have three frequency intervals for the ring breathing mode: 500-660, 1050-1100 and 600-750 cm^{-1} for light substituent, heavy substituent and mixed substituent according to literature [23]. For the title compound, the ring breathing mode of tri-substituted benzene ring is assigned at 1100 cm^{-1} theoretically. with a PED 04 44% and the IR intensity and Raman activity values are low. The ring breathing mode of para-substituted phenyl

rings with entirely different substituent are expected in the range 780-880 cm^{-1} according to literature [23] and in the present case, this is confirmed by the bands at 824 cm^{-1} and 783 cm^{-1} by PED analysis with PEDs 42 and 48%. For the mode at 783 cm^{-1} the IR intensity is less than 10%.

The in-plane CH bending modes of the phenyl rings are assigned as 1018 cm^{-1} (IR), 1277, 1188, 1162, 1020 cm^{-1} (DFT) for PhI, 1106 cm^{-1} (Raman), 1236, 1103, 1094 cm^{-1} (DFT) for PhII and 1002 cm^{-1} (Raman), 1293, 1174, 1161, 1000 cm^{-1} (DFT) for PhIV as expected [21]. The out-of-plane CH bending modes of the phenyl rings are assigned at 928 cm^{-1} (IR), 928 cm^{-1} (Raman), 947, 930, 822, 797 cm^{-1} (DFT) for PhI, 875 cm^{-1} (IR), 880 cm^{-1} (Raman), 895, 880, 773 cm^{-1} (DFT) for PhII and at 956 cm^{-1} (IR), 959, 841, 829, 818 cm^{-1} (DFT) for PhIV [21].

4.2 Frontier Molecular analysis

Using frontier molecular orbital analysis characterization of intra molecular charge transfer through conjugated paths can be explained through the donor – acceptor groups [24]. In the present case the HOMO and LUMO energies are -8.136 and -5.272 eV, respectively. The ionization energy $I = -E_{HOMO}$ = 8.136eV and electron affinity $A = -E_{LUMO}$ = 5.272eV and energy gap = 2.864 eV. The global chemical descriptors are given as hardness $\eta = (I-A)/2$, chemical potential $\mu = -(I+A)/2$ and electronegativity index $\omega = \mu^2/2\eta$ [25]. In the present case η, μ and ω are 1.432, -6.704 and 15.693 respectively. In the HOMO-LUMO plot, the HOMO is localized over the acetamido group, benzoxazole, trisubstituted phenyl ring and the phenyl ring attached with the tert-butyl group, while the LUMO is over the tertbutyl phenyl ring, tert-butyl group, benzoxazole ring and trisubstituted phenyl ring. This shows the charge transfer in the molecular system through the conjugated paths.

4.3 Molecular Electrostatic Potential Map

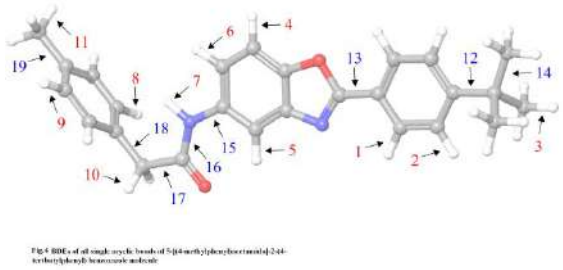
MEP is used for predicting sites in studies of biological recognition and hydrogen bonding interactions and relative reactivity's towards electrophilic attack [26]. The different values of the electrostatic potential are represented by different colors and increases in the order red < orange < yellow < green < blue. Red indicates the strongest repulsion while blue represents the strongest attraction. From the MEP plot it is clear that the carbonyl group and nitrogen atom in the benzoxazole moiety are the strongest repulsion regions and NH group is the strongest attraction centers.

4.4 Nonlinear Optical properties

The polarizability, first and second hyperpolarzabilities of the title compound are respectively, 5.4051×10^{-23}, 1.328×10^{-30} and -603.64×0^{-37} esu. The reported value of a similar derivative is 1.37×10^{-30} esu [27] and in the present case the first hyperpolarizability of the title compound is 10.22 times that of the standard NLO material urea [28]. From the values of hyperpolarizabilities of the title compound, we can conclude that the title compound and its derivatives are good objects for further research in nonlinear optical analysis.

4.5 Reactive and degradation properties based on autoxidation and hydrolysis

Molecular modeling provides important results thanks to which forced degradation experiments can be significantly rationalized and optimized [29]. Namely, there is clear correlation between the mechanism of autoxidation and BDE for hydrogen abstraction. Concretely, if the BDE for hydrogen abstraction is in the proper interval then particular molecule location can be considered as possible starting point for the mechanism of autoxidation. Concerning the proper interval of BDE values it is important to know that all peroxy radicals have similar BDE values (87-92 kcal/mol) which can be considered as independent of the chemical surrounding [30]. This implies that if the BDE for hydrogen abstraction at some location is in this interval it can be considered that autoxidation mechanism is possible. However, the study of Wright et al. [31] have shown that autoxidation mechanism is the most probable for molecule where BDE for hydrogen atom is in the interval between 75 and 85 kcal/mol. Beside calculations of BDE for hydrogen abstraction it is also useful to calculate BDE values for the remaining single acyclic bonds since these indicate the weakest bonds, and thus the locations where degradation could start. BDE values for all single acyclic bonds are presented in Figure.



Bond	BDE [kcal/mol]
1	118.94
2	115.63
3	107.62
4	119.23
5	121.88
6	119.70
7	105.84
8	116.95
9	116.96
10	92.68
11	93.96
12	88.91
13	125.71
14	70.52
15	93.50
16	83.09
17	70.18
18	95.33
19	102.92

Results presented in Figure indicate that it is not likely for MPATB molecule to be prone to autoxidation mechanism since all calculated BDE values for hydrogen abstraction are higher than 92 kcal/mol, although there are two bonds (denoted with 10 and 11) with BDE values close to 92 kca/mol. This further indicates that MPATB molecule is stable in the open air and in the presence of oxygen. Of the remaining single acyclic bonds there are two with the lowest BDE values, around 70 kcal/mol each. Those bonds are denoted with numbers 14 and 17 and they could be the locations where degradation could start.

In order to investigate which atoms of MPATB molecule have pronounced interactions with water molecules, we have calculated RDF as obtained after MD simulations. RDF, g(r), gives the probability of finding a particle in the distance *r* from another particle [32]. According to the calculated RDFs there are five carbon atoms and four non-carbon atoms with significant interactions with water molecules. Carbon atoms with significant interactions with water molecules are C1, C5, C11, C47 and C53. Of these five, three of them, C1, C5 and C53 have lower peak distances (between 3.5 Å and 4 Å), than atoms C11 and C47 (that have peak distances between 4.5 Å and 5.0 Å). On the other side two carbon atoms with the highest g(r) values are carbon atoms C11 and C53. The fact that carbon atoms C47 and C53 have pronounced interactions with water molecules is very important because the BDE value for the abstraction of nearby hydrogen atom is close to 92 kcal/mol. This further indicates that autoxidation mechanism for MPATB molecule is hard to be expected since oxidation and hydrolysis could compete at the mentioned molecule location. Concerning the non-carbon atoms the most important RDF is calculated for hydrogen atom H37, for which the highest g(r) value is somewhat higher than 0.9, while the peak distance is located at below 2 Å. Other atoms with significant interactions with water include hydrogen atom H7, nitrogen atom N35 and oxygen atom O39. Oxygen atom O39 has the highest g(r) value of almost 1.0, while its peak distance is located at around 2.7 Å. The importance of nitrogen atom N35 lies in the fact that this atom is also recognized as important reactive center according to the ALIE results.

4.6 Molecular docking studies

Based on the structure of a compound, PASS (Prediction of Activity Spectra) [33] is an online tool which predicts different types of activities. PASS analysis of the title compound predicts thymidylate synthase activity with probability to be active (Pa) value of 0.754. Thymidylate synthase (TS) is a key enzyme in the synthesis of 2'-deoxythymidine-5'-monophosphate, an essential precursor for DNA biosynthesis. For this reason, this enzyme is a critical target in cancer chemotherapy [34]. Thus we choose thymidylate synthase and used as target for docking study. High resolution crystal structure of thymidylate synthase was downloaded from the RSCB protein data bank website with PDB ID: 3TMS. All molecular docking calculations were performed on Auto Dock-Vina software [35]. Amino acid Trp101 forms two π-sigma, π-π T-shaped, π-alkyl interaction with CH_3 group, phenyl rings respectively. Phe149 forms π-π T-shaped and Tyr164 forms π-stacked interactions with phenyl ring. Ser131 shows H-bond with benzoxazole ring. The docked ligand forms a stable complex with thymidylate synthase) and got a binding affinity value of

-8.5kcal/mol. These studies show that the title compound can be used for developing new anti-cancerous drug.

5. Conclusion

5-[(4-methylphenyl)acetamido]-2-(4-tertbutylphenyl)benzoxazole was synthesized and characterised by experimental and theoretical methods. The structure, vibrational wave numbers, frontier molecular orbital, MEP, NLO and NBO analysis of the title compound is carried out by DFT level using the B3LYP/6-311++G(d,p) basis set. The stability and intermolecular interaction have been interpreted by NBO analysis. The title compound binds at the active site of the substrate by weak non-covalent interactions and the amino acid Trp101 forms two π-sigma, π-π T-shaped, π-alkyl interaction with CH_3 group, phenyl rings respectively; Phe149 forms π-π T-shaped and Tyr164 forms π-stacked interactions with phenyl ring. Ser131 shows H-bond with benzoxazole ring.

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Oral Presentation III

THE MUD CRAB, *SCYLLA SERRATA* - AN INDICATOR OF HEAVY METAL POLLUTION LOAD OF ASHTAMUDI LAKE, KOLLAM, KERALA.

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Introduction

The second largest backwater lake in Kerala Ashtamudi Lake is well known for its houseboats and backwater resorts. Ashtamudi wetland was included in the list of wetlands of international importance. The lake is splendid with a variety of finfishes and shellfishes. Ashtamudi Lake, is prone to several kinds of toxic aquatic pollutants. Among the wide range of pollutants, heavy metals play a prime role in disturbing the delicate balance of the ecosystem. The nature of heavy metal contamination in the water and sediment samples of Ashtamudi lake has been reported by Razeena *et al.,* (2012) Sherly *et al.,* (2015) , Suma *et al.,*(2012) and many others. Many organisms especially fishes are harmed when heavy metals accumulate inside them. Pollutants especially heavy metals can be transferred through the upper classes of the food chain once accumulated by an aquatic organism and paves way for biomagnifications. Fishes especially shellfish do not have any mechanism to prevent bioaccumulation, which makes it as a good indicator to the problem of heavy metal pollution. Studies on the bioaccumulation of various pollutants in different organs of fishes of Ashtamudi lake has been extensively studied by Chinnadurai *et al.,*(2016), Sherly *et al.,*(2015), Razeena *et al.,*(2014) and many others. The mud crabs of genus Scylla are exceptionally important due to their large size, better nutritive value and can export in live condition, hence it receives great demand in the domestic as well as export market. The main aim of the present study was to determine the accumulation status of selected heavy metals on the targeted fish species - *Scylla serrata,* of Ashtamudi Lake, Kollam.

Materials and methods

Three study sites site 1- Kureepuzha, site 2 - Perumon and site 3- West Kallada of Ashtamudi were selected for the present study. *Scylla serrata* of about 3 to 6 cm in carapace width and 220 to 270 gm weight (60 numbers) were collected from each study site for one year (February – 2016 to January 2017). The live specimens after brought to the laboratory were clearly washed with tap water to remove mud. The specimens were further identified using standard identification keys for the conformation of the species (FAO, 1995).

Muscles were dissected out, taken into petridishes and kept in a hot air oven. The temperature was maintained at 600^{o}C for a period of 48 –72 hours. Samples were fine powdered using mortar and pestle after complete drying. 0.5 gm of dried powder of each tissue samples were then digested in open beakers on a hot plate by adding nitric acid and perchloric acid in (4:1) ratio. After that the samples were kept on hot plate and the temperature gradually allowed to rise to 60 ^{o}C continue adding both acids in (4:1) ratio till the sample become colourless. The digested samples were allowed to cool. Then transferred to 25 ml volumetric flasks, and made up to mark with de-ionised water. The digests were store in plastic bottles for the analysis of Cadmium, Chromium, Copper, Lead and Zinc using an atomic absorption spectrophotometer (AAS , Pinaacle 900H) as described by APHA (1998). Metal concentrations were calculated in mg/kg. Data analysis in the tissue samples was performed using statistical package of SPSS 22. Significant differences between heavy metals concentration in various sites, determined using One- Way analysis of variance (ANOVA) followed by Fisher's LSD (Least significantly difference) post hoc test . The level of significance was $p < 0.01$.

Results and Discussion

The mean values of Cadmium at site 1 and 2 were 1.104 mg/kg and 1.5 mg/kg respectively. The maximum permissible limit of Cadmium in fresh water crabs according to FAO was 0.5 mg/kg. The samples collected from site 1 and 2 exceeds this limit with respect to their mean values. In case of site 3 the values were not detected .For Chromium, the allowable limit is 0.2 mg/kg. In the present study, the Site 1showed a mean value of 1.60 mg/kg and site 2 with a mean value of 1.0 mg/kg, which was also found exceeded the FAO limit; whereas for site 3 the values were not detected. The maximum permissible limit of Copper in fresh water crabs according to FAO was 30 mg/kg and the samples of the three study sites are safe with respect to this limit. The respective mean values of Copper for site 1, 2 and 3 were 6.85 mg/kg, 5.3 mg/kg, and 2.7 mg/kg. The maximum allowable limit for Lead is 0.5 mg/kg whereas the present investigation showed a mean value of 8.14 mg/kg at site1 and 2.8 mg/kg at site 2. The permissible limit for Zinc is 40 mg/kg and all the study sites are safe concerning Zinc. The values for site 1, 2 and 3 were 15.50 mg/kg, 13.0 mg/kg, and 8.2 mg/kg respectively.

The accumulation of heavy metals in the muscle samples of *Scylla serrata* of site 1 were in the decreasing order of Zn > Cu > Pd> Cr >Cd. The decreasing order of heavy metal accumulation in site 2 samples was Zn > Cu > Pd > Cd > Cr . With respect to the Site 3 samples, the most abundant element was Zinc followed by Copper, and all other three heavy metals - Cadmium, Chromium and Lead was not detected in muscle samples of *Scylla serrata* . The decreasing order of heavy metal accumulation with respect to sites is site1 < site2< site3. Comparison of the elemental analysis in the muscle samples of *Scylla serrata* of the three study sites with reference to FAO and their respective inferences was depicted in table 1. The heavy metal analysis in the muscle samples of *Scylla serrata* of three study sites with respect to their mean values are shown in figure.

The results of the One way analysis of variance (ANOVA) showed that selected heavy metals such as Cadmium (F = 17.820), Chromium (F = 130.748), Copper (F = 119.606), Lead (F = 58.955) and Zinc (F = 52.526) were found to be different in their values with respect to the sites and showed significance at 1% level (p< 0.01). The results of the Fisher's LSD (Least significant difference) Post hoc multiple comparisons further reveals that site 1 and 2 significantly differ from site 3 with respect to the accumulation of heavy metal Cadmium. With respect to heavy metals Copper and Lead , site 1 significantly differ with site 2 and site3; whereas for the heavy metals Chromium and Zinc, all the three sites were found to be significantly different among each other (Table 2)

Conclusion

Above mentioned results further reveals that the samples of *Scylla serrata* collected from site 1- Kureepuzha are more polluted with heavy metal contamination when compared with other sites. The decreasing order of heavy metal accumulation with respect to sites is site1 < site2< site3. The analysis of the statistical results confirms the pollution status of the Lake.The study further reveals that the bioaccumulation levels on *Scylla serrata* with respect to Cadmium, Chromium and Lead at site 1 and 2 are significantly higher. Hence, this study is very relevant in relation to the pollution status of the Lake as a whole.

Table 1: Comparing the elemental analysis in the muscle samples of *Scylla serrata* with international standard

Heavy metals	FAO limits (mg/kg)	Present study – mean values (mg/kg)			Inference
		Site1	Site 2	Site3	
Cadmium	0.5	1.10	1.5	Not detected	Site 1 and 2 above permissible limit
Chromium	0.2	1.60	1.0	Not detected	Site 1 and 2 above permissible limit

Copper	**30**	**6.85**	**5.3**	**2.7**	**All sites below permissible limit**
Lead	**0.5**	**8.14**	**2.8**	**Not detected**	**Site 1 and 2 above permissible limit**
Zinc	**40**	**15.50**	**13**	**8.2**	**All sites below permissible limit**

Figure showing the heavy metal accumulation in the muscles of *Scylla serata* of three study sites.

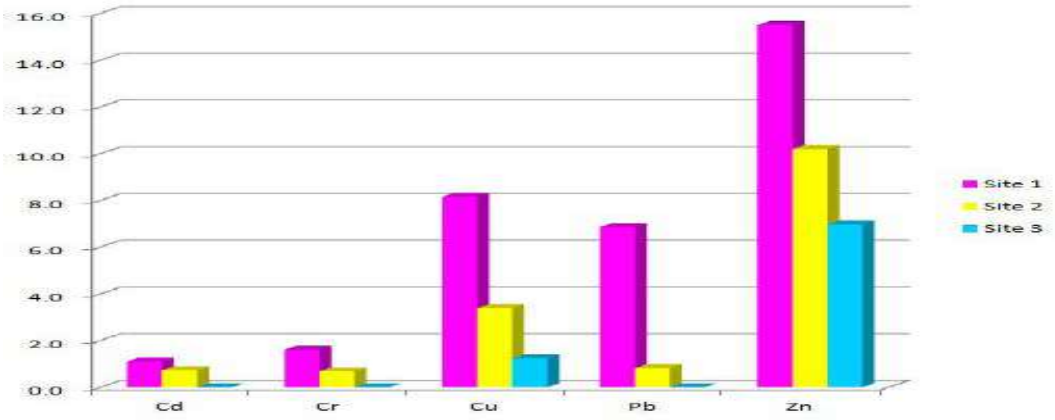

Table 2: Analysis of variance (One-Way ANOVA) of heavy metals of the muscles of *Scylla serata* comparing three sites of the Ashtamudi Lake.

	Study sites				
Heavy metals	**Site 1 (Mean ± SD)**	**Site 2 (Mean ± SD)**	**Site 3 (Mean ± SD)**	**F value comparing study sites**	**P Value**
Cadmium	**1.104 ± 0.608** [a]	**0.725 ± 0.515** [a]	**.00 ± .00** [b]	**17.820**	**< 0.001***
Chromium	**1.604 ± 0.386** [a]	**0.675 ± 0 .171** [b]	**.00 ± .00** [c]	**130.748**	**< 0.001***
Copper	**8.141 ± 1.471** [a]	**3.383 ± 1.105** [b]	**1.225 ± 0.616** [b]	**119.606**	**< 0.001***
Lead	**6.850 ± 2.730** [a]	**0.816 ± 1.045** [b]	**.00 ± .00** [b]	**58.955**	**< 0.001***
Zinc	**15.50 ± 2.489** [a]	**10.191 ± 2.461** [b]	**6.958 ± 0.701** [c]	**52.526**	**< 0.001***

* = p < 0 .01, The mean difference is significant at 1% level; SD – Standard deviation

[a, b, c] - Means within rows with differing subscripts are significantly different using Fisher's LSD post hoc test.

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Oral Presentation IV

Adsorptive removal of methyl red from aqueous solution using kaolinite: Kinetics and Isotherm studies

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ABSTRACT

The contamination of surface water by anionic dyes released from the effluent of textile industries is a major environmental concern. Removal of methyl red (MR) by kaolinite clay was studied in a batch system. The effect of pH, contact time and temperature were optimized for the maximum removal efficiency. The optimal removal was observed at pH 4.0, the percentage of adsorption was found to be 84.5%. Adsorption isotherm were modelled by using Langmuir and Freundlich model and found that Langmuir model fitted well with the isotherm data. Thermodynamics studies also indicates that adsorption followed exothermic and spontaneous one. Kinetics studies also revealed that Pseudo second order fitted well than Pseudo first order by using the equilibrium data.

Keywords: Adsorption, methyl red, kaolinite, isotherm

1. INTRODUCTION

Dyes which are discharged from several industries cause not only aquatic pollution but also their limit (<1mg/L) cause severe carcinogenic and mutagenic effects on humans [1,2]. The removal of these carcinogenic dyes is very difficult due to their non-biodegradability. Methyl red (MR) is a mono-azo anionic dye ($C_{15}H_{15}N_3O_2$) soluble in water and widely used in textile and other industries causing harmful effects such as skin irritation, and digestive tract irritation, if inhaled [3]. Numerous methods are available for the treatment of dyes before discharge into the water bodies, among them adsorption has attained world wide attention due to low cost and simple operation. Recently the usage of clay minerals in adsorption as an adsorbent due to high cation exchange capacity is well established. So we used kaolinite as an adsorbent for Methyl red (MR) removal from aqueous phase. Kaolinite is a 1:1alumino silicate crystalline clay mineral with stacked pairs of tetrahedral silica sheets and octahedral alumina sheets. So the aim of the present study is to treat MR with kaolinite to enhance adsorption of MR from aqueous system.

2. EXPERIMENTAL PROCEDURE

2.1 Materials and Methods

The adsorbent material used in this study was kaolinite (English Indian Clay Ltd) from Kochuveli. Methyl Red (Merck) was directly purchased and used. Other reagents such as HCl and NaOH were also obtained from Merck and were used as received. De-ionized water is used in the entire study for methyl red (MR) solution preparation.

2.2. Batch Adsorption Experiments

The stock solution of MR was prepared by dissolving accurately weighed amount of MR in milli Q water (1000 mg L^{-1}). Batch adsorption experiments were conducted by agitating 0.1g kaolinite clay with 50 mL of MR of different concentrations prepared using double distilled water from stock solution. The pH of the solution was adjusted using different concentrations of HCl and NaOH solutions. The solution was then subjected to constant shaking in a temperature controlled digital water bath shaker. At predominant time intervals, the supernatant solution was pipetted by using 1mL micropipette and analysed for residual MR concentration. The amount of MR

adsorbed on the surface was calculated as,

$$q = \frac{\left[\left(C_0 - C_A\right)\right]V}{m} \qquad (1)$$

where q is the amount of MR adsorbed onto unit amount of the adsorbent in mg/g; C_o and C_A are the initial and equilibrium MR solution concentration (mg/L) respectively; V is the volume of the aqueous phase (mL); and m is the weight of the adsorbent (g).The results obtained were modelled using Lagergren Pseudo first order and Pseudo second order. The pH, adsorbent dose, equilibrium time is kept at optimum value. Langmuir and Freundlich isotherm models were also interpreted by the isotherm data.

3. RESULTS AND DISCUSSIONS

3.1 Effect of pH on initial MR adsorption

The influence of pH on the adsorption of MR onto Kaolinite was investigated at different pH ranging from 2.0 to 8.0 for an initial concentration of 15 mg/L. The percentage of dye adsorption on the adsorbent increased with increase in pH and reaches a maximum at pH 4.0 and then decreases. For an initial concentration of 15 mg/L, maximum adsorption 84.5% (4.20 mg/g) for kaolinite clay was obtained.

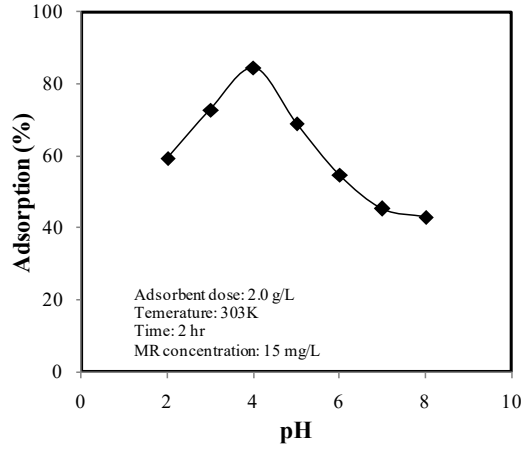

Fig.1. Effect of pH on the adsorption of MR onto kaolinite clay

In general, especially in the acidic pH region, the adsorbent surface is normally positively charged which in turn favour the adsorption of negatively charged dye anions through electrostatic force of attraction. The experimental results also showed that the optimum pH for the adsorption of MR on kaolinite was 4.0. But in the case of higher pH, the surface is enriched with negative charge and subsequently may be repelled by the negatively charged MR molecules. According to the concept of surface complex formation, the adsorption reaction between the MR molecule and solid surface can be best described by the following equation (2) and (3)

$$S^+ + MR^- \leftrightarrow S - MR \qquad (2)$$

$$S - OH + MR^- \xrightarrow{H^+, -H_2O} S - MR \qquad (3)$$

Where, S^+ and SOH are polar sites on the surface of the adsorbents.

3.2 Adsorption kinetics studies

In order to establish the kinetics of MR adsorption, Lagergren first order and second order kinetic models were studied.

The pseudo-first-order kinetic model equation is as follows,

$$\log\left(q_e - q_t\right) = \log q_e - \frac{k_1 t}{2.303} \qquad (4)$$

The pseudo-second-order kinetic model equation is as follows,

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (5)$$

Where q_e and q_t are the amount of MR adsorbed at equilibrium and at time t respectively in mg/g, k_2 is the equilibrium rate constant of pseudo-second-order adsorption (g/mg/min). Values of $k_2$and qe were calculated from the plot of t/q_t against t. The results from the graph is shown in Table 1

3.3 Adsorption Isotherm studies

The equilibrium studies were performed to evaluate the adsorption capacity of the adsorbents using different initial concentrations of MR from 15 to 100 mg/L.

The Langmuir isotherm equation for adsorption is represented as

$$\frac{C_e}{q_e} = \frac{1}{bQ^0} + \frac{C_e}{Q^0} \qquad (6)$$

The Freundlich isotherm equation for adsorption is represented as

$$\log q_e = \log K_F + \frac{1}{n}\log C_e \qquad (7)$$

Table 1: Kinetic parameters for adsorption of MR on kaolinite clay

Models	Parameters	Initial concentrations (mg/L)			
		25	50	75	100
Pseudo first order	K_1 (min^{-1})	0.0023	0.0138	0.0023	0.0138
	q_e (mg/g)	3.47	8.93	4.40	10.30
	R^2	0.271	0.968	0.058	0.864
Pseudo second order	K_2 (g/mg/min)	0.0094	0.0025	21.2766	0.0019
	q_e (mg/g)	6.45	18.51	0.016	28.57
	R^2	0.9300	0.9930	0.9760	0.9930

Table 2: Langmuir and Freundlich constants for the adsorption of MR onto clay.

Adsorbent	Temperature (°C)	Langmuir isotherm			Freundlich isotherm		
		Q^o (mg/g)	b (L/mg)	R^2	K_F (mg/g)	n (L/mg)	R^2
Kaolinite	20	81.30	0.02	0.924	2.74	1.65	0.991
	30	75.18	0.03	0.962	4.69	4.97	0.644
	40	46.86	0.01	0.926	1.07	2.08	0.865
	50	34.60	0.01	0.978	1.15	2.43	0.536

where q_e is the amount of MR adsorbed per unit weight of the adsorbent in mg/g, C_e is the equilibrium MR solution concentration in mg L^{-1}.Q^0 and b is obtained by plotting C_e/q_e versus C_e.Q^0 and b are Langmuir constants related to the adsorption capacity in mg/g and intensity of adsorption in L/mg respectively (Fig.2). K_F and 1/n are Freundlich constants related to adsorption capacity in mg/g and energy of adsorption in L/mg respectively. By plotting log q_e versus log C_e, the values of K_F and 1/n were calculated from graph. The results from the graph is shown in table 2.

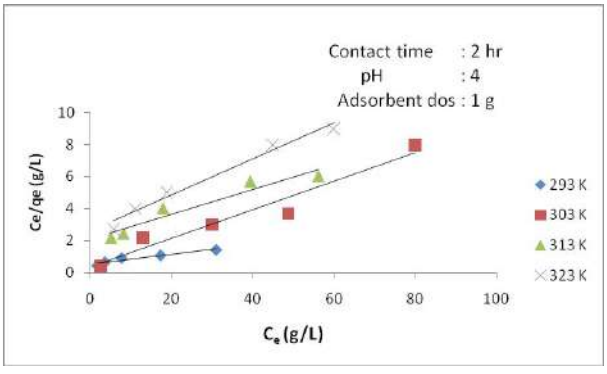

Fig.2: Langmuir plot for the adsorption of MR on clay

3.4 Thermodynamics parameters

Inorder to study the spontaneity and feasibility for the adsorption of MR onto kaolinite clay, thermodynamics studies were conducted using the parameters standard Gibb's free energy (ΔG^0), standard enthalpy change (ΔH^0), standard entropy change (ΔS^0) under optimized conditions: Temperatures (20,30, 40,50 ^{0}C), adsorbent dosage: 2.0 g/L, contact time: 2 hr, pH: 4.0.

Thermodynamic parameters were calculated by using the following equations

$$\Delta G^0 = - RT \ln k_0 \qquad (8)$$

$$\ln k_0 = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \qquad (9)$$

were k_0 is the unit less equilibrium constant, T is the absolute temperature of the solution in Kelvin and R is the Universal gas constant with a value of 8.314 J/mol/K. ΔH^0 and ΔS^0 were calculated from the slope and intercept of Van's Hoff plots of $\ln K_0$ versus 1/T.

The negative value of ΔG^0 (-3.52, -4.28, -4.51, -2.53 KJ/mol) for (different temperatures

(20, 30, 40, 50 ^{0}C) implies the spontaneity and feasibility for the adsorption of MR onto kaolinite.The positive value of ΔH^0 (-11.12 kJ/mol) indicates the adsorption is an exothermic one. The positive value of ΔS^0 (24.04 kJ /mol/K) indicates the increased affinity of MR onto kaolinite [4].

4. Conclusions

The results showed that kaolinite is an efficient adsorbent for the rapid removal of MR from aqueous solution. A maximum adsorption capacity of about 81.30 mg/g was obtained for a temperature 20^{0}C. The experimental data were best fitted with Pseudo second order and Langmuir isotherm model. Thermodynamic studies also revealed exothermic and spontaneous nature of adsorption.

Acknowledgements

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Oral Presentation V

DFT and molecular dynamics investigation of 1-(3-Chloro-4-fluorophenyl)-3-[3-(trifluoromethyl)phenyl]thiourea (ANF-2)

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Abstract:
In the present study, the HOMO and LUMO analysis is used to determine the charge transfer within the molecule. The first hyperpolarizability of the title compound is 48.09 times that of the standard NLO material urea. The maximum negative region is localized over the C=S group and 1,3-dsubstituted phenyl ring and the maximum positive region is localized on NH groups indicating a possible site for nucleophilic attack. Average local ionization energies have been mapped to the electron density surface in order to detect molecule sites where electrons are least tightly bound. Other possible reactive centers of the title molecule have been detected by calculation of Fukui functions. In order to investigate the possibility for autoxidation and hydrolysis of investigated molecule, we have calculated bond dissociation energies and radial distribution functions. Charge hopping properties of electrons and holes have been assessed using the Marcus semi-empiric approach and the results were compared with urea and thiourea molecules. The docked ligand forms a stable complex with prostaglandin E synthase and has a binding affinity value of -6.5kcal/mol and the title compound can be a lead compound for developing new analgesic drug.

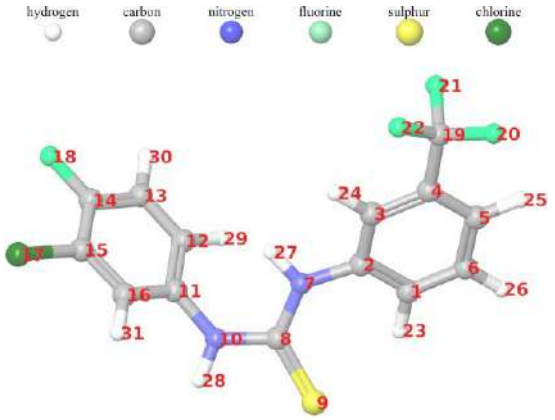
Keywords: DFT; Thiourea; ALIE; BDE; RDF; Molecular docking.

1. Introduction

Biological activities of 1,3-disubstituted thiourea derivatives have been the subject of various studies. Within this group, the highest antibiofilm [1], antiviral [2] and tuber-culostatic [3] potency was observed for thioureas containing electron-withdrawing (*e.g.* halogen) functionalities at the *ortho-*/*para-* position of the aromatic ring [4]. Pharmaceutical molecules based on the biologically active molecules are constantly polluting all types of waters and present great danger for the aquatic organisms [5]. Although various methods for purification of water are available, degradation of mentioned type of compounds is still difficult and inefficient employing conventional approaches. Scientific community is for some time committed to development of improved methods for their degradation and advanced oxidation processes are seen as both economical and efficient alternative [6]. Understanding in details reactive properties of biologically active molecules is prerequisite for the improvement of methods for their degradation. From the experimentation aspect this can be tedious task so DFT calculations and molecular dynamics (MD) simulations are frequently employed to initially assess the reactive and degradation properties. Namely, by calculation of bond dissociation energies (BDE) can show molecule sites prone to autoxidation and indicate bonds that are the weakest, while MD simulations indicate the influence of solvent to the investigated molecule. The calculation of these quantities has been conducted in this work as well.

2. Computational Details

Firstly, conformational analysis has been conducted with the MacroModel program as implemented in Schrödinger Materials Science Suite 2015-4 [7]. By its default settings total of 96 structures have been detected and all of these structures have been optimized with Jaguar 9.0 [8] program, also as implemented in Schrödinger Materials Science Suite 2015-4 at B3LYP/6-31G(d) level of theory in order to refine geometries and chose five structures with the lowest energies. Five structures with lowest energies have been further optimized at B3LYP/6-31G(d,p) level of theory with finer grid density and increased integral accuracy.



Finally, the lowest energy structure has been chosen for detailed investigation of reactive properties. Jaguar 9.0 [7] program has been used for DFT calculations of average local ionization energy (ALIE) surfaces, Fukui functions and BDE. For these purposes a B3LYP exchange-correlation functional [9] has been used with 6-311++G(d,p) basis set for ALIE surface, 6-31+G(d) for Fukui functions and 6-311G(d,p) for BDE. Radial distribution functions (RDF) have been calculated after MD simulations performed with Desmond [10] program, also as implemented in Schrödinger Materials Science Suite 2015-4. For MD simulations OPLS 2005 force field [11] and NPT ensemble class have been used. Simulation time was 10 ns, while cut-off radius was 12 Å. System was modeled by placing one ANF-2 molecule in the cubic box with ~3000 water molecules.Solvent was treated with simple point charge (SPC) model [12]. Intramolecular noncovalent interactions have been investigated using the method of Johnson et al. [13]. To simulate the amorphous phase and in such way to obtain possible pair configurations 32 molecules of ANF-2 were treated with MD simulations with OPLS 2005 force field in the cubic box with simulation time of 10 ns. Same methodology was applied for the obtaining of amorphous phase for urea and thiourea molecules, but with 256 molecules placed in cubic box, due to the fact that these molecules are much smaller. Again, cut-off radius of 12 Å was used.

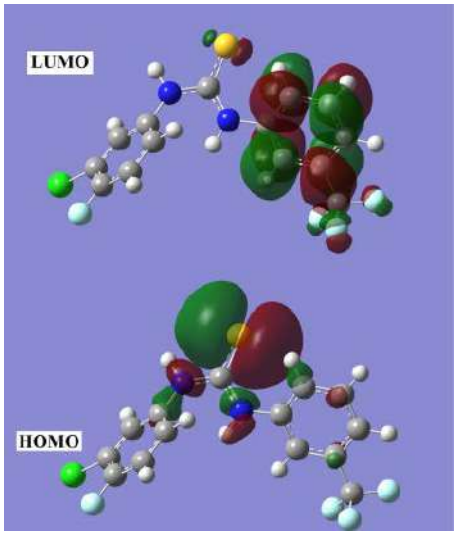
3. Results and discussion

3.1 Nonlinear optical properties

Nonlinear optical effects is due to the interaction of electromagnetic fields in various media to produce new fields altered in frequency, phase, amplitude or other propagation characteristics [14]. For the title compound, the polarizability, first hyperpolarizability and second hyperpolarizability are respectively, 3.3945 × 10^{-23}, 6.2517 × 10^{-30} and -24.627 × 10^{-37} esu and these values of the investigated molecule clearly reveal that they have nonlinear optical behavior with non-zero values. The reported value of the first hyperpolarizability of phenyl thiourea derivatives is 1.86 × 10^{-30} esu [15] and the first hyperpolarizability of the title compound is 48.09 times that of the standard NLO material urea [16].

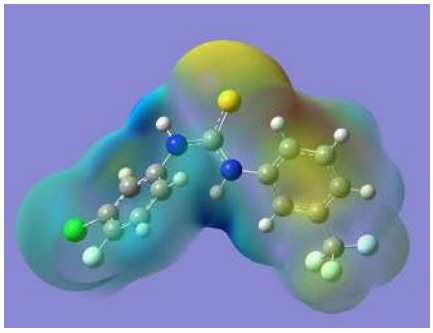
3.2 Frontier molecular orbital analysis

HOMO and LUMO energy values are very important parameters for quantum chemistry and HOMO is the outermost orbital, tends to give electrons and act as an electron donor while the LUMO accepts electrons [17]. According the B3LYP/6-31G(d,p) method, the HOMO and LUMO energy values are -7.325 and -4.551eV.



The ionization energy and electron affinity can be expressed as: I= $-E_{HOMO}$ = 7.325, A = $-E_{LUMO}$ = 4.551eV [18]. The hardness η and chemical potential μ are given the following relations η = (I-A)/2 and μ = - (I+A)/2, where I and A are the first ionization potential and electron affinity of the chemical species [19]. For the title compound, HOMO-LUMO energy gap = 2.774eV, Ionization potential, I = 7.325eV, Electron affinity A= 4.551eV, global hardness η = 1.387eV, chemical potential μ = -5.938eV, global electrophilicity index = $\mu^2/2\eta$ = 12.711eV.

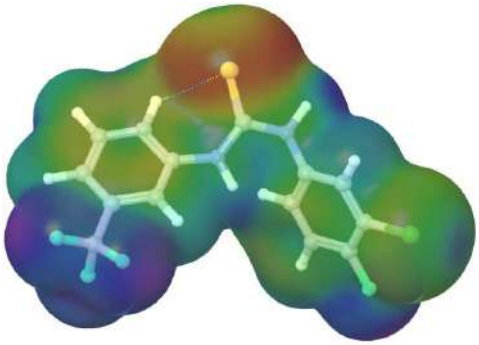
3.3 Molecular Electrostatic Potential



MEP map illustrates the charge distribution of molecules three dimensionally and it gives the reactive sites of the molecule. This map gives the visualization of variably charged regions of a molecule and the knowledge of the charge distribution is used to determine how the molecules interact with one another and physiochemical property relationships [20]. In the MEP map the different values of the electrostatic potential at the surface are represented by different colors with potential values increases in the order red < orange < yellow < green < blue. The red, orange and yellow regions of the MEP are negative potential regions related to electrophilic reactivity. The maximum negative region is localized over the C=S group and 1,3-dsubstituted phenyl ring and the maximum positive region is localized on NH groups indicating a possible site for nucleophilic attack. These sites give information about the region from which the compound can has intermolecular interactions with most reactive sites for both electrophilic and nucleophilic attack.

3.4 ALIE surfaces and Fukui functions

ALIE values have been mapped to the electron density surface and the representative surface of ANF-2 molecule has been presented in Figure. This useful quantum molecular descriptor was introduced by Sjoberg et al. [21] and determines the molecule locations where



electrons are most easily removed, i.e. locations that are possibly prone to electrophilic attacks. ALIE is defined as sum of orbital energies weighted by the orbital densities according to the following equation:

$$I(r) = \sum_i \frac{\rho_i(\vec{r})|\varepsilon_i|}{\rho(\vec{r})}, \qquad (1)$$

where $\rho_i(\vec{r})$ represents the electronic density of the *i*-th molecular orbital at the point $\vec{r}$, ε_i represents the orbital energy and $\rho(\vec{r})$ is the total electronic density function.

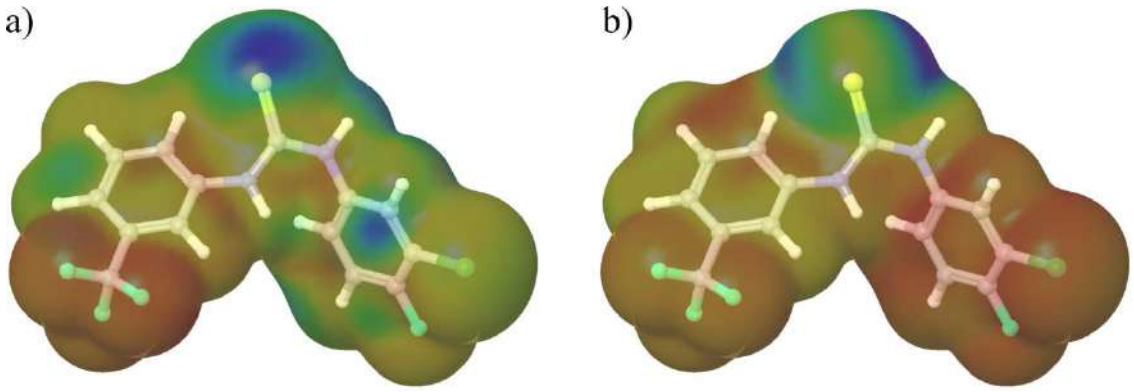
Results in Figure indicate that one location is significantly prone to electrophilic attacks. That is sulfur atom S9 and for the removal of electrons in its near vicinity 165 kcal/mol of energy is necessary. On the other side the electrons are the most tightly bound in the near vicinity of hydrogen atoms H28 and H31. These locations could have significant interactions with water molecules, which is going to be confirmed by MD simulations and calculated RDFs. In Figure one detected intra-molecular non-covalent interaction has been illustrated, between sulfur atom S9 and hydrogen atom H23.

To further determine which locations of the title molecule could be possible reactive centers we will refer to Fukui functions. This descriptor shows how electron density throughout the molecule changes with addition or removal of charge, allowing one to locate areas prone to electrophilic or nucleophilic attacks. Finite difference approximation is used in Jaguar program for calculation of the Fukui functions according to the following equations:

$$f^+ = \frac{\left(\rho^{N+\delta}(r) - \rho^N(r)\right)}{\delta}, \qquad (2)$$

$$f^- = \frac{\left(\rho^{N-\delta}(r) - \rho^N(r)\right)}{\delta}, \qquad (3)$$

where *N* denotes the number of electrons in the reference state of the molecule and δ represents the fraction of electron, which is set to be 0.01 [22].



Color code in above Figure is as following. Purple color is positive color and in case of f^+ function it indicates molecule areas where electron density increases with the addition of charge. On the other side, red color is negative color and in the case of f^- function it shows molecule areas where electron density decreases with the removal of charge. Results presented in above Figure indicate that concerning Fukui f^+ function there are two locations where electron density increases with the addition of charge and therefore prone to electrophilic attacks. Beside sulfur atom S9, f^+ function recognizes carbon atom C16 as possibly prone to electrophilic attacks. On the other side, f^- function indicates the rest of the molecule possibly prone to nucleophilic attacks, as red color is distributed across the molecule.

3.5 Natural Bond Analysis

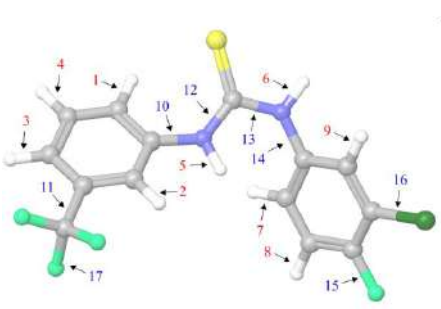
The natural bond orbital (NBO) calculations were performed using NBO 3.1 program [23] to understand various second-order interactions in the molecular system. The second-order perturbation theory analysis of Fock-matrix in NBO basis shows strong intra-molecular hyper conjugative interactions are formed by orbital overlap between n(S), n(N), n(Cl), n(F) and σ*(C-N), π*(C-C), σ*(C-S), σ*(C-F) bond orbital which result in intra-molecular charge transfer causing stabilization of the system.

The various important intra-molecular hyper conjugative interactions are: C_8-S_9 from N_7 of $n_1(N_7)\rightarrow\sigma^*(C_8\text{-}S_9)$ which increases electron density 0.48288e and weakens the respective bonds C_8-S_9 leading to stabilization of 68.90kJ/mol; N_7-C_8 from S_9 of $n_2(S_9)\rightarrow\sigma^*(N_7\text{-}C_8)$ which increases the electron density 0.06207e and weakens the respective bonds N_7-C_8 leading to stabilization of 12.09kJ/mol; C_8-S_9 from N_{10} of $n_1(N_{10})\rightarrow\sigma^*(C_8\text{-}S_9)$ which increases electron density 0.48288e and weakens the respective bonds C_8-S_9 leading to stabilization of 67.42kJ/mol; C_{14}-$C_{15}$from Cl_{17} of $n_3(Cl_{17})\rightarrow\pi^*(C_{14}\text{-}C_{15})$ which increases the electron density 0.4698e and weakens the respective bonds C_{14}-C_{15} leading to stabilization of 13.09kJ/mol; C_{14}-C_{15} from F_{18} of $n_2(F_{18})\rightarrow\pi^*(C_{14}\text{-}C_{15})$ which increases electron density 0.43698e and weakens the respective bonds C_{14}-C_{15} leading to stabilization of 21.60kJ/mol; C_{19}-F_{22} from F_{20} of $n_3(F_{20})\rightarrow\pi^*(C_{19}\text{-}F_{22})$ which increases the electron density 0.10312e and weakens the respective bonds C_{19}-F_{22} leading to stabilization of 12.72kJ/mol; C_{19}-F_{20} from F_{22} of $n_3(F_{22})\rightarrow\sigma^*(C_{19}\text{-}F_{20})$ which increases the electron density 0.06207e and weakens the respective bonds C_{19}-F_{20} leading to stabilization of 12.60kJ/mol.

The natural hybrid orbital with higher energies are $n_2(S_9)$, $n_3(Cl_{17})$, $n_3(F_{18})$, $n_3(F_{20})$, $n_3(F_{21})$ and $n_3(F_{22})$. The energy values of these orbital are respectively, -0.19884, -0.33765, -0.42637, -0.42376, -0.41733 and -0.42439 a.u. The p-characters are nearly 100% and low occupation numbers for these orbital are 1.87411, 1.92492, 1.91319, 1.93051, 1.93139 and 1.93148. The orbital with lower energies, high occupation numbers are: $n_1(S_9)$, $n_1(Cl_{17})$, $n_1(F_{18})$, $n_1(F_{20})$, $n_1(F_{21})$ and $n_1(F_{22})$ with energies, -0.69927, -0.93461, -1.04846, -1.05905, -1.05996, -1.06075 a.u. and p-characters, 17.93, 17.66, 30.30, 29.17, 99.98 and 29.05%) and high occupation numbers, 1.98601, 1.99362, 1.98857, 1.98707, 1.98756, and 1.98711. Thus, a very close to pure p-type lone pair orbital participates in the electron donation to the $n_1(N_7)\rightarrow\sigma^*(C_8\text{-}S_9)$, $n_2(S_9)\rightarrow\sigma^*(N_7\text{-}C_8)$, $n_1(N_{10})\rightarrow\sigma^*(C_8\text{-}S_9)$, and $n_3(Cl_{17})\rightarrow\pi^*(C_{14}\text{-}C_{15})$ interactions in the compound.

3.6 Degradation properties based on autoxidation and hydrolysis

It is important to understand degradation properties of potential drug candidates in order to be able to develop safe pharmaceutical formulations [24]. One solution for this are forced degradation studies which are long and expensive procedures. Luckily, principles of molecular modeling offer possibilities to initially assess the degradation properties by DFT calculations and MD simulations. DFT calculations can be used to calculate BDEs (Figure) which indicate which molecule locations are candidates for the start of autoxidation process. On the other side MD simulations can be used to calculate RDF thanks to which influence of solvent molecules to each atom of investigated compound can be investigated.

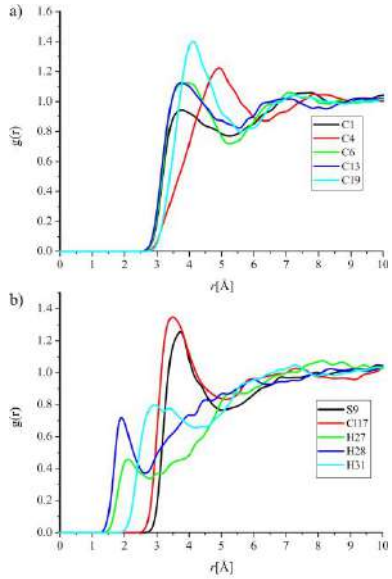


Bond	BDE [kcal/mol]
1	111.90
2	118.90
3	119.50
4	118.10
5	91.79
6	91.96
7	117.14
8	120.06
9	120.02
10	93.37
11	107.01
12	69.65
13	69.42
14	89.46
15	125.99
16	89.94
17	114.00

Formation of radical of organic biologically active molecule occurs with formation of peroxy radical and reaction can proceed only if the formed peroxy radical is able to abstract hydrogen atom from another molecule [25]. On the other side, hydrogen atoms can be abstracted only at certain molecule locations, where BDE values have adequate values. According to the work of Wright et al. [26] for BDE values ranging from 75 to 85 kcal/mol the sensitivity of molecule towards the mechanism of autoxidation is the highest. However, the BDE values ranging from 87 to 92 kcal/mol should also be taken into account since BDE values of all peroxy radicals are in this range and are practically independent of chemical surrounding.

Beside BDE values for hydrogen abstraction it is also useful to know BDE values of the rest of the acyclic bonds because this data can indicate which bonds are the weakest and where degradation could also start. Obtained results concerning the BDE values indicate that investigated ANF-2 molecule could be sensitive towards the mechanism of autoxidation as the lowest BDE value for hydrogen abstraction is below 92 kcal/mol (bonds denoted with numbers 5 and 6). This value is close to the upper border value, but on the other side BDE values of the rest of the single acyclic bonds indicate that the weakest bonds are adjacent bonds denoted with numbers 12 and 13. Thus, according to the BDE values, degradation process could start in the near vicinity of nitrogen atoms.

RDF, $g(r)$, is the probability of finding a particle in the distance r from another particle and results concerning the interaction of ANF-2 molecule with water molecules are presented in Figure below.



In Fig.a it can be seen that five carbon atoms have significant interactions with water molecules. Carbon atoms C6 and C13 are very similar in terms of maximal g(r) values and peak distances which have approximate values of 1.1 and 3.5 Å, respectively. Carbon atom C1 is also similar to them, but with lower maximal g(r) value which has the value of around 0.9. Carbon atom C4 has the longest peak distance of almost 5 Å, while its maximal g(r) value is 1.2. In the case of carbon atoms, the highest maximal g(r) value has been calculated for atom C19, around 1.4, while its peak distance is around 4 Å. As expected, much more pronounced interactions with water molecules were calculated for non-carbon atoms. From the aspect of peak distance the most important RDFs are calculated for hydrogen atoms H27

and H28, with values of below 2 Å. On the other side the highest maximal g(r) values have been calculated for sulfur and chlorine atoms, with values of around 1.3, but with higher peak distances, somewhat higher than 3.5 Å.
Hydrogen atoms H27 and H28 are important reactive centers from the aspect of RDFs so it could be expected that at these locations autoxidation mechanism is competing with hydrolysis. Since BDE values are significantly high, it is expected that investigated molecule is stable to the influence of open air and oxygen.

3.7 Charge hopping properties between ANF-2 molecules

Investigated ANF-2 molecule is based on the thiourea molecule. Thiourea molecule is analogues molecule to urea, which is used as standard NLO material and for the comparison of calculated hyperpolarizabilities. Therefore, beside hyperpolarizability, in this work we have calculated also some optoelectronic properties of ANF-2 molecule and compared it with urea and thiourea.We have decided to calculate quantities which principally determine the charge hopping dynamics between molecules. Those quantities are electron and hole reorganization energies (λ_- and λ_+ respectively) and transfer integral (t). According to the Marcus semi-empiric approach these two quantities determine the charge hopping rate (k_{IT}) by the following expression:

$$k_{IT} = \frac{4\pi^2}{h}\frac{1}{\sqrt{4\pi\lambda k_B T}} t^2 \exp\left[\frac{-\lambda}{4k_B T}\right] \quad (4)$$

Determination of the k_{IT} is very useful as it is directly proportional to the mobility of charge carriers and diffusion coefficient. Thus, calculation of k_{IT} allows one to initially assess the charge hopping properties of the investigated molecules. The larger the reorganization energies and lower the charge coupling is, the higher will be the values of k_{IT} . Once the possible pairs of ANF-2 molecules were obtained by MD simulations, calculations of t have been performed for 60 randomly chosen pairs (half of the total number of pairs) and the average values have been taken. Same procedure was conducted for obtaining charge hopping rates for urea and thiourea molecules. Results show that reorganization energy of electrons is the lowest in the case of ANF-2 molecules, thus indicating that charge hopping could be the highest in the case of this molecule. Indeed, calculated k_{IT}^- indicate that the highest value was obtained precisely in the case of ANF-2 molecule – about three times higher than the corresponding values of urea and thiourea molecules. On the other side, reorganization energy of holes for ANF-2 molecule is much higher than corresponding values of urea and thiourea molecules, resulting in one order of magnitude lower k_{IT}^+ , comparing with urea and thiourea. Finally, the difference between k_{IT}^- and k_{IT}^+ is the lowest in the case of precisely ANF-2 molecules. This result is important as difference in mobilities of charge carriers should not be to too high due to the recombination.

3.8 Molecular docking

Based on the structure of a compound, PASS (Prediction of Activity Spectra) [27] is an online tool which predicts different types of activities. PASS analysis of the title compound predicts activities prostaglandin E synthase activity with probability to be active (Pa) value of 0.638. Microsomal prostaglandin E synthase is a membrane-bound terminal enzyme that exhibits the inhibitor of analgesia [28]. High resolution crystal structure of prostaglandin E synthase was downloaded from the RSCB protein data bank website with PDB ID: 4YL3 and all molecular docking calculations were performed on Auto Dock-Vina software [29]. The 3D crystal structure of aryl hydrocarbon receptor was obtained from RCSB Protein Data Bank and the protein was prepared for docking by removing the co-crystallized ligands, waters and co-factors and the Auto Dock Tools (ADT) graphical user interface was used to calculate Kollman charges and polar hydrogen's. The docking protocol predicted the same conformation as was present in the crystal structure with RMSD value well within the reliable range of 2Å [30]. Amongst the docked conformations, one which binds well at the

active site was analyzed for detailed interactions in Discovery Studio Visualizer 4.0 software. The ligand binds at the active site of the substrate by weak non-covalent interactions. Amino acid Val105 forms interactions like π-sigma and π-alkyl with phenyl ring and alkyl with CF_3. Leu104 shows halogen and alkyl interaction with CF_3. Val108 forms alkyl interaction with CF_3 and Leu132 exhibit π-alkyl interaction with phenyl ring. The docked ligand forms a stable complex with prostaglandin E synthase and got a binding affinity value of -6.5kcal/mol. Thus the title compound can be a lead compound for developing new analgesic drug.

4. Conclusion

The nonlinearity of the title molecule is due to the extended π-electron delocalization over the thiourea group and hence the title molecule and its derivatives are good objects for further studies in nonlinear optical studies. Sulfur atom is recognized to be possibly prone to electrophilic attacks by both ALIE surfaces and Fukui f^+ function. Additionally, Fukui f^+ function also recognizes carbon atom C16 as possibly prone to electrophilic attacks. Only one intramolecular noncovalent interaction has been detected for the title molecule. Although there are two BDE values below 92 kcal/mol, for abstraction of hydrogen atoms H27 and H28, it is hardly to expect that this molecule is sensitive towards autoxidation because mentioned two hydrogen atoms have also pronounced interactions with water molecules so competition with the influence of hydrolysis could be expected. The lowest BDE values of the rest of the single acyclic bonds have been calculated for the ones involving nitrogen atoms, so it could be expected that degradation process starts there. Investigation of optoelectronic properties indicate that ANF-2 molecules have better charge hopping properties of electrons, comparing to urea and thiourea molecules. From the molecular docking studies, the title compound binds at the active site of the substrate by weak non-covalent interactions; amino acid Val105 forms interactions like π-sigma and π-alkyl with phenyl ring and alkyl with CF_3; Leu104 shows halogen and alkyl interaction with CF_3; Val108 forms alkyl interaction with CF_3 and Leu132 exhibit π-alkyl interaction with phenyl ring.

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Oral Presentation VI

Gold Cross-linked Molecularly Imprinted Conducting Polymer Decorated on Functionalized Carbon Nanotubes for Electrochemical Sensing of Sudan I

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ABSTRACT
Sudan I (1-phenyl azo -2-naphthol), synthetic colorant widely used in cosmetic, paint, textile and wood industries. Sudan dyes are categorized as class 3 carcinogens by the International Agency for Research on Cancer (IARC) and are also considered as a possible genotoxic carcinogen and mutagen to humans[1]. Molecular imprinting technique produce a polymeric network owing to specific binding site, has become a sensitive and a sensitive tool for the determination of chemical or biological species with predetermined recognition capacity. The conducting polymers utilized in the imprinting technique found to improve the sensitivity of the sensor. The coupling of gold nanoparticles (Au NPs) with molecular imprinted polymer (MIP) has been used in generating interconnection networks[2]. The present work involves the synthesis of multiwalled carbon nanotube (MWCNT) based molecularly imprinted conducting polymer (MICP) sensor for the trace determination of Sudan I in real samples. Thiophene functionalized MWCNTs (MWCNT@SiO_2-Th) were synthesized by the reaction between aminated MWCNTs with thiophene-3-acetic acid. MICP was synthesized by in-situ oxidative polymerization of MWCNT@SiO_2-Th with thiophene acetic acid (TAA) as monomer in presence of Sudan I using thiophene-gold complex as cross linker. Organized material was characterized by FTIR, XRD, SEM, TEM and XPS analysis. The resulting material was drop casted on glassy carbon electrode which then was used for determination of Sudan I by cyclic voltammetric and differential pulse voltammetric techniques. The MICP sensor demonstrated a high selectivity, sensitivity and stability. A lower detection limit (1.8 nM) and a dynamic linear range obtained during DPV analysis can concluded to the applicability of the sensor in the analysis of Sudan I from real samples.

Keywords: Sudan I, Electrochemical sensing, Molecular imprinting

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Oral Presentation VII

A green approach for the synthesis of polysaccharide based hydrogel for controlled release of tetracycline hydrochloride

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Abstract
The *in situ* loading study of tetracycline hydrochloride in acrylamide grafted β-cyclodextrin was carried out by microwave assisted free radical polymerization using ceric ammonium nitrate as redox initiator. The stability of tetracycline hydrochloride at pH 2.4 and 7.4 was studied. The *in vitro* release study shown that the maximum release of drug was at pH 2.4.

Keywords:Tetracycline hydrochloride; β – cyclodextrin ;Acrylamide; Controlled release

Introduction

Natural polymers are preferred for medical application over synthetic polymers because of their biodegradability, lowcost, easy availability andnontoxicity [1,2].However, theypossessthe drawbacks such as uncontrolled hydration, microbialcontamination,and drop in viscosity during storage etc. The chemical composition of natural and synthetic polymers modifies the chemical properties of these natural polymers by hybridization. A grafted copolymer is a macromolecular chain with one or more species of block connected to main chain as side chain(s) having the main polymer backbone commonly referred to as the trunk polymer and branches of another polymeric chain emanating from different points along its length [3].

Cyclodextrins are a family of cyclic oligosaccharides composed ofα-(1, 4) linked glucopyranose subunits.Cyclodextrins have been found as potential candidates because of their ability to alter physical, chemical and biological properties of guest molecules through the formation of inclusion complexes. Cyclodextrin molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes.β-cyclodexrin based hydrogels are widely used in biomedical applications as drug delivery systems because they increase the aqueous solubility, stability and bioavailability of drugs.β-cyclodextrinare known to be rarely hydrolyzed and only a small fraction of them is absorbed in the passage through stomach and small intestine. At the same time,β-cyclodextrin has several adverse effects like nephrotoxicity and low aqueous solubility due to the relatively strong binding of the β-cyclodexrin molecules in the crystal state. This can be avoided by the grafting copolymerization.

The microwave assisted grafting has been widely applied in variety of polysaccharides, namely gellan gum[2],alginate[4], guar gum[5], xanthan gum[6] and many more. In the present study, the grafted copolymerization of acrylamide on to β – cyclodextrin was carried out by the microwave irradiation using ceric ammonium nitrate as a redox initiator.

The Ce(1V) induced grafted copolymerization of vinyl monomers onto polysaccharide substrate [7, 8].The main constraint of graft copolymerization is the formation of concurrent homopolymer resulting in low grafting yield. Apart from the redox initiator induced graft polymerization, microwave assisted grafted copolymerization can also been employed. The microwave irradiation is characterization by rapid transfer of energy in the bulk of reaction mixture. The microwave assisted graft copolymerization requires a very short reaction time and proceeds even in the absence of any redox initiator [9].

Present study explores the application cyclodextrin based hydrogel for controlled release study of tetracycline hydrochloride. Tetracycline hydrochloride (TCH) is broad spectrum antibiotic, but it is mainly active against gram negative bacteria. Tetracycline can be effectively used against cancer, rheumatoid arthritis, osteomyelitis etc. Tetracyclines are less toxic in nature, but these are adsorbed

very poorly in human body. Therefore, a controlled drug vehicle is needed for the static bioavailability of drug. So many studies have been reported on the controlled delivery of TCH to the human body.

Materials and methods

Materials

Analytical grade of tetracycline hydrochloride (TCH) was purchased from Sisco Research Laboratories Pvt. Ltd., Maharashtra,India. β – cyclodextrin (β- CD) was purchased from Tokyo Chemical Industry CoLtd., Tokyo, Japan. Acrylamide (AAM) was purchased from Merck Life Solence Pvt. Ltd., Mumbai, India. Ceric ammonium nitrate (CAN) was obtained from MerckSpecialties Pvt. Ltd., Mumbai, India. Acetone (density = 0.788-0.792 g/mL) was bought from Spectrochem Pvt. Ltd., Mumbai, India. All other chemicals used were of reagent grade. Throughout the experiment, Millipore water was used.

Preparation of TCH loaded polymer by in situ method.

In situ polymerization in the context of drug delivery implies the development of drug delivery systems within the polymerization mixtures.TCH loaded copolymer were prepared according to the following method. About 5 mg TCH dissolved in 120 mL water, to this adds 1g β-CD. 5g of AAM was mixed with 30 mL of water and added to the above and stirrer for 1 h.300 mg of CAN dissolved in 30 mL water and added to the above dispersion. The dispersion was irradiated by microwave for 2 min. It was left for overnight and then precipitated using acetone. It was further washed with 30% aqueous ethanol to remove unreacted monomer and other reagents. Thegrafted polymer then dried at 40 °C to a constant weight and converted to fines[10].

TCH stability study at pH 2.4 &pH 7.4

The stability of TCH was studied at pH 2.4& pH 7.4.Sample of TCH (5 mg) was incubated at 37°C in 100 mL of phosphate buffer (pH 7.4) and in 100 mL of citrate buffer (pH 2.4). At a scheduled time intervals, sample were withdrawn from each solution and assayed by UV spectrophotometer at 276nm in order to measure the ampicillin concentration.

Swelling study of polymer.

A small previously weighed piece of the material (W_1)was immersed in 50 mL buffer(pH2.4&pH7.4) and left to swell for 2h then, the swollen piece was recovered [11] and excess water was removed carefully with a tissue paper and reweighed(W_2) .The swelling index can be calculated as:

Swelling index = $W_2 - W_1$

Where W_1 and W_2 are the weight of swollen and dry polymers.

In-vitro drug release study

About 0.1 g drug loaded polymer was put in different pH conditions of 2.4 of citrate buffer&7.4of phosphate buffer. Placed in a water bath shaker at a stirring speed of 100 rpm maintained at a constant temperature of 37^0C.The concentration of drug released at a particular time intervals can be measured by UV spectrophotometer at 276nm.

Result and discussion

In the copolymerization of AAM-g-β-CD, ceric ammonium nitrate is a common reagent employed to initiate the free radical graft polymerization. At first ceric ions attack on β-CDand form β-CD-ceric complexes. Theceric (1V) ions in the complexes are then reduced to ceric(III) ion by oxidizing hydrogen atom and thereby creating a new free radical on to β-CD backbone. A critical amount of free radical is required for the free radical formation. The grafting of AAM on to β-CD is effected by having free radical reacted with the monomer unit via covalent bond. The reaction is terminated through combination of two free radical. During the in situ copolymerization, ampicillin is enter in to the grafted polymer .The drug made only weak interaction with the polymer. The reaction sample can be subjected to M.W irradiation to induce rapid energy transfer in its bulk there by shortening the reaction time.M.W is considered as a catalyst which synergies with ceric ion in graft polymerization.

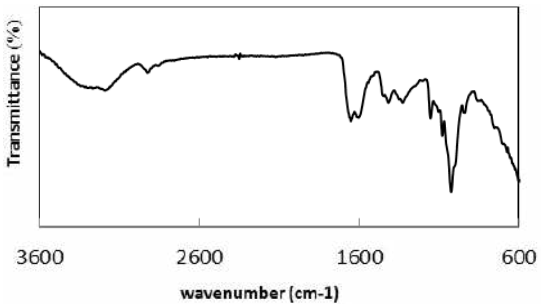

Fig.1. FTIR of acrylamide-cyclodextrin hydrogel

In FTIR, vibration frequency at 1030-1082 cm^{-1} was observed in the spectra of β-CD, which was characteristic of a C-O-C vibration [7]. The band at 1628 cm^{-1} was due to the first overtone of the O-H bending. Marked changes were observed in the spectra of cyclodextrin-acrylamide polymer compared to β-CD. The bands at 1651 and 1602 cm^{-1} were attributed to amide-I (C-O stretching) and amide-II (N-H bending) conferred by AAM[5]. The peak at 3188-3499 $cm^{-1}$was explained due to the overlap of N-H stretching band of amide group and O-H stretching band. A shoulder at around 1450 cm^{-1} was due to the C-N stretching vibration and a peak at 1022 cm^{-1} due to CH-O-CH_2 group which occurred during grafting reaction

Grafted copolymerization of vinyl monomer increased swelling power due to the introduction of free hydrophilic groups. Due to these hydrophilic groups, stronginterchangehydrogen bonding takes place between the grafted side chains of acrylamide. The 3-D network that can hold more water in it. From the swelling study it is observe that the maximum swelling was at pH 2.4 (335%) and minimum at pH 7.4 (178.8%).The maximum release of drug was at pH 2.4.This is due to the maximum swelling index of polymer at this pH.

The stability study of TCH was also conducted and stability of TCH depended on the pH of the medium. Stability study was carried out at two different pH of 2.4 and 7.4 (Figure not shown). Among these two pH conditions the rapid degradation was found to occur at a pH of 2.4. After 48 hrs, about 30% of drug was get burnt. Whereas for pH=7.4, degradation was occurring slowly and even after 48 hrs, about 82% of the drug was retained.

In-vitro release study was performed at phosphate buffer solution pH 7.4 and citrate buffer solution pH 2.4.Releases of drug at certain intervals are measured by UV-spectrophotometer. The results are presented in Fig.2.There was only 4.5% release was occurred at the pH 7.4.But at pH 2.4 there was a controlled release of drug was takes place in an interval of1800 minute. Release mechanism of drug is controlled by diffusion as well as erosion mechanism.

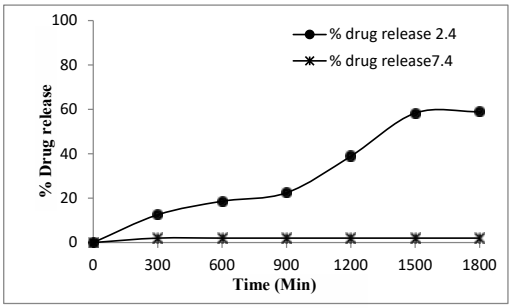

Fig.2. *In Vitro* release kinetics of TCH

To determine the mechanism of drug release, the initial portion of % drug release Vs. time profile have been fitted to the empirical equation proposed by Ritger and Pepas(1987). $M_t/M_\infty = Kt^n$, where M_t/M_∞ is the fraction of drug release at time t,K is the kinetic rate constant and n is the diffusional exponent characterizing the mechanism of drug release. Here n =0.38, ic anomalous / non Fickian type of diffusion is occur [12].In non –Fickian / anomalous transport, both diffusion as well as macro molecular relaxation time scales are similar and both will control the overall rate of penetrant absorption.Non-Fickian release is described by two mechanism-coupling of drug diffusion and polymer relaxation. The net cumulative effect of drug's solubility influenced by its structure, molecular weight and other physical parameters.

Conclusion

TCH was loaded on AAM-β-CD through in situ method by the microwave irradiation using ceric ammonium nitrate as a redox initiator. The maximum in vitro release of TCH occurs at pH 2.4.And also the maximum swelling index of polymer showed in the same pH.The release of drug followed non-Fickian diffusion controlled drug release process.

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Oral Presentation VIII

Understanding the Citric Acid–Urea Co-Directed Microwave Assisted Synthesis and Ferric Ion Modulation of Fluorescent Nitrogen Doped Carbon Dots: A Turn On Assay for Ascorbic Acid

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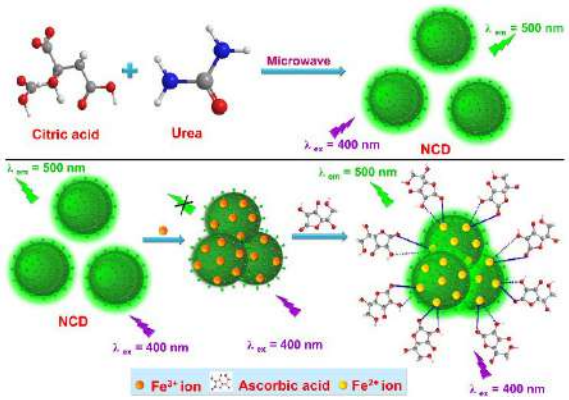
Abstract: Herein, nitrogen doped carbon dots (NCDs) were synthesised from citric acid and urea *via* a previously reported microwave assisted route. The NCDs shows emission maximum at 500 nm on excitation at 400 nm. The fluorescence of NCDs decreases slightly with increase in basicity of solution up to pH 7.5 and then increases again after pH 8.5, along with a blue-shift in tested alkaline pH. This pH dependent blue-shift indicates the presence of both carboxyl↔carboxylate and phenol↔phenolate prototropic equilibrium in NCDs. Due to the special interaction of these phenolates and carboxylates on NCDs surface with di- or tri- valent heavy transition metal ions; it is demonstrated that ferric ion (Fe^{3+} ion) can quench the fluorescence of NCDs. This Fe^{3+} induced static quenching of NCDs is a collaborative effect of inner filter effect, aggregation and ferromagnetism. However, Ascorbic acid (AA) can recover the fluorescence of Fe^{3+} quenched NCD with detection limit as low as 96 μM. This detection strategy has good selectivity towards AA over other antioxidants, saccharides, proteins and neurotransmitters. Furthermore, (spiked) human serum and (spiked) human urine were analysed and found good recovery percentage.



Oral Presentation IX

Carrageen strengthened pyrolysed rice husk filter in heavy metal pollution mitigation: Removal of Pb(II) and Cu(II) from aqueous medium

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Abstract
The present is study focused on the preparation of an active biomaterial for the expulsion of metal ions preferably Pb(II) and Cu(II) from solution. The pyrolysed rice husk powder (pRH) and carrageen strengthened pyrolysed (pRH CG) were taken as the adsorbents to proceed with the adsorption experiments and to compare the rate of intake of Pb(II) and Cu(II) metal ions by the materials in aqueous media. The pH 5, Contact time 60 m and T = 30°C were found to be the optimized adsorption parameters to accure the maximum rate of adsorption for both the adsorbents. The adsorption capacity of the pRH for Pb(II) and Cu(II) were found to be 51.28 and 50.14 mg/L respectively, and for pRH CG were 69.55 and 54.22 mg/L respectively for an initial concentration of 100 mg/L. Both the adsorption processes follow the pseudo-second order reaction kinetics and the Langmuir adsorption model was best fitted in explaining both the adsorption mechanisms. The two reactions are spontaneous and exothermic in nature. Thus the study reveals that the pRH CG has improved adsorption efficiency than the pRH to irradiate the Pb(II) and Cu(II) ions from waste water.

Introduction
Water pollution is a major problem in the global context which requires ongoing evaluation and revision of water resource policy at all levels (international down to individual aquifers and wells). It has been suggested that water pollution is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily [1,2]. An estimated 580 people in India die of water pollution related illness every day. The presence of heavy metals in the environment is a major concern due to their toxicity for many life forms. Especially the contamination of water bodies by toxic heavy metals through the discharge of industrial wastewater is a huge issue to be addressed. Cu, Zn, Cr, Cd, etc are harmful wastes produced by industry that arise a risk of contaminating water resources. Organic pollutants are susceptible to biological degradation, while heavy metals will not degrade into harmless end products and can cause various diseases and disorders [3,4]. Major sources of Pb can be dyes, gasoline, insecticides, tobacco smoke and hair colouring. Lead is a naturally occurring bluish-gray metal present in small amounts in the earth's crust. Lead has many different industrial, agricultural and domestic applications.

Agricultural waste material can be used as a potential adsorbent for sequestering heavy metal ions from aqueous solutions. It is highly efficient, low cost and renewable source of biomass can be exploited for heavy metal remediation. The potential of RH for the removal of heavy metals from aqueous solution was reviewed and conveyed that the most explored metals included Cd, Pb, Zn, Cu, Co, Ni, and Au [5]. The effectiveness of Rice Husk(RH) for removing chromium from effluent was investigated by Sumathi et al through batch experiments and compared the adsorption capacity of RH with sawdust, coir pith, and charcoal. The sawdust exhibited a higher adsorption capacity, followed by coir pith [6]. A detailed study of lead(II) adsorption with pH indicated a positive trend with increment in the pH variation [7] The maximum adsorption was ~87.75% at a pH of 4.6 (0.5, and an initial concentration of 30 mg/L. The effects of initial concentration, temperature, adsorbent loading,

and pH were investigated for an optimized condition for Pb adsorption and under the optimum conditions, the lead uptake was reported to be 8.60 mg/g [8]. Arsenic removal with the use of Rice husk ash has been investigated [9-11]. Rice husk ash can be used as an adsorbent for removal of arsenic from water. They reported that 10 g/L of RHA dosage can remove arsenic by 5-12%. Feng et al., [12] investigated the adsorption capacity of rice husk ash for the removal of lead and mercury in aqueous stream. The main finding of this research was that the finer the RHA particles used, the higher the pH of the solution, the more lead and mercury are absorbed by rice husk ash. Srivastava et al., [13] has made an attempt to use rice husk ash (RHA), a waste obtained from the rice husk-fired furnaces, as an adsorbent in the removal of cadmium (Cd (II)) and zinc (Zn (II)) ions from binary systems. It is an effective adsorbent for the removal of Cd (II) and Zn (II) metal ions from aqueous solution.

In the present study carrageen supported pyrolysed rice husk powder is used for the removal of Pb(II) and Cu(II) from aqeous media. Carrageenans are a family of linear sulfated polysaccharides and contain the repeating disaccharide, 1,3-linked-β-D-galactopyranosyl, and the 1,4- linked-α-D-galactopyranosyl sugar residue. When sulphate groups are introduced in the place of hydroxyl group in disaccharide unit, yields different carrageenan types [14,15]. Carrageenans are large, highly flexible molecules that curl to form helical structures. This gives them the ability to form a variety of different gels at room temperature. They are widely used in the food industry, for their gelling, thickening, and stabilizing properties [16,17].

2.1 Materials

All chemicals used were of analytical grade and obtained from Merck India Ltd. Experimental solutions were made in Millipore water having conductance 0.064 μS. The stock solution is 1000 mg/L of Pb, Cu metal ion solution, which is prepared by dissolving accurately weighed Pb, Cu nitrates in Millipore water and made up in a 1000 ml standard flask. The desired concentrations of experimental metal ions solutions were prepared by diluting the stock solution with Millipore water using standard flasks.

2.1. Preparation of adsorbent

Rice husk samples are collected from rice mill and ground to size down the husk material. Rice husk is pyrolyzed and made in to a foam structure using carrageenan. To develop the foam structure, pyrolyzed rice husk (pRH) is ground well in a planetary mill and then mixed with carageenan in water media at 70^0C in 3:1 ratio. The low viscous mixture is then poured in to the mould of pvc pipe and allow to cool at room temperature. Then it will be taken to the freeze drier to get rid of the water and will result in a green foam. This composite foam in cylindrical structure is brought to the inert tube furnace to get it pyrolyzed and final structure will be a pyrolyzed done.

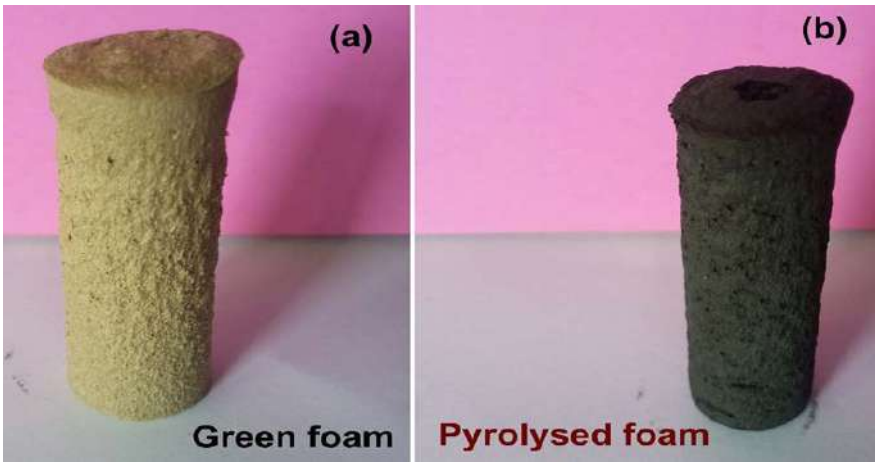


Fig. 1. Cylindrical foam as seen in figure 1(B):(b). Adsorption studies of both pRH and pRH CG were

2.2. Characterization Tools

The X-ray powder diffraction pattern of the material were taken from Philips XPERT-PRO diffractometer with CuKα radiation of wavelength 1.5406 A^0 at a scanning speed of 2°/min, in the range of 2θ = 10°–80°. The surface morphology of the synthetized material and its adsorption amid changes were portrayed by using TESCAN VEGA 3 LMU Scanning electron microscope with tension voltage of 15 KV and current 30 mA at 25°CThe concentration of metal ions was collected from Metrohm 797 VA Computrace Volta metric trace metal analyzer and Perkin Elmer PinAAcle 900H Atomic absorption spectrophotometer.

2.3. Batch Adsorption Experiments

The optimum condition for the adsorption of Heavy metals Pb and Cu was confirmed by the column experiments. Experimental solutions of above metals were prepared from a stock solution (1000 mg/L) with varying concentrations in deionized water. To perform column adsorption, experiment the adsorbent filled columns are passed with varying concentrations of the metal ions and allowed to stand for 0 - 1.5 hr. The pH was adjusted by using HNO_3 and NaOH. 1 ml solution was eluted from the column at definite intervals of time. The amount of metal ions adsorbed on the solid surface was calculated by using equation

$$q = \frac{\left[\left(C_0 - C_A\right)\right]V}{m} \qquad (1)$$

Where q, is the amount of Pb(II) and Cu(II) were adsorbed per unit mass of bentonite (mg g^{-1}); C_0 and C_A are initial and equilibrium concentration (mg L^{-1}) respectively; V, is the volume of the aqueous phase (mL) and m, the mass of the adsorbent (g).

3. Result and Discussion

3.1 XRD

X-ray diffraction analysis of the powdered pyrolyzed rice husk shows a broad peak at 2Θ value of 21 to 24 0, which is the characteristic of the presence of amorphous silica and amorphous carbon present in the pyrolyzed rice husk system.

3.2 Microstructural analysis

Fig 2: (a) and (b) are pyrolyzed carrageenan and ground pyrolyzed rice husk. (c) and (d) are micrographs of ground pyrolyzed rice husk composite with carrageenan as the matrix. (e) and (f) are micrographs of raw rice husk dispersed in carrageenan matrix.

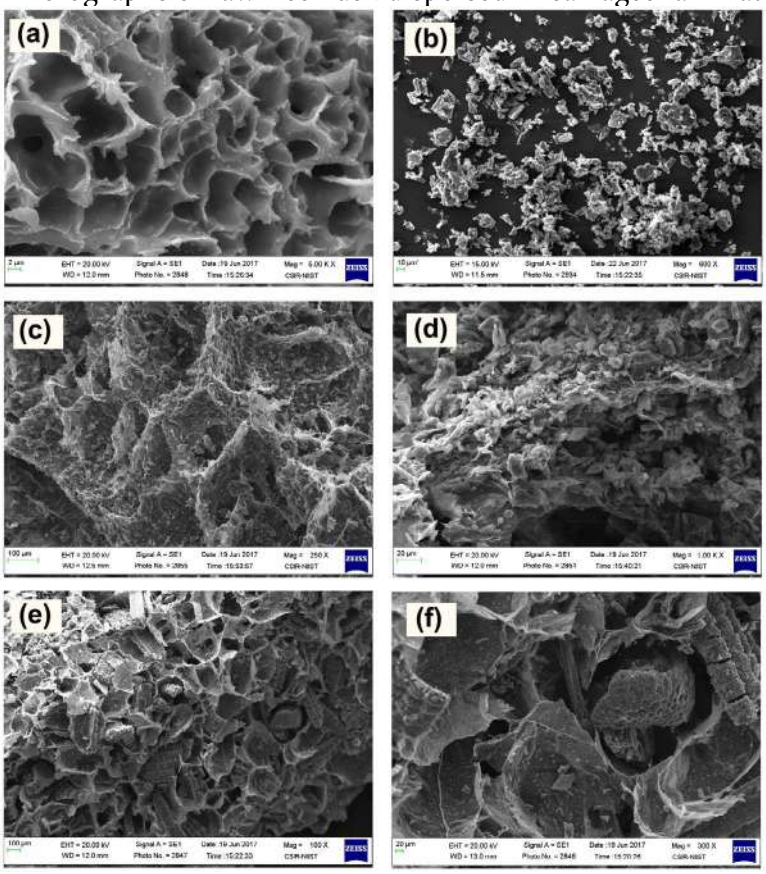


Fig (3a) shows freeze dried carrageenan after pyrolysis having lot of pores. Fig (3b) is that of pyrolyzed rice husk after grinding. The remaining SEM micrographs are indicating the composite between these two. The fractogram (3c) and (3d) are that of ground prolysed pRH – carrageenan composite while (3e) and (3f) are fractogram of green foam after pyrolysis. In the later case the grinding of rice husk is avoided. While we used ground pRH we have a well dispersed system compared to that with the ungrounded version.

3.3 Effect of various parameters on adsorption

3.3.1 Effect of solution pH

The pH factor is very important in the adsorption process as the adsorption of metal ions onto solid was markedly affected by the solution pH because it influences the metal chemistry in aqueous media as well as the surface chemistry of the adsorbent (37) As a result, the rate of adsorption will vary with the pH of the aqueous solution. In the present study the adsorption behavior of Pb (II) and Cu(II) on pRH and pRH CG has been investigated at different pH ranging between 3 and 7. The percentage of metal ions adsorption on to pRH and pRH CG increased with increase in pH and reaches a maximum at pH 5 and then decreased. At an initial concentration of 10 mg/L, maximum metal ions adsorption of 88.1 and 85.7 % for Pb (II) and Cu(II) onto pRH and 96.1 and 88.5 % of Pb and Cu on to pRH CG was observed at pH 5.

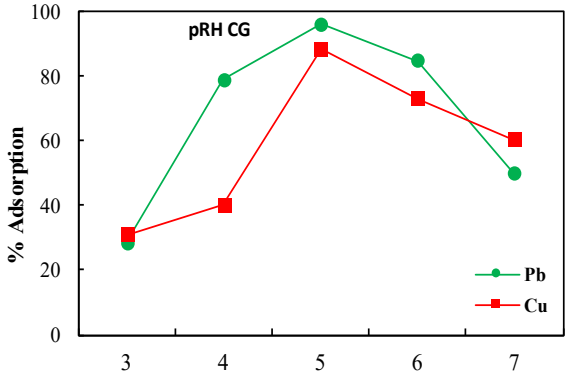
3.3.2 Effect of contact time

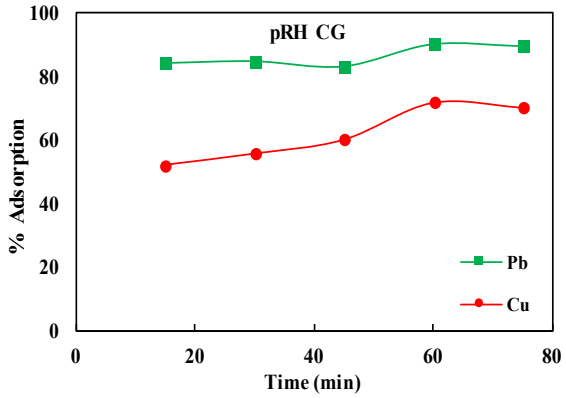
Adsorption is an equilibrium process and their action time is one of the important factors influencing the adsorption of metal at the solid-liquid interface. Removal of Pb (II) and Cu(II) by pRH and pRH CG are carried out at pH 5 for initial concentrations 10 mg/L. In order to estimate the adsorption behavior of adsorbent accurately, it is important to allow significant time for the experimental solution to reach equilibrium. The uptake of Pb (II) and Cu(II) onto pRH and pRH CG as a function of time and the result clearly indicate that the adsorption of metal ions reached equilibrium at 60 minutes beyond which there may not be further increase in the adsorption process, and thus it was fixed as the optimum contact time. The adsorption rapidly increased in the first minute after which adsorption slowly approaches towards equilibrium.

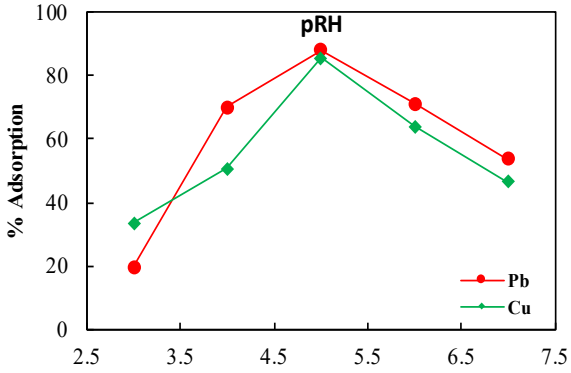
With a bare surface initially, the available surface area is very large compared to the density of Pb (II) ions and consequently, the rate of adsorption was very high. However, with the increase in coverage, the fraction of the bare surface rapidly diminished and Pb (II) ions had to compete among themselves for the adsorption sites. This result in slowing down of the interaction and the rate now becomes predominantly dependent on the rate at which Pb (II) ions are transported from the bulk to the adsorbent-adsorbate interface. The kinetics of the interaction is thus likely to be dependent on different rate process as the interaction time increases.

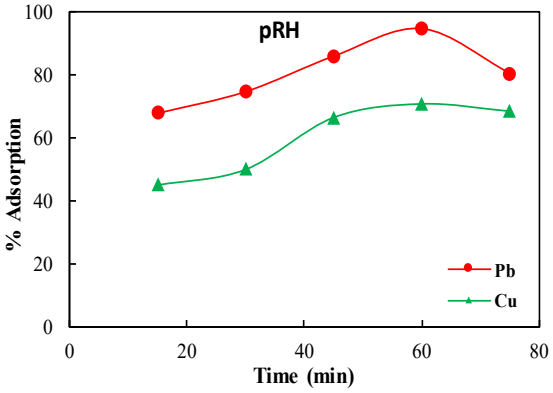
3.3.4 Effect of temperature

Temperature has a direct influence on the adsorption of Pb (II) on pyrolyzed rice husk. The adsorption experiments were performed in the temperature range 20-50 °C for different initial concentrations 10, 25, 50, 75, 100 and 125mg/L at a constant adsorbent dose of 0.1 g/L and at a pH of 5. The result shows that the equilibrium adsorption capacity decreased with the rise in temperature from room temperature and the maximum adsorption of Pb (II) and Cu(II) on to pRH and pRH CG was observed at 30°C.



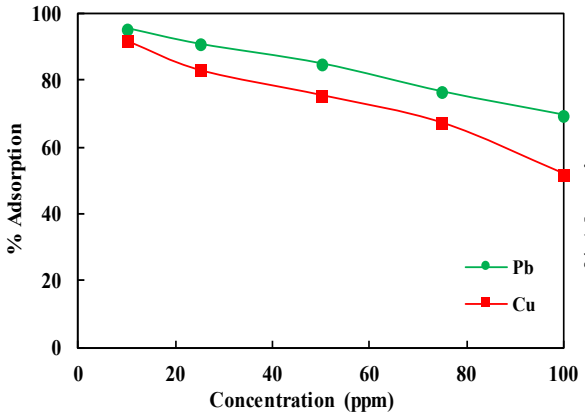


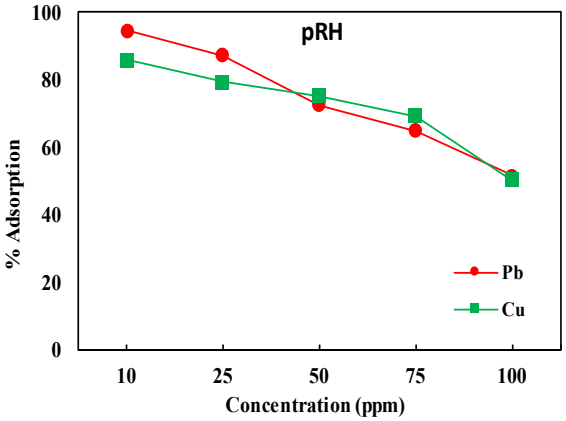




3.3.3 *Effect of initial concentration*

The effect of initial concentration can be carried out by preparing an adsorbent-adsorbate solution with fixed adsorbent dose and different initial concentrations (10, 25, 50, 75, 100 and 125 mg/L) for different time intervals and shaken until equilibrium. The percentage removal of Pb (II) is highly dependent on the initial amount of metal ion concentration. Fig.4 shows that the adsorption of metal ion decreases with increase in initial concentration. At low concentration there will be unoccupied active sites on the adsorbent surface and when the initial concentration increases, the active sites required for adsorption of the metal ion decreases.





3.4 *Adsorption isotherm*

The relationship between the amount of a substance adsorbed at constant temperature and its concentration in the equilibrium solution is called the adsorption isotherm. The study of isotherm is highly needed to find out the effectiveness of the adsorbent material for the removal of solutes from aqueous solutions. Moreover, it is helpful in optimizing an adsorption system for the removal process. In the present study, the equilibrium data obtained from adsorption studies were applied to Langmuir and Freundlich isotherm models. The linear form of Langmuir isotherm can be written as:

$$\frac{C_e}{q_e} = \frac{1}{bQ^0} + \frac{C_e}{Q^0} \qquad (4)$$

The isotherm constants are presented in Table 1. The essential feature of Langmuir isotherm can be expressed by RL, a dimensionless constant referred to as equilibrium parameter which indicates the type of adsorption is given by the

expression

$$R_L = \frac{1}{1 + bC_0} \qquad (5)$$

Where b is the Langmuir constant, C_o is various concentration of phosphate solution. The value of R_L indicates the type of the isotherm to be unfavorable ($R_L > 1$), linear ($R_L = 1$), favorable ($0 < R_L < 1$), or irreversible ($R_L = 0$). From table 2. the experimental values of K_F and 'n' for adsorption of Pb(II) and Cu(II) onto pRH and pRH CG suggests that the adsorption favorable for a physical mechanism. The higher regression coefficient is observed for Langmuir plot indicating that Langmuir model will be best to study the adsorption of Pb(II) and Cu(II) onto PRH and pRH CG. From the experiment data the RL values were found to be between 0 and 1 for all initial concentration of Pb(II) and Cu(II) solution at 10 mg/L and thereby obeying the Langmuir isotherm model.

Conclusion

The present study concludes that pRH and pRH CG can be used as an inexpensive, sustainable, reusable, and environment-friendly treatment option for successive removal of Pb(II) and Cu(II) from contaminated drinking water. Apart from light weight and easy portability of the foam structure, another good aspect about them is in designing suitably sized portable water purifying system. They can be easily installed on a waterline in a cylindrical form or it can be made to a small capsule form in accordance with the system requirement.

Acknowledgements

We are thankful to Dr. T.N. Prakash, Director, NCESS and former Director, V.M. Tiwari for providing laboratory and knowledge resource facilities. The instrumental facilities in NCESS under SWQM (Sea Water Quality Monitoring) Programme funded by ICMAM, Ministry of Earth Sciences, is also acknowledged.

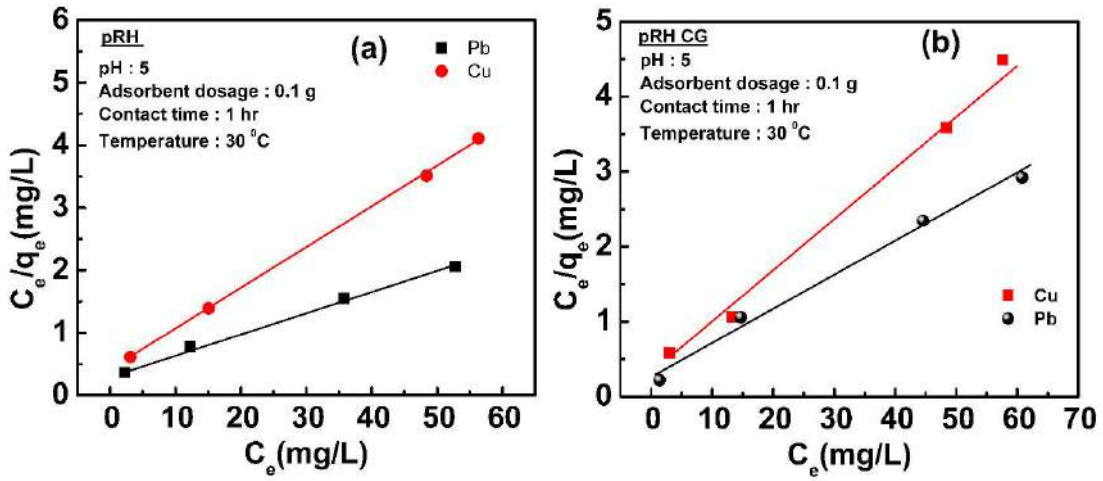


Fig 5: Langmuir plot for the adsorption of Pb(II) and Cu(II) onto pRH and pRH CG at 30°C

Table 1 Isotherm constants

Materials		Langmuir model				Freundlich model		
	Metal ions	Q_0 (mg/g)	b(mg/L)	R^2	R_L	K_f (mg/g)	n(mg/L)	R^2
pRH	Pb (II)	20.1568	0.0995	0.9960	0.2498	1.9065	2.1702	0.7758
	Cu (II)	15.3751	0.1592	0.9993	0.2899	1.7552	2.8947	0.8291
pRH CG	Pb (II)	24.1944	0.2856	0.9858	0.1978	2.1566	3.2136	0.8981
	Cu (II)	22.2717	0.1658	0.9863	0.2130	1.7750	3.0637	0.7486

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Oral Presentation X

Investigation of the reactive properties of a thiourea derivative by spectroscopic and DFT calculations

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[a] *Fatima Mata National College, Kollam*

ABSTRACT
Thiourea derivatives display remarkable antitubercular, antiviral, antimalarial and anti-inflammmatory activities. A thiourea derivative, 1-(4-chloro-3-nitrophenyl)-3-(3,4-dichlorophenyl) thiourea has been synthesized and spectroscopic characterization has been performed by FT-IR and FT-Raman techniques. The aforementioned spectra was theoretically obtained which were then compared with experimentally obtained data. The molecular structural parameters and vibrational wave numbers of the title compound have been investigated theoretically using Gaussian09 [1] with B3LYP/6-31G(d,p) basis set. Potential energy distribution is calculated for the normal modes of vibrations using GAR2PED program[2]. Beside spectroscopic characterization the aim of this study also encompassed detailed computational investigation of global and local reactive properties. The NLO analysis, NBO analysis, frontier molecular orbital analysis and MEP are done with the help of Gaussian software . Important local reactivity properties have also been obtained by analysis of molecular electrostatic potential (MEP) and local average ionization energy (ALIE) surfaces.
The vibrational spectral analysis of the title compound is reported. For the title compound, the N-H, C-N, NO_2, CCl, C=S and the phenyl rings vibrational modes are all in good agreement with the reported values of similar derivatives. The natural bond orbitals (NBO) calculations shows the strong interaction $n_1(O_{20})\rightarrow\pi^*(N_{19}\text{-}O_{21})$has the highest E(2) value 93.53 kJ/mol. Almost 100% p-character was observed in lone pairs of S_{10}, Cl_{18}, Cl_{17}, O_{20}, O_{21} and Cl_{24}.
The Homo-Lumo analysis are frequently used to initially indicate the interaction of molecule with other species. It is evident from the frontier molecular orbital plot that there is a charge transfer within the molecular system from the thiourea group to the nitro substituted phenyl ring.
NLO properties of the title compounds has been calculated and the dipole moment is 6.7768 Debye, polarizability is 3.4876×10^{-23} e.s.u, and the first and second order hyperpolarizabilities are 8.4096×10^{-30} and -30.880×10^{-37} e.s.u. Here, the first hyperpolarizability of the title compound is 64.69 times that of the standard NLO material urea [3] . The C-N bond lengths in the title compound are in between a single and double bond and hence there is an extended π-electron delocalization over the thiourea group which is responsible for the nonlinearity of the system.
MEP analysis is also reported. The different values of the electrostatic potential are represented by different colors and potential increases in the order of red < orange < yellow < green < blue. The red, orange and yellow regions of the MEP are negative potential regions related to electrophilic reactivity. From the MEP map of the title compound, the maximum negative region is localized over the C=S group and oxygen atoms and the maximum positive region is localized on NH groups and nitrogen atom of NO_2 group indicating a possible site for nucleophilic attack. ALIE results show that the most important molecule site from the aspect of electrophilic attacks is characterized by low ALIE value (S_{10}) and the molecule site where electrons are most tightly bound are characterized by high ALIE value. The molecular docking studies reveal that docked ligand form stable complexes with the target protein and show inhibitory activity against it.

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Oral Presentation XI

ADSORPTION OF METHYLENE BLUE BY BIOCHAR-DERIVED FROM PLANT-BIOMASS

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Abstract
In this work, biochar derived from banana stem has been employed for the removal cationic dye, methylene blue from aqueous solutions. Sorption characteristics of the as-fabricated low cost biochar for the removal of dye was studied in batch conditions. Biochar exhibited good sorption performance over the pH range 9. Langmuir and Freundlich adsorption isotherm models were fitted to the experimental data. From the regression analysis, Freundlich was found to be the best fit model suggesting multilayer adsorption.

Keywords: Biochar, Methylene blue, adsorption isotherms

Introduction

Over the past few decades increased use of synthetic dyes led to a major reason for environmental pollutions. They have complex aromatic molecular structures that make them more stable and more difficult to biodegrade. The solubility of dyes in water effluents possesses serious risks to crop, aquatic life and human health. Different separation techniques like precipitation, ion-exchange, adsorption, coagulation / flocculation, ozonation, membrane separation and liquid- liquid extraction have been used to remove dye from wastewater. Adsorption process is considered to be an effective separation technique compared to other methods for wastewater treatment in terms of cost, simplicity of design and high adsorption capacity

In recent time, researchers are focusing on the production of low cost naturally available agricultural solid waste based adsorbents. However,to produce effective high capacity adsorbents comparative to commercial activated carbon researchers paved more attention to biochar. Biochar is a porous, carbon-residue derived from the thermal conversion of waste biomass under limited oxygen or anaerobic condition [1]. "Biochar" is arecently coined term emerging in conjunction with the renewable fuel Soil amelioration, and carbon sequestration. So far the most standardized definition of biochar is regulated by International Biochar Initiative (IBI) guidelines, which states that 'the biochar is a solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment' [2].It can be utilized as an adsorbent for the removal of toxic contaminants from wastewaters or polluted soils [3–8].Relatively high levels of matrix-bound carbon in biochar, along with a high degreeof porosity and large surface area, helps to play vital roles in the adsorption of heavy metals and other pollutants from contaminated environments [9,10,11,12].

The presence of functional groups on the surface of biochars impart adsorption potential for toxic substances, (As), nickel (Ni), copper (Cu), cadmium (Cd), and lead (Pb) in heavy metal contaminated soils [13,14]. Therefore, possible reductions in sources may be accomplished if biochar is present in the soil. However, biochar properties are highly variable and biochar quality is also influenced by the feedstock materials and pyrolysis conditions. The skeletal structureof biochar consists mainly of carbon and minerals of different pore sizes. Micropores are responsible for surface area and high absorptive capacity, while mesopores are important for liquid-solid adsorption processes, and macrospores areimportant for aeration, hydrology, movement of roots, and bulk soil structure. The size and pattern of pores in biochar depends on the composition of the feedstock materials and the temperature adopted during biochar formation. The porous structure of biochar is composed of numerous aromatic compounds and other functional groups that are produced from lignin-based biomasses.

Due to it abundant availability, economic feasibility and the presence of various functional groups that were useful to produce biochar composite, in this study we have chosen banana stem waste as precursor for the production of biochar . We demonstrate facile fabrication of a eco-friendly biochar using banana stem . The adsorption behaviour of cationic dye on to magnetic biochar were studied under different experimental conditions using batch method. The operating parameters such as pH, initial concentration and contact time were investigated in detail. The adsorption equilibrium was evaluated by Langmuir and Freundlich isotherm models. The prepared biochar was characterized by various analytical techniques and studied the physio-chemical properties of the fabricated composite.

2. Materials and Methods

2.1 . Materials

Biomass waste banana stem were collected from a farm in kollam. Methylene blue was chosen as the adsorbate for the study because of its potent toxicity to water resources . For the purpose, methylene bluewas purchased from E Merck Indian. Other reagents solution wereused for the present study were also procured from E Merck India. The reagents used in the study were all analytical grade reagents.

2.2. Biochar preparation

Banana stem was properly washed, cut into small pieces, dried, powdered and sieved before the use. 10 g of the powdered banana stem was weighed and transferred to silica crucible. The crucible was kept in a preheated muffle furnace, where nitrogen flow was maintained for 15 minutes continuously. The material was completely dried at 350 °C. for 12h. During the process hemicelluloses undergoes limited volatilization and carbonization [15].After heating for the pre-planned time, the crucible was transferred to a nitrogen filled dessicator. The char was again powered, sieved and weighed.

2.3. Adsorption experiments

Adsorption experiments were performed in a batch reactor to determine the adsorption potential of thebiochar sample produced in the laboratory. To determine the effect of pH, 2 g/L of the biochar was added to the Erlenmeyer flasks containing 50 mL of 10 mg/L and 25 mg/L of methylene blue solution. The pH of the solutions were adjusted from 6.0 to 9.0 using suitable concentrations of HNO_3 and NaOH. The samples were then placed in a shaking incubator at 180 rpm and 30 °C for 4 h.

2.4 Adsorption isotherm models

In this work, the biochar was blended with 8 sets of methylene blue solutions of which concentrations were ranging from 10 mg/L to 200mg/L at 30 °C in a shaking water bath at 180 rpm. All the samples were equilibrated for 24 h and the equilibrium concentrations were determined using UV-Visible spectrophotometer at λmax 668 nm. The equilibrium data were then fitted into Langmuir and Freundlich isotherm models fig (1&2).

The Langmuir isotherm model, which assumes homogeneous monolayer sorption, is written as:

$$\frac{C_e}{q_e} = \frac{1}{Q_0 b} + \frac{C_e}{Q_0}$$

Where Q_0 (mg/g) is the maximum sorption capacity. b is the Langmuir constant related to adsorption capacity and adsorption rate. When $1/q_e$ is plotted against 1/Ce, a straight line with slope $1/Q_0$ and intercept $1/Q_0b$ is obtained.

The Freundlich isotherm model, which assumes heterogeneous adsorptive energies on the adsorbent surface is written as:

$$log q_e = log K_F + \frac{1}{n} log\, c_e$$

where K_F and n are the Freundlich constants related to adsorption capacity and intensity, respectively.

3. Result and Discussion

3.1 Effect of solution pH

The sorption of 10 and 25 mg/L of methylene blue onto the biochar dose of 2 g/L at different pH varying from 6.0 to 9.0 was studieddatas shown in Table1. It was found that the sorption waslow at lower pH. After that, the sorption percentage gradually increased reaching a maximum value of 99.2% and 98.1% for 10 mg/L and 25 mg/L at pH 9.0..The equilibrium pH lowered after adsorption, indicating the release of H^+ into the solution. Therefore the possible mechanism for

the sorption was a cation exchange mechanism. The polar functional groups like carboxylic groups on the biochar surface may exchange protons with the cations in the solution. The surface of prepared carbon samples contains an excess of H^+ ions competing with cationic methylene blue for adsorption sites, which reduces adsorption at lower pH. These acidic sites were deprotonated and surface becomes negatively charged at alkaline pH which strongly attracts the cationic dye and increases adsorption capacity [16]. Apart from the cation exchange mechanism, there is a possibility that the electron rich graphene surface on the char exerts electrostatic attraction for the cations in the solution. The char surface being porous, an intraparticle diffusion of cations may occur which get trapped very well in these pores.

3.2 Adsorption isotherms

The sorption onto thebiochar is influenced by the structural and chemical properties of the sorbent surface as well as the sorbate. Adsorption also depends on the pore size distribution, specific surface area, polarity and functionality of the biochar surface. A better understanding of the sorbent –sorbate interaction is made possible from the isotherm studies.

The concentrations 10. 25, 50, 100,150 and 200 mg/L of methylene blue solution were chosen for the isotherm study with an adsorbent dose of 2 g/L. The calculated isotherm parameters are given in Table 2 The sorption data fits very well with the Freundlich model with high regression coefficient. The Freundlich constant n, was found to be less than one, suggesting that the adsorption sites on the biochar is not homogeneous.

Conclusion

This study demonstrates the facile fabrication of biochar from low-cost abundant banana stem waste. Taking advantage of surface structure and functionalities of the biochar, the isotherm studies of the adsorption of methylene blue on tobiocharwas conducted. The optimum pH for the adsorption of methylene blue was found tobe9.0 .Thevariation in the amount adsorbed from 37.4 to 11.9 mg g^{-1} respectively indicates that it is effective to carry out the adsorption with 2g L^{-1} sorbent dose than with higher dosages. The regression coefficient was found to be higher for Freundlich isotherm model, suggesting that the adsorption sites on the magnetic biochar were not homogeneous.

Acknowledgement

The authors are expressing sincere gratitude to The Head, Department of Chemistry, Fatima Mata National College, Kollam for providing laboratory facilities. The corresponding authorthanks UGC, New Delhi for financial assistance in the form of Minor Research Project .The authors sincerely acknowledge the services rendered by CUSAT(Kochi) for their assistance in the characterization of the samples.

Table1 : Effect of adsorbent dose on the adsorption of 100 mg/L methylene blue solution

pH	Adsorption (%)
6	74.0
7	88.4
8	93.2
9	95.3

Table 2: The isotherm parameters for the adsorption of methylene blue onto biochar

Isotherm Constants	Pb(II)
Freundlich	
K_F	12.5
1/n	0.273
R^2	0.997
Langmuir	
Q^0 (mg/g)	32.05
b	0.30
R^2	0.5512

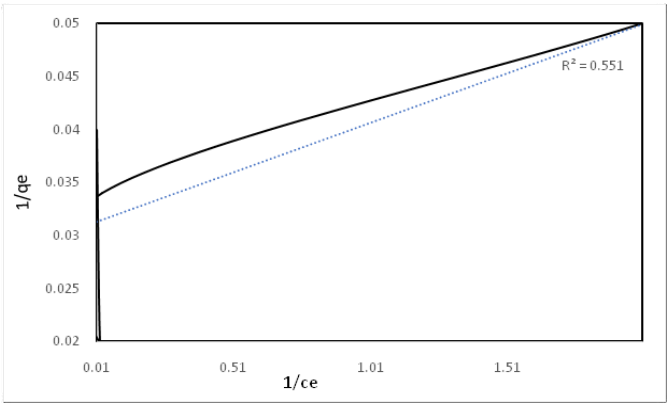

Fig.1 Langmuir adsorption isotherm

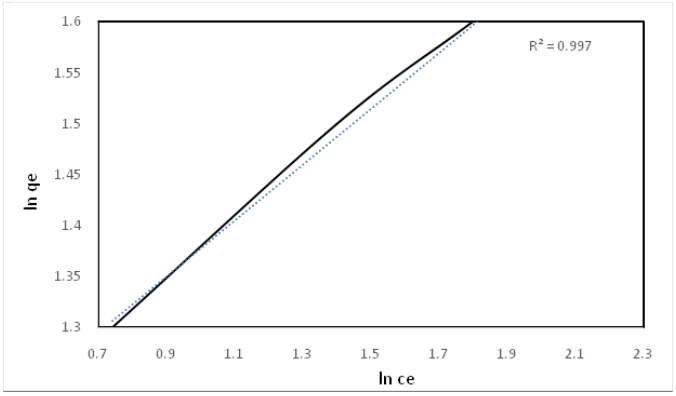

Fig 2. Freundlich adsorption isotherm

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Oral Presentation XII

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING COLEUS AROMATICUS LEAF EXTRACT AND EVALUATION OF ITS ANTIBACTERIAL PROPERTIES

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ABSTRACT

The present work reports the green synthesis of zinc oxide nanoparticles using *coleus aromaticus leaf* extracts by a simple and ecofriendly route. The formation of zinc oxide nanoparticle is confirmed by characterization technique like Ultraviolet-Visible Spectroscopy (UV-Vis), Photo Luminescence(PL), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction Spectroscopy (XRD) and Scanning Electron Microscopy (SEM). The ultraviolet-visible spectroscopy results show peak in between 300-400nm, which is the characteristic peak of ZnO NPs. From FTIR measurements we can understood biomolecule can act as capping agent and thereby stabilizes the synthesized zinc oxide nanoparticles. The photoluminescence spectroscopy result shows peak in between 350-650nm, which is the characteristic peak of ZnO NPs. The XRD result confirms the hexagonal wurtzite structure and the size of the synthesized nanoparticle is 21.04nm.The SEM image shows that ZnO NPs possess nanocomb structure and the average size was found to be 10-50nm. The antibacterial activity of the synthesized zinc oxide nanoparticles was tested with Escherichia coli, Enterobacter, Bacillus subtilis, staphylococcus aureus, pseudomonas aeruginosa. Organisms such as Escherichia coli, Bacillus subtilis, Staphylococcus aureas shows considerable effect with synthesized zinc oxide nanoparticles.

INTRODUCTION

Zinc oxide is one of the important metal oxides with a band gap of 3.37 eV. It is an inorganic semiconductor having hexagonal wurtzite structure and is also known for its non- toxicity, high photo sensitivity stability and large excitation binding energy [1]. The use of plant extracts can be considered as an greener alternative to the physical and chemical methods for the production of nanoparticles. Green mediated synthesis of ZnO NPs using plants extracts such as Aloe vera [2], Ocimum Tenuiflorum [3], Punica granatum [4], Moringa oleifera [5], Cassia fistula [6] etc have been reported. ZnO NPs find wide variety of application in the field of medicine, optics, catalysis, food technology etc [7]. The biosynthesized ZnO NPs possess high antibacterial,antioxidantandanti-inflammatory properties [8-13]. The present study deals with the synthesis of ZnO nanoparticles by biological method from the leaf extract of Coleus Aromaticus,its characterization and evaluation of its antibacterial properties

MATERIALS AND METHODS

Materials

Analytical grade $ZnNO_3$ was obtained from Sigma – Aldrich chemicals and used as received. Coleus aromaticus leaves were collected from local areas in Alappuzha district in Kerala, India. Distilled water was used to perform the experiments.

Microorganisms used for antibacterial study

Five pathogenic bacterial stains produced from microbial type culture collection (MTCC,Chandigarh, India) were employed in the present study to investigate the antibacterial properties of the synthesized zinc oxide nanoparticles. Enterobacter, Escherichia coli, Bacillus subtillis, Staphylococcus aureas and Pseudomonas aeuroginosa were used as the test pathogens for the antibacterial study.

Methods

Preparation of leaf extract

Coleus Aromaticus leaves were collected and washed several times with water to remove the

dust particles and then removed the residual moisture. The extract used for the reduction of zinc ions (Zn^{2+}) to zinc oxide nanoparticles was prepared by placing 50g of washed dried fine cut Coleus Aromaticus in 250ml glass beaker along with 100ml of distilled water. The mixture was heated for 60 minutes until the colour of the aqueous solution changes from watery to light yellow. The extract was cooled to room temperature and filtered using filter paper.

Preparation of zinc oxide nanoparticles

For the synthesis of zinc oxide nanoparticles 50mL of Coleus Aromaticus leaf extract was taken and boiled to 60-80^{o}C using a stirrer heater. 5g of Zinc nitrate was added to the solution as the temperature reaches 60^{o}C. This mixture is then boiled until it reduces to a deep yellow colored paste. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400^{0}C for 2 hours. A light white colored powder was obtained and this was carefully collected and packed for characterization purposes. The material was mashed in a mortar and pestle so as to get a finer nature for characterization.

CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES

UV –VIS ANALYSIS

The reduction of zinc ions was monitored by measuring the UV–Vis spectrum of the reaction mixture by using a UV–Vis spectrophotometer (Shimadzu model) in the wavelength ranging from 300–650 nm.

FTIR ANALYSIS

FTIR spectra of the biosynthesized ZnO NPs was taken by mixing 2mg of ZnO NPs with 200mg of spectroscopic grade KBr. FTIR spectra were recorded using a Nicolet 520P spectrometer with detector at 4000-400 cm^{-1}resolution and 20 scans per sample.

XRD ANALYSIS

The phase variety and grain size of synthesized zinc oxide was determined by X-ray Diffraction Spectrometry. The synthesized ZnO NPS were studied at a voltage of 30KV and current of 20MA with a scan rate of 0.03^{0}/s. In this work BRUKER D5005 diffractometer is used. Different phases present in the synthesized samples were determined using X'pert high score software with search and match facility. The particle size of the prepared samples was determined by using Scherer's equation as follows:

$D = 0.9\lambda / \beta Cos\ \theta$

Where D is the crystal size, λ is the wavelength of X-ray, θ is the Bragg's angle in radian and β is the full width at half maximum of the peak in radian

PHOTOLUMINISCENCE ANALYSIS

PL spectroscopy is a widely used technique for characterization of the optical and electronic properties of molecules and semiconductors. In this work JASCO spectrometer is used for the analysis

SCANNING ELECTRON MICROSCOPY

SEM analysis was done by Vega3 Tuscan SEM machine. Thin film of the sample was prepared on the carbon coated copper grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

ANTI MICROBIAL ACTIVITY

Bacterial stains were employed to investigate the antibacterial properties of the biosynthesized ZnO NPs. The organisms such as Escherichia coli MTCC 585, Enterobacter, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeroginosa were used to study the antibacterial property. Bacterial stains were streaked on nutrient agar and the single pure culture were streaked on nutrient agar slants and stored at 4^{O}C to keep the stains viable. For this Nutrient agar (for the test and storage) and nutrient growth (sub culture) are used.

RESULTS AND DISCUSSION

UV-VIS ANALYSIS

Optical properties of the prepared zinc oxide nano structure sample were revealed by UV-Vis spectroscopy. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. As the leaf extract is heated with zinc nitrate solution to give light white powder indicating the formation of ZnO NPs**.** The characteristic peak of ZnO nanoparticle in the

UV – Vis absorption spectra is between 300-400nm range. The green synthesized ZnO NPs show an absorption peak at 350nm.

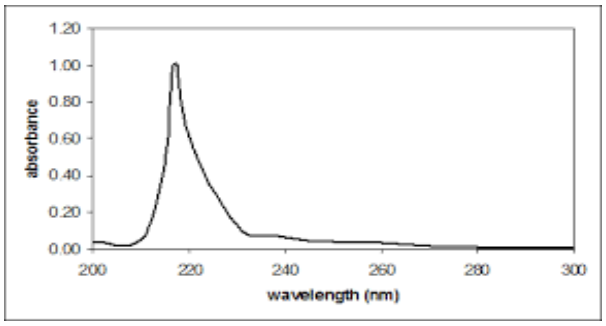

Fig 1: UV-Vis spectra of zinc nitrate

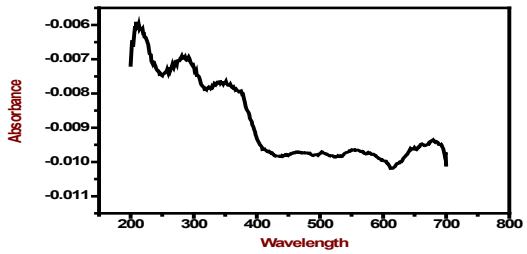

Fig2 : UV- Visible spectra of leaf extract

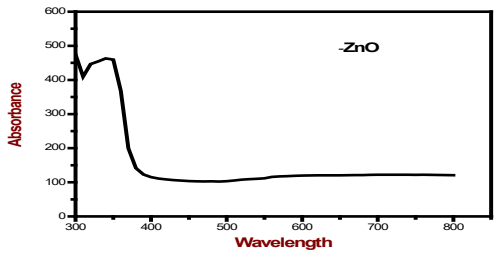

Fig 3 : UV-visible spectra of ZnO nanoparticles

FTIR ANALYSIS

FTIR measurements were carried out to identify the biomolecule for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of ZnO NPs in figure shows that the band in 3411.30 cm^{-1}corresponds to O-H stretching. The peak in 2469.39 cm^{-1} shows P-H bonds corresponding to phosphine. 2174.39 cm^{-1} corresponds to C=C=O stretching. The C=O stretching corresponds to 1557.44 cm^{-1}. 1409 cm^{-1} corresponds to CH_2 bending. C-O stretching correspondence to1117.2 cm^{-1} P-OR ester give the peak at 1020cm^{-1}. =CH_2 stretching gives in 865.44 cm^{-1}. C-H bending is obtained at 654 cm^{-1}.The most important peak of Zn-OH rocking is seen at 548.98 cm^{-1}. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having the above mentioned functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of ZnO NPs in the aqueous medium. Carbonyl groups proved that flavanones or terpenoids were absorbed on the surface of the metal nanoparticles. Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or p-electrons in the absence of other strong ligating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in the reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids. These issues can be addressed once the various fractions of the coleus aromaticus leaf extract are separated, identified and individually assayed for reduction of the metal ions.

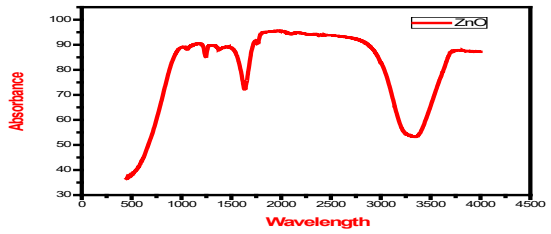

Fig 4: FTIR of Leaf Extract

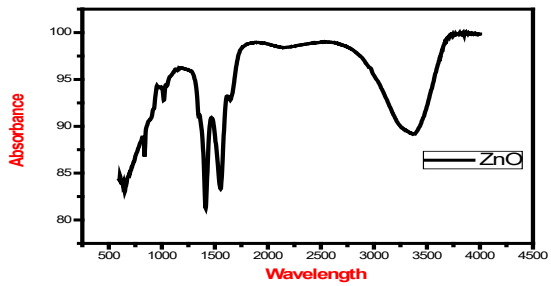

Fig5: FTIR of zinc oxide nanoparticle

XRD ANALYSIS

The diffraction intensities were recorded from 3^0 to 80^0 at 2Θ angles. The figure reveals ten intense peaks in the whole spectrum of 2Θ value ranging from 3^0 to 80^0 corresponding to ten diffraction facets of ZnO nanoparticles. A number of Bragg reflections corresponding to the (100), (002), (101), (102), (110), (103), (200) ,(200), (112), (113) sets of lattice planes were obtained. This corresponds to the Hexagonal Wurtzite structure of ZnO NPs. The average size of the ZnO NPs calculated using Scherer's formula was 21.04 nm. Hence from the XRD data it is clear that ZnO NPs formed using leaves of coleus aromaticus were essentially crystalline in nature.

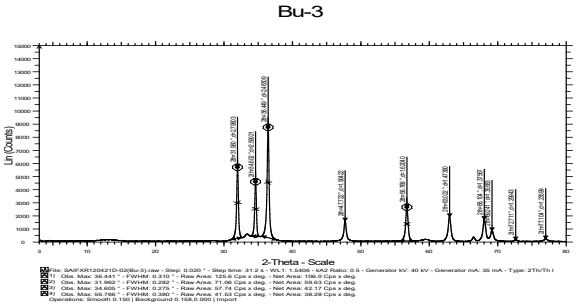

Fig6: XRD pattern of zinc oxide nanoparticles

PHOTOLUMINESENCE ANALYSIS

From the PL analysis, two important peaks are obtained. The first one corresponds to ZnO NPs, which is nearly 400nm range and the second one corresponds to the capping agent. The PL spectra of ZnO nanopowder samples are obtained over wavelength range 350-650nm on irradiating at wavelength 320nm because the excitation energy of the specimen is 320nm. From the spectra, the absorption peak is obtained at386nm corresponding to the excited state in the bulk ZnO. Oxygen has tightly bound 2p electrons and zinc has tightly bound 3d electrons, which sense the nuclear attraction efficiently, which give violet luminescence at 408nm, it is attributed to the transition from conduction band to deep holes. The acceptor states and donor states support the emission at wavelength 439nm (Blue band –I) which decrease with annealing temperature. Recombination of a photo generated hole with an electron occupying the vacancy give band at 483nm. 496nm peak is originated from near conduction band edge to deep acceptor level

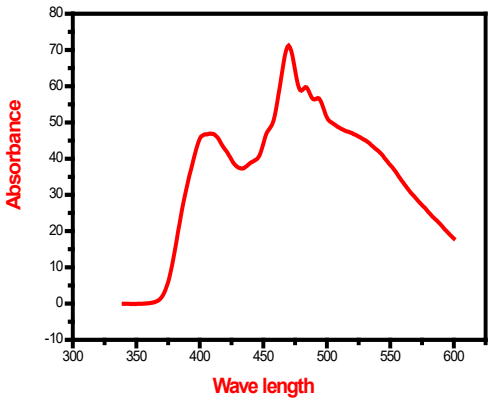

Fig7: PL spectrum of ZnO

SEM ANALYSIS

The morphological studies of synthesized zinc oxide nanoparticles are done by SEM analysis. Zinc oxide has different surface morphologies like nanorods, nanocombs, nanobelts, nanospirals, nanospring and spherical by various authors by adapting different preparation techniques. The SEM analysis revealed that the ZnO NPs were predominantly nanocomb in shape. The SEM image was recorded at 2µm range to find the individual particles. The obtained zinc oxide were observed to be nanocomb with average size between 10-50nm.

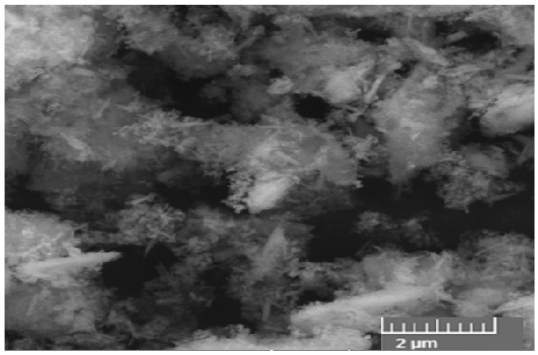

Fig8: SEM image of zinc oxide nanoparticles

ANTIBACTERIAL ANALYSIS

The agar disc diffusion method was employed for screening of antibacterial activities of ZnO NPs. Distilled water is used as a negative control with no inhibition zone and Streptomycin is used as a positive control with clear zone inhibition. The inhibition zone was observed after 24h incubation at 37^0C (**fig 9**:). Result shows that the zinc oxide has considerable antibacterial activity. The antibacterial activity of different solution containing zinc oxide nanoparticle demonstrated that both

Enterobacter, E.coli, Bacillus subtillis, S.aureas and Pseudomonas aurgenosa were inhibited by different solutions with different extent. The synthesized nanoparticles are has considerable effect on E.coli, Basillus subtillis, S.aureas. **Table 1** gives the antibacterial activity of ZnO NPs. At the end of this antimicrobial screening test, it is confirmed that biologically synthesized ZnO NPs possess effective antibacterial activity. Therefore the synthesized ZnO NPs can cover a large domain of medical and food technologies.

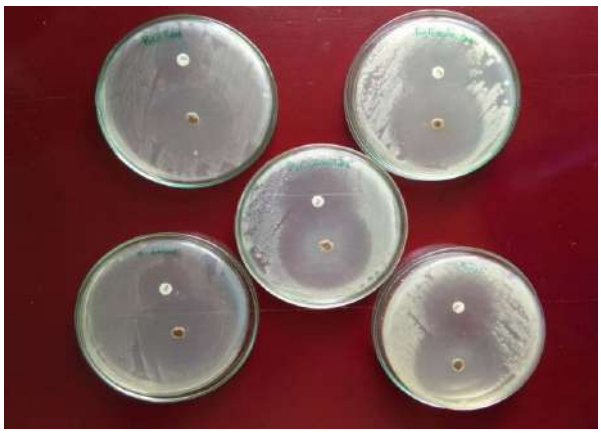

Fig9: Antibacterial activity of zinc oxide against Bacillus, Enterobacter, pseudomonas, E.coli, S.aureas.

CONCLUSION

This study reports green mediated synthesis of ZnO NPs by treating zinc ions with coleus aromaticus leaf extract at room temperature without using any harmful chemicals. The fact is confirmed by ultraviolet- visible absorption spectroscopy(UV-Vis), Fourier Transform Infrared spectroscopy (FTIR) ,X-ray diffraction spectroscopy (XRD), Photoluminescence spectroscopy (PL), Scanning electron microscopy(SEM). The Ultraviolet-Visible spectroscopy results show peak in between 300-400nm which is the characteristic peak of ZnO NPs. From FTIR measurements we can understand biomolecule can act as capping agent and thereby stabilize the synthesized zinc oxide nanoparticles. XRD result shows the hexagonal wurtzite structure of zinc oxide nanoparticles and size of nanoparticle is found to be 21.04nm. The SEM results show that it has nanocomb like structure and the average size is in between 10-50 nm range. PL-studies studies shows four peaks at 386nm,439nm, 483nm and 496nm. The first peak corresponds to ZnO NPs and the other three correspond to capping agent which confirms the bio sensing property of ZnO. Antibacterial activity of the zinc oxide was tested with five bacteria stain and it shows considerable effect on S.aureas, Bacillus subtillis and Escherichia.coli. At the end of this antibacterial screening test, it is confirmed that the biologically synthesized zinc oxide nanoparticle possess effective antibacterial property. Thus, synthesized zinc oxide nanoparticle can cover a large domain of application in the field of medical, food etc.

Micro organisms	Sample (Zone inhibition)	Streptomycin (zone inhibition)
Enterobactin	4.8	5
E.coli	4.5	3
Bacillus subtilis	3.8	2.4
Stephylococcus aureas	4.1	3
pseudomonas	3.7	4

Table 1 : Antibacterial activity of zinc oxide nanoparticles

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Oral Presentation XIII

Hydroxyquinoline derivatives with bromine and iodine atoms: Theoretical investigation by DFT calculations, MD simulations and molecular docking studies

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Abstract
In the present work, DFT characterization and molecular docking studies of of 5,7-dibromo-8-hydroxy quinoline (DBHQ (**1**)) and 5,7-diiodo-8-hydroxy quinoline (DIHQ(**2**)) have been obtained theoretically. The HOMO-LUMO plots in the title molecules show the charge transfer in the molecular system through the conjugated paths. The electrophilic and nucleophilic sites are revealed from the molecular electrostatic potential maps. Thanks to the DFT calculations global and local reactive properties of title compounds have been obtained. MD simulations provided insights into the reactivity with water and with selected proteins. The molecular docking studies reveal that the ligands bind at the active site of the macromolecule and could restrict or block the functioning of Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS), there by acting as antiprotozoal agents.

Keywords: DFT; Quinoline; ALIE; RDF; Docking.

Introduction

Derivatives of quinoline are pharmaceutically and biologically important heterocyclic molecule containing a benzene ring and pyridine ring fused together at nearby two side carbon atoms and are widely used as a source to synthesis of numerous drugs, anti-bacterial, anti-filarial, anti-malarial, anti-fungal, cardiovascular, anti-tuberculosis and as receptor agnosists [1-3]. Quinoline derivatives are used as NLO molecules, optical switching devices, photographic sensitisers and electrochemical sensing devices [4]. Taking into account the importance of computational studies for investigation of reactive properties of various organic molecules [5], in the present study of quinoline derivatives we have also performed DFT and MD studies. Global reactive properties have been investigated by visualization of frontier molecular orbitals and by calculation of well-established quantum molecular descriptors. Surfaces of molecular electrostatic potential (MEP) and average local ionization energies (ALIE) have been obtained in order to assess the reactive properties based on the charge distribution, while Fukui functions also served for identification of possibly important reactive molecular sites. Understanding of degradation properties of pharmaceutical molecules is of great importance from the ecological aspects, since natural weather conditions are usually not enough for their degradation [6]. Oxidative processes are of great importance for degradation of organic molecules [7] and in this regard we have also calculated bond dissociation energies (BDE) for hydrogen atoms, since these quantities are connected with molecule's sensitivity towards autoxidation mechanism. Hydrolysis mechanism is also important since pharmaceutical molecules eventually end up in some type of water. Therefore, in order to understand molecules stability in water, MD simulations have been performed with the latest OPLS3 force field.

2. Computational Details

Jaguar 9.4 [8] program and Desmond [9] program have been also used for computational investigation of new quinoline derivatives. Namely, Jaguar was used for DFT calculations, while Desmond was used for MD simulations, both as implemented in Schrödinger Materials Science Suite 2016-4. B3LYP exchange-correlation functional [10]

has been employed for DFT calculations with Jaguar, with 6-311++G(d,p), 6-31+G(d,p) and 6-311G(d,p) basis sets, for the calculations of ALIE, Fukui functions and BDEs, respectively. OPLS3 [11] force field was employed for MD simulations. Simulation time was set to 10 ns, while temperature was set to 300 K. Pressure was 1.0325 bar, while cut off radius was 10 Å. System was of isothermal–isobaric (NPT) ensemble class, with simple point charge (SPC) model [12] used for the description of solvent. System was modeled by placing of one target molecule into the cubic box with ~2000 water molecules. The method of Johnson et al. [13] was used, as implemented in Jaguar program, for the determination and characterization of noncovalent interactions. Maestro GUI [14] was used for the preparation of input files and analysis of results in the case of Jaguar and Desmond programs.

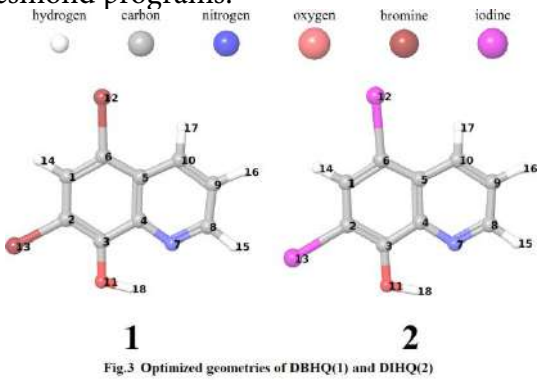
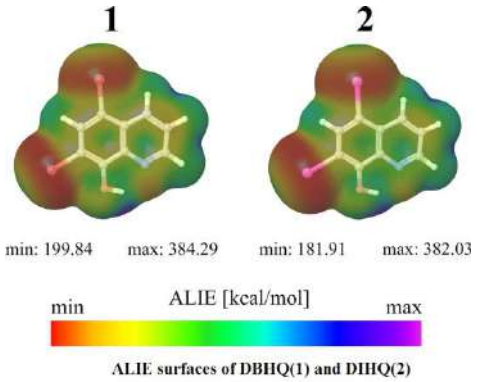


Fig.3 Optimized geometries of DBHQ(1) and DIHQ(2)

3. Results and discussion

3.1 ALIE surface, Fukui functions and noncovalent interactions

We have mapped ALIE values to the electron density surface in order to clearly detect molecule sites where electrons are least tightly bonded and therefore the molecule sites that are prone to electrophilic attacks [15]. Representative ALIE surfaces of two investigated quinoline derivatives have been presented in Figure. ALIE surfaces of DBHQ(**1**) and DIHQ(**2**) provided in Figure indicate that locations of bromine and iodine atoms are characterized by the lowest ALIE values and therefore it can be concluded that these molecule sites are prone to electrophilic attacks. However, it can be also seen that the lowest ALIE value of DIHQ(**2**) is much lower, for ~18 kcal/mol, than the lowest ALIE value of DBHQ(**1**), thus indicating that quinoline derivative with iodine atoms is much more sensitive towards electrophilic attacks. Maximal ALIE values (~382 kcal/mol) are in both cases located in the near vicinity of hydrogen atom of OH group, indicating location where electrons are the most tightly bonded to the molecules. It is also interesting to note that the lowest ALIE value of derivative with iodine atoms is practically matching the lowest ALIE value of the pristine quinoline, which we have reported in our previously submitted paper [16].



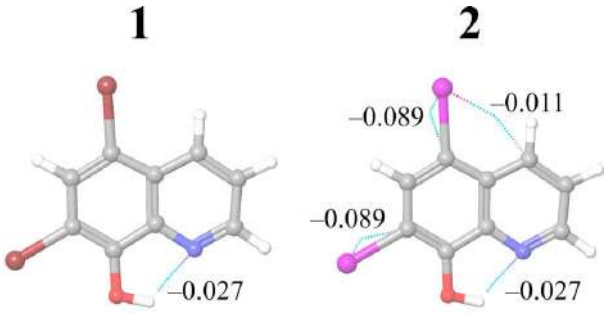
ALIE surfaces of DBHQ(1) and DIHQ(2)

Analysis of electron density between atoms of DBHQ(**1**) and DIHQ(**2**) reveals formation of several noncovalent interactions (Figure) within quinoline derivative with iodine atoms. Namely, in the case of DIHQ(**2**) four noncovalent interactions have been determined, with the strongest one being between iodine and adjacent carbon atoms (with corresponding strengths of –0.089 electron/bohr^3). Other two noncovalent interactions in the case of DIHQ(**2**) involve iodine I12 and carbon C10 atoms, which is the weakest noncovalent interaction, and nitrogen N7 and hydrogen H18 atoms. Only one noncovalent interaction formed in the case of DBHQ(**1**), between nitrogen N7 and H18 atoms, with the same strength as the corresponding noncovalent interaction in the case of DIHQ(**2**).

In this study Fukui functions have been calculated according to the following equations:

$$f^{+} = \frac{\left(\rho^{N+\delta}(r) - \rho^{N}(r)\right)}{\delta}, \qquad (2)$$

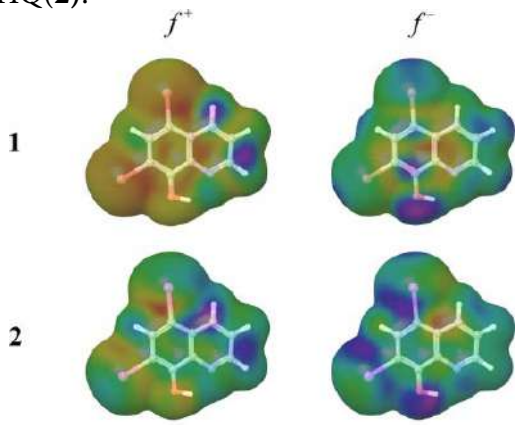
$$f^{-} = \frac{\left(\rho^{N-\delta}(r) - \rho^{N}(r)\right)}{\delta}. \qquad (3)$$



where N stands for the number of electrons in reference state of the molecule, while δ stands for the fraction of electron which default value is set to be 0.01 [17]. The values of calculated Fukui functions have been mapped to the electron density surface, in order to visualize locations where electron density increased/decreased after the addition/removal of charge (Figure). Positive color in Figure in the case of Fukui f^+ functions is the purple one, and indicates molecule sites where electron density increases after the charge addition. On the other side negative color is the red one and in the case of f^- functions indicates molecule sites where electron density decreased after the removal of charge. In terms of position of positive color in the case of f^+ functions it can be seen that quinoline derivatives are very similar. Namely, in both cases purple color of f^+ function is located at two specific sites of the nitrogen containing six member ring (carbon atoms C8 and C10), designating them as the electrophilic molecule sites where electron density increases after the addition of charge. On the other side, although distribution of positive color in the case of f^- function differs significantly, the location of negative color which determines where electron density decreased after the removal of charge is again practically the same for both quinoline derivatives. Namely, in the case of f^- function for both moelcules negative color is located in the near vicinity of carbon atom C5.

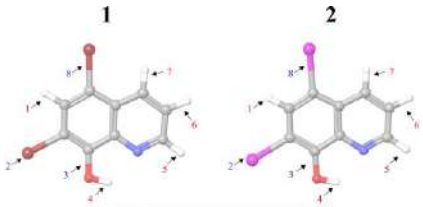
3.2 Reactive and degradation properties based on autoxidation and hydrolysis

Computational investigations of organic molecules based on DFT calculations and MD simulations are of great importance for the understanding of their reactive properties [18]. Taking into account how oxidation reactions are important as degradation pathways of pharmaceuticals and organic materials, in this work we have calculated BDE for hydrogen abstraction (H-BDE), since this quantity can indicate whether some organic molecule is sensitive or not towards autoxidation mechanism [19]. H-BDE values between 70 and 85 kcal/mol indicate sensitivity towards autoxidation mechanism. H-BDE values between 85 and 90 kcal/mol could also be of importance for autoxidation mechanism, but should be treated with caution, while H-BDE values lower than 70 kcal/mol are not appropriate for autoxidation mechanism [5]. Figure contains information about BDE values for all single acyclic bonds of DBHQ(**1**) and DIHQ(**2**).



Fukui functions a) f^+ and b) f^- of the DBHQ(1) and DIHQ(2)

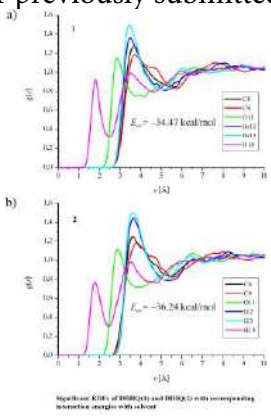
H-BDE values provided in Figure indicate that both newly synthetized quinoline derivatives are highly stable towards autoxidation mechanism. This also indicates that their degradation under natural conditions is hard and imposes the necessity of advanced oxidation processes for their efficient removal. The lowest H-BDE value of ~93 kcal/mol for both derivatives is located on the hydrogen atom of OH group, however this is still higher than the upper border level of 90 kcal/mol. All other H-BDE values are much higher than the desired values and indicate that these molecules are stable in open air and in the presence of oxygen. Concerning the BDE values of the rest of the single acyclic bonds it is also evident that BDE values for the abstraction of iodine atoms are significantly lower (6–7 kcal/mol) than the BDE values for the abstraction of bromine atoms.



Bond number	1	2
1	118.23	116.28
2	72.19	65.00
3	119.48	119.35
4	93.35	93.30
5	112.60	112.58
6	118.97	118.89
7	117.82	117.00
8	71.54	63.39

Besides oxidation reactions, hydrolysis is also important mechanism for the degradation of organic materials. Stability of organic molecules in water by explicit inclusion of water molecules can be computationally investigated thanks to the MD simulations. After MD simulations atoms with pronounced interactions with water can be determined by calculation of the radial distribution functions, which also has been done in this work, Figure.

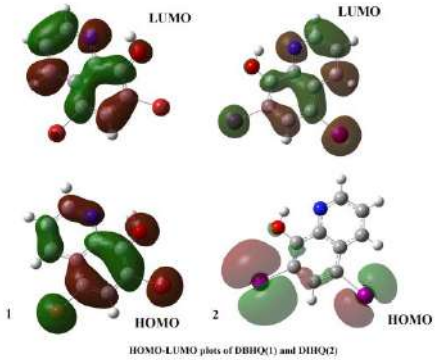
In cases of both newly synthetized quinoline derivatives hydrogen atoms (H18) of OH group have the most pronounced interactions with water molecules. RDFs of these atoms are characterized with the two distinct solvation spheres. The first maximal g(r) values for the RDF of H18 in both cases are located at distance of around 1.7 Å. Other atoms of both derivatives with significant interactions with water molecules are oxygen atoms (O10), bromine/iodine atoms, and carbon atoms C8 and C9. In general, interaction energies of DBHQ(**1**) and DIHQ(**2**) with water according to MD simulations are very similar, further indicating that both of these derivatives have very similar stability in water. However, both of these quinoline derivatives are having higher interaction energies with water than pristine quinoline, for which we calculated interaction energy in our previously submitted article [16].



3.3 Nonlinear optical properties

Nonlinear optics explains the interaction of electromagnetic fields in various materials to produce new electromagnetic fields, altered in wavenumber and other physical properties of the molecular systems [20]. The calculated polarizability of DBHQ(**1**) and DIHQ(**2**) are 2.397×10^{-23} and 2.2882×10^{-23} esu. The dipole moments of DBHQ(**1**) and DIHQ(**2**) are respectively, 3.5783 and 3.8456 Debye. The fist order hyperpolarizabilities are 6.8987×10^{-30} and 8.3872×10^{-30} for DBHQ(**1**) and DIHQ(**2**) which are comparable with the reported values of similar derivatives and these values are 53.07 and 64.52 times that of the standard NLO material urea [21]. The theoretically predicted second order hyperpolarizabilites are -8.062×10^{-37} esu for DBHQ(**1**) and -9.311×10^{-37} esu for DIHQ(**2**). Hence the title compounds and its derivatives are good objects for further studies of nonlinear optical properties.

3.4 Frontier molecular orbital analysis

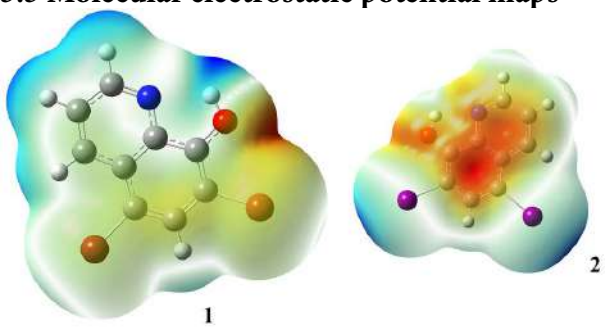


HOMO-LUMO plots of DBHQ(1) and DIHQ(2)

Frontier molecular orbitals are investigated in order to understand global stability and reactive properties of the title compounds. Visualization presented in Figure indicates the importance of iodine and bromine atoms, as HOMO is practically completely delocalized in the near vicinity of these atoms. This result designates iodine and bromine atoms to act as electron donor during the interactions with other molecules. HOMO is delocalized over the entire region of DBHQ(**1**) and except for the ring PhII of DIHQ(**2**).On the other side LUMO orbital is mainly delocalized over the entire rings of DBHQ(**1**) and DIHQ(**2**).Using information on the energies of HOMO and LUMO, useful and frequently used quantum-molecular descriptors such as the ionization

energy and electron affinity can be calculated according to the following simple relations: $I = -E_{HOMO}$, $A = -E_{LUMO}$, $\eta = (-E_{HOMO} + E_{LUMO})/2$ and $\mu = (E_{HOMO} + E_{LUMO})/2$ [22]. Parr et al. [23] proposed the global electrophilicity power of a ligand as $\omega = \mu^2/2\eta$. For the title compounds, energy difference between HOMO and LUMO, HOMO-LUMO gap, are equal to 2.873 eV for DBHQ(**1**) and 0.779 eV for DIHQ(**2**). Ionization potential, I, and electron affinity, A, are calculated to be 8.144 eV, 5.271 eV and 5.880, 5.101 eV for DBHQ(**1**) and DIHQ(**2**), respectively. The values of HOMO-LUMO gap and global hardness (η = 1.4365 for DBHQ(**1**) and 0.3895 eV for DIHQ(**2**) are almost the same as in the case of other similar derivatives that we have previously investigated [24]. Although the stability parameters of these derivatives are practically the same, there are significant differences in the values of chemical potential and global electrophilicity. Also, the calculated electrophilicity of the DBHQ(**1**) and DIHQ(**2**) molecules are 15.66 and 38.701 eV, which is significantly lower than the value of electrophilicity of derivative in the work of Rajeev et al. [24], with the values of 28.29 and 24.40 eV, meaning that the title molecules are much more stable.

3.5 Molecular electrostatic potential maps



MEP plots of DBHQ(1) and DIHQ(2)

Molecular electrostatic potential (MEP) simultaneously displays molecular shape, size and electrostatic potential in terms of colour grading. MEPs map has been found to be a very helpful tool in the analysis of the correlation amide molecular structures with its physiochemical property relationship, including biomolecules and drugs [25]. It provides a visual technique to comprehend the relative polarity of the molecule as shown in Figure. Different values of the electrostatic potential are represented by various colours; red<organge<yellow<green<blue. In the MEP maximum negative region represents the site for electrophilic attack indicated by red colour while the maximum positive region represents nucleophilic attack indicated by blue colour. From the MEP plot of the title compound it is clearly seen that oxygen and ring groups are most electronegative region suitable for electrophilic attack and hydrogen atoms are most electropositive region suitable for nucleophilic attack.

3.6 Natural Bond Orbital (NBO) Analysis

The natural bond orbitals (NBO) calculations were performed using NBO 3.1 program [26] as implemented in the Gaussian09 package at the DFT/B3LYP level. The strong interaction $n_2O_{11} \rightarrow \pi^*(C_3\text{-}C2)$ has the highest E(2) value 37.87 kJ/mol and a very strong interaction has been in $n_1N7 \rightarrow \sigma^*(C5\text{-}C_4)$ with an energy of 10.49 kJ/ mol for DBHQ(**1**) and the strong interaction $n_1C5 \rightarrow \pi^*(C9\text{-}C10)$ has the highest E(2) value 59.36 kJ/mol and a very strong interaction has been in $n_1N7 \rightarrow \sigma^*(C9\text{-}C8)$ with an energy of 10.14 kJ/mol for DIHQ(**2**). Almost100% p-character was observed in π bonding of C_1-C6,C3-C2 and the lone pairs of n_2O_{11}, n_3Br13 and n_3Br12 for DBHQ(**1**) and in π bonding of C6-C1,C3-C2 and the lone pairs of n_1C5, n_3I12 and n_3I13 DIHQ(**2**).

3.7 Molecular docking studies

Protozoal organisms are one of the leading agents of mortality in humans [27]. Two leading protozoal organisms viz.; Plasmodium falciparum and Entamoeba Histolytica cause Malaria and amoebiasis respectively [28]. PASS [29] is an online tool to predict the biological activity spectrum of a compound. PASS analysis of the compounds predicts the title ligands to be Antiprotozoal (Amoeba) agents with P_a score of 0.96 and 0.91. To further establish their antiprotozoal activity, we decided to carry out molecular docking studies of the compound against Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) [PDB ID: 3QGT]. The PDB structure 3QGT [30] was selected for docking as the reported structure has been established from X-ray

Crystallographic data with a good resolution of 2.3 Å. Further the enzyme has an attached co-crystallized inhibitor so has a well defined binding site which could be targeted. Molecular docking has recently been used as a convenient tool to get insights into the molecular mechanism of protein ligand interactions [31]. All docking calculations were performed on AutoDock-Vina software [32]. The 3D crystal structure of PfDHFR-TS was obtained from Protein Data Bank. Before docking the ligands, the protein was prepared by removing co-crystallized waters, ligands and co-factors. The AutoDockTools graphical user interface was used to calculate Geisteger charges, add polar hydrogen and partial charges using Kollman united charges. The active site of the enzyme was defined to include residues of the active site within the grid size of 40×40×40 Å. The ligand was prepared for docking by minimizing its energy at B3LYP/SDD level of theory. The most popular algorithm, Lamarckian Genetic Algorithm (LGA) available in Autodock was employed for docking. The docking protocol was tested by docking the co-crystallized inhibitor onto the enzyme catalytic site which showed perfect synergy with the co crystallised ligand with RMSD close to zero. Amongst the docked conformations the best scored conformation predicted by AutoDock scoring function was visualized in DSV, LigPlot and Pymol softwares for ligand–protein interactions.

The molecule binds at the catalytic site of the substrate by weak non-covalent interactions. Amino acid Ile164 forms hydrogen bond with the oxygen atom of hydroxy group attached to ligand DIHQ(2). Phe58 forms π- π interaction with benzene ring of the ligand. DBHQ(1) forms one H-bond with NDP and one with Ile164 in addition to π- π interaction with Phe58. Amino acids Phe58 Asp54 Ile112 Ile164 and NDP surround the ligand molecules and hold it by non-covalent and hydrophobic interactions. Docking scores of -6.7 and -6.3 kcal/mol for DBHQ(1) and DIHQ(2) respectively. These results reveal that the ligands bind at the active site of the macromolecule and could restrict or block the functioning of Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS), there by acting as antiprotozoal agents.

4. Conclusions

The nonlinear optical properties are also predicted theoretically and the calculated NLO properties of the title compounds are greater than that of urea and therefore the title compounds are good objects for further studies in nonlinear optics. The molecular calculations like natural bond orbitals, HOMO-LUMO and molecular electrostatic potential surface were also performed. ALIE surfaces indicate that introduced bromine and iodine atoms are the molecule sites with the lowest ALIE values and therefore the molecule sites that are prone to electrophilic attacks. It is interesting that the lowest ALIE value in the case of derivative with iodine atoms (DIHQ(**2**)) is practically the same as the lowest ALIE value of pristine quinoline. Derivative DIHQ(**2**) is characterized by four intra-molecular noncovalent interactions, among which the strongest are the ones including iodine and the adjacent carbon atoms. Fukui functions indicate rather similar situation for both derivatives, showing that the possibly important reactive sites could be carbon atoms C5, C8 and C10. H-BDE values indicate that the investigated derivatives are stable towards the autoxidation mechanism, while RDFs indicate that the hydrogen atom H18 of the OH group is having the most pronounced interactions with water molecules. Molecular docking studies suggest that the title compounds could restrict or block the functioning of Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS), there by acting as antiprotozoal agents.

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Oral Presentation XIV

Synthesis of Chemosensors from Silver nano

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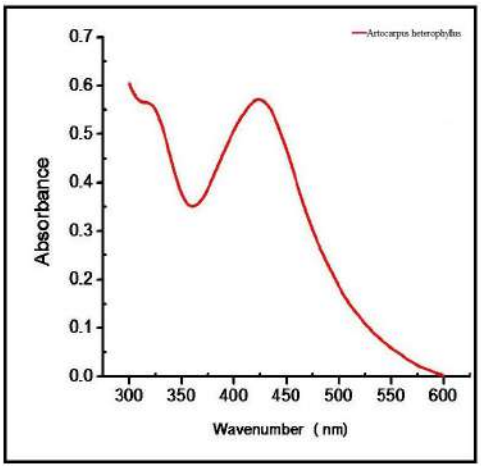
Abstract
silver nanoparticles were green synthesized using different plant substrates like *Biophytum sensitivum* and *Artocarpus heterophyllus* were characterized with Surface Plasmon resonance in ultraviolet spectroscopy (UV–vis) and FTIR. The bio-synthesized silver nanoparticles were changes its color, accompanying the broadening of SPR band upon the addition of mercuric ions into the medium and hence paved way towards the detection of mercury and hence led towards the development of cost effective colorimetric sensors.

Introduction
Chemosensors that are broadly employed in heavy metal ion detection have considerably gained attention because these metals play imperative roles in living systems and have a brutally toxic impact on the environment [1, 2]. Because of its widespread distribution in air, water and soil and since it is a toxic element that exists in metallic, inorganic, and organic forms, mercury is regarded as one of the most dangerous metals that pose a serious threat to humanity. It can cause several developmental delays and health problems that can damage the brain, nervous system, kidneys, and endocrine system [3]. Therefore, it is vital to be able to detect and measure the level of Hg^{2+} in both environmental and biological samples under aqueous conditions. The usual synthetic pathway for nanoparticles preparation was substituted by a novel green one pot synthesis using plant extracts. Hitherto ample literature is available in the synthesis of silver due to its wide range of industrial applications [4-9]. But the current work focuses on some selected medicinal plants which are common in the outskirts of kerala. The plants selected varied from medicinal plants. The plants *Biophytum sensitivum* and *Artocarpus heterophyllus* usually found in the premises of every household. Unlike the other plant, *Biophytum sensitivum* is an important ingredient used in various ayurvedic medicines as well as home medications. Though sensors were developed previously for the detection of heavy metals like mercury [10-16]. The major objective of the present work is to effectively utilize these plants towards the synthesis of a very important material namely nano silver which has already occupied a premier position in the arena of nanotechnology.

Experimental
The plants selected for the study include *Biophytum sensitivum* and *Artocarpus heterophyllus* were collected from the campus premises. The extract was prepared using the leaf and stem of *Biophytum sensitivum*, leaf of *Artocarpus heterophyllus*. Silver nitrate used were of analytical reagent grade obtained from Bombey refinery private Ltd. Bioreduction of Ag^{+} was carried out using the prepared extract and the bioreduction was carefully monitored by a UV visible spectrometer and FTIR studies in the 10;1 ratio of extract and Silver nitrate. The nano silver prepared was further used to detect the presence of mercury and the process of mercury detection was also monitored.

Results and Discussion
The presence of silver nanoparticles were confirmed by the SPR and FTIR spectrums.
According to proposed mechanism, it is seen that upon the addition of $HgCl_2$solutionto the fresh unmodified solution of silver nanoparticles, mercury (II) ion attach themselves to the surface of the nanoparticles thereby detaching the stabilizer compounds which were earlier present on the surface. It was due to the presence of this stabilizer molecule that the nanoparticles were earlier stable in solution. Owing to a redox reaction between silver and mercury ions , the zero valent silver nanoparticles transformed themselves into univalent silver ions thereby

changes their color. The Orange-red colour exhibited by the nanoparticles were due to the surface Plasmon resonance. After reduction there was no stable nanoparticles or zero valent silver atom present in the medium to exhibit the novel phenomenon of SPR and the colour changes.



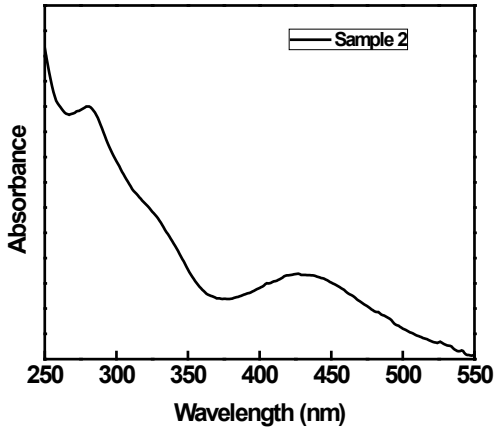
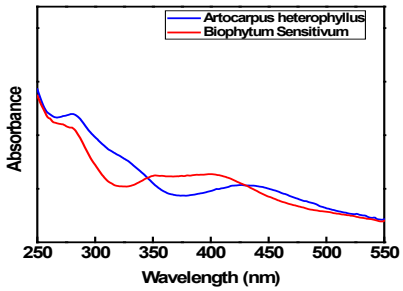


Figure 1: UV spectrum of the nano silver particles obtained by the bioreduction of silver nitrate using Biophytum sensitivum and Artocarpus heterophyllus plant precursors.

After the addition of mercuric ions into the freshly prepared nanoparticles, the colour of the solution faded. The fading of colour was correlated with the shifting of the SPR band in the UV spectrum. Figure 2 shows the curves obtained after the addition of mercuric ions. The results clearly substantiate the detection of mercury by the nanoparticles.



(a)

(b) (c)

Figure 3: (a)UV spectrum of the nanoparticles showing the shift of SPR band after the addition of mercuric ions (b) addition of Mercuric ion to Biophytum sensitivum (c) Mercuric ion to Artocarpus heterophyllus

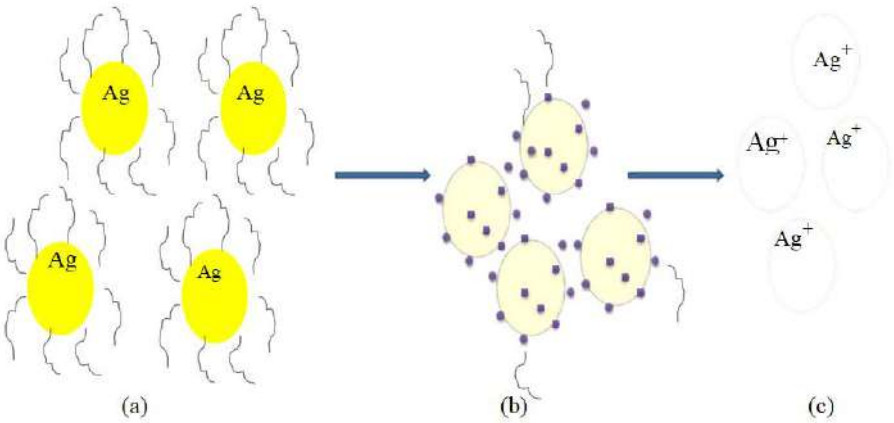


Figure :2 The schematic representation of the detection of mercury (a) Stabilization of silver nanoparticles by the biological stabilizers (b) Replacement of stabilizer by mercuric ions (c) Formation of silver ions after redox reaction

FTIR has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules which is used to search the chemical composition of the surface of the silver nanoparticles and identify the biomolecules for capping and efficient stabilization of the metal nanoparticles . There were many functional groups present which may have been responsible for the bio-reduction of Ag+ ions. The band intensities in different regions of the spectrum for plant extract and silver nanoparticles were analyzed and are shown in Figure

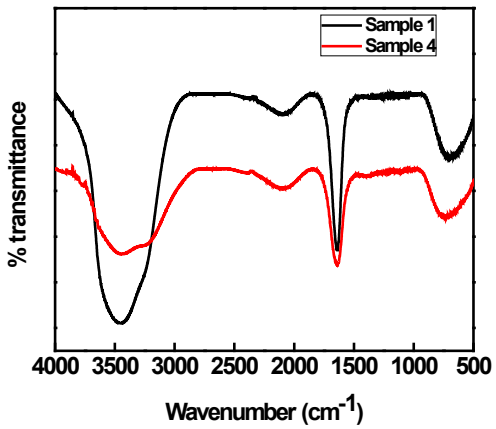


Figure 4: FTIR spectrum of Biophytum sensitivum (sample1) and Artocarpus heterophyllus (sample 4)

FTIR spectrum shows different major peak positions at 3464, 2073, 1639, 868 and 628 cm-1. The similarities between the spectra with some marginal shifts in peak position clearly indicate the presence of the residual plant extract in the sample as a capping agent to the silver nanoparticles. The peak located at 1639 cm-1 could be assigned to C=O stretching or amide bending [17]. The broad and intense peak at 3464 cm-1 corresponds to OH stretching vibrations of phenol/carboxylic group present in extract. It showed peak in the range of 628 cm-1 relating to the alkyl halides band especially the C-Cl bond [18]. Therefore, it may be inferred that these biomolecules are responsible for capping and efficient stabilization of synthesized nanoparticles.

Conclusion

The work presents a novel green synthesis for the preparation of nano silver from plants that are common in the household premises of kerala. Additionally it also aims towards the development of chemical sensors for the detection of mercury in a cost effective manner. Moreover the convergence of green chemistry and nanotechnology has made the entire process more sustainable and ecofriendly.

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Oral Presentation XV

PHOTOCATALYTIC HYDROGEN EVOLUTION BY WATER SPLITTING USING HETERO BIMETALLIC METAL ORGANIC FRAMEWORK

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Abstract
Successful evolution of hydrogen by water splitting is reported using newly synthesized heterobimetallic MOFs of cobalt and nickel as photocatalysts. The MOFs have been synthesized with terephthalic acid by solvothermal method. The MOFs have been characterized using FTIR, PXRD, TEM, EDS and TGA. The activity has been compared with that of single metallic MOFs of cobalt and nickel and also using 2-aminoterephthalic acid. Photocatalytic activity has been analysed using UV-visible and photoluminescence spectroscopy.

Introduction

MOFs have been rapidly emerging as efficient photocatalysts due to their tunable optical properties. MOFs have been extensively explored for their optical properties and are now rationalized with metal centres and linkers in terms of their substituents (Matthew B. Chambers et al., 2017).Transition metals have been found as proficient catalysts for hydrogen evolution. Organic linkers in MOFs can be tuned with suitable substituents for efficient hydrogen evolution. Here in the present study we aimed to generate a highly efficient MOF photocatalyst with 2-amino terephthalic acid as organic linker and cobalt and nickel as its metal centre. The catalytic property of cobalt and nickel in the clusters of MOF can enhance the photocatalytic activity and amino functionalized organic linker can increase the efficiency of its antenna property. The tuning of optical property by incorporation of cobalt and nickel in to the cluster and amino functionalisation together are novel for photocatalytic hydrogen evolution.

Materials and Methods

Nickel Sulphate, Cobalt nitrate, terephthalic acid, 2-aminoterephthalic acid, ethanol, triethylamine, DMF were the chemicals used. Terephthalic acid and 2-aminoterephthalic acid were used as the ligands. FT-IR, PXRD, TEM, EDS, TGA, UV-Visible, Photoluminescence spectroscopy and photocatalytic analysis were used for the characterisation and confirmation of photocatalytic activity of MOFs.

Results and Discussion

1. Characterisation of MOFs

1.1. Bonding characterisation of CoNi@ NH_2BDC photocatalyst using FT-IR spectral Analysis

FT-IR spectra of MOFs of Co@BDC, Ni@BDC, CoNi@BDC, Co@NH_2BDC, Ni@NH_2BDC, CoNi@NH_2BDC are shown in figure 1. The absorption bands between 3440cm^{-1}– 3300 cm^{-1} corresponds to O-H stretching frequency of coordinated water molecules. Absorption peaks at 1629 cm^{-1} and 1369 cm^{-1} belong to stretching vibrations of OCO in organic linker, indicate the presence of terephthalic acid function moiety in the MOFs. The asymmetric stretching of C=O was red shifted to 1596 cm^{-1} and symmetric stretching of C=O to 1370 cm^{-1} (Zhicheng Zhang et al., 2014). The C=O shifted to 100-110 cm^{-1} lower frequency. This demonstrated the coordination generated by cobalt and nickel with COO- group of 2- amino terephthalic acid in amino functionalised MOFs and terephthalicacid.

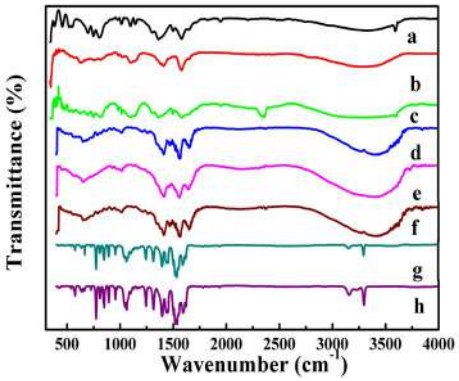

Fig 1. FT- IR spectra of a) Co@BDC b) Ni@BDC c) CoNi@BDC d) Co@NH_2BDC e)Ni@NH_2BDC f) CoNi@NH_2BDC g) 1Co0.5Ni@NH_2BDC h) 1Ni0.5Co@NH_2BDC

1.2. Thermal stability of the metal organic framework photocatalyst

The thermal stability and the weight loss of the photocatalytic metal organic framework can be studied from TG-DTA analysis. TG of the MOFs were performed in the temperature range 100-800 °C with a heating rate of 100 °C/min. TG-DTAs of amino functionalised and normal MOF photocatalyst is shown in fig 4.2.

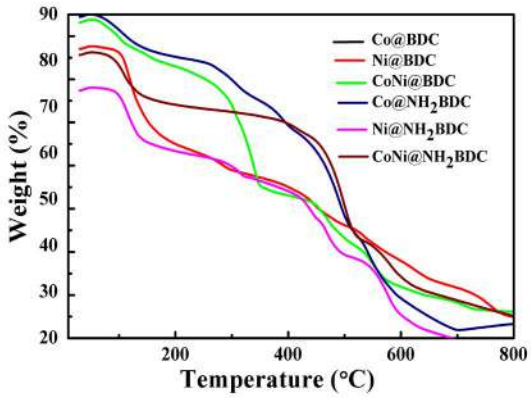

Fig 2. TG-DTA thermogram of MOF photocatalyst

All the unimetallic metal organic framework were stable around 300 °C. Bimetallic metal organic framework photocatalyst was stable around 400 °C while aminofunctionalised was stable arond 410 °C. Unimetallic Co@BDC and Ni@BDC decompose in three stages. The first step between 250-280 °C and is due to the removal of water molecules. Co@BDC and Ni@BDC exhibit a rapid weight loss above 300 °C indicating the fast decomposition of the MOF structure. The second step decomposition occurs between 320-330 °C and this could be due to elimination of –COOH group from the framework. The third step degradation occurs between 425-435 °C which corresponds to phenyl group. The final product after heating upto 800 °C will be cobalt oxide or nickel oxide. Bimetallic CoNi@BDC was stable around 400 °C. The thermogram plot shows weight loss above 400 °C and it corresponds to the organic part. A weight loss at 350 °C may be due to the removal of –COOH group. A rapid weight loss at 410 °C corresponds to the collapse of organic framework and the final product is a mixture of oxides of cobalt and nickel. Amino functionalised unimetallic Co@NH_2BDC and Ni@NH_2BDC were also stable around 300 °C and exhibited a quick weight loss indicating the complete decomposition of organic part. Amino functionalised bimetallic CoNi@NH_2BDC was stable around 450 °C. A rapid weight loss is seen above 450 °C.

1.3. Compositional characteristics of synthesised MOF photocatalyst

EDX analysis was performed to study the composition of elements in the synthesised MOFs. EDX spectra of the prepared MOF photocatalyst are shown in the figure 3. EDX spectra show the presence of expected elements in the MOFs indicating the successful synthesis of MOFs.

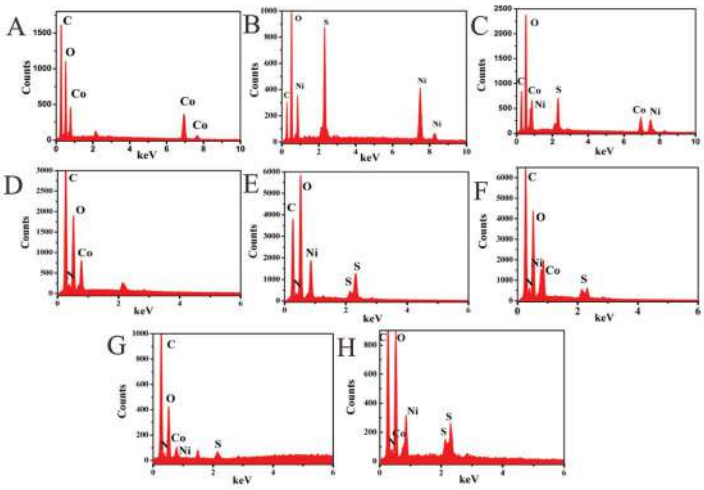

Fig 3. EDX spectra of A) Co@BDC B)Ni@BDC C) CoNi@BDC D)Co@NH_2BDC E) Ni@NH_2BDC F) CoNi@NH_2BDC G) 1Co0.5Ni@NH_2BDC H)1Ni0.5Co@NH_2BDC

1.4. Powder XRD Analysis

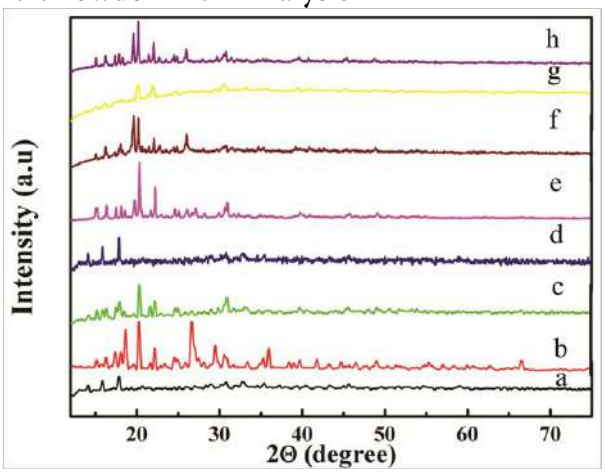

Fig 4. XRD pattern of a) Co@BDC b) Ni@BDC c) CoNi@BDC d) Co@NH_2BDC e) Ni@NH_2BDC e) CoNi@NH_2BDC f) 1Co0.5Ni@NH_2BDC g) 1Ni0.5Co@NH_2BDC photocatalyst autoclaved at 150 °C.

X-ray diffraction analysis can be used to study the crystalline characteristics and diffraction angles of the catalysts. XRD pattern also give information regarding the phase purity of the photocatalysts. Figure 4 shows the XRD pattern of MOF photocatalysts. CoNi@BDC prepared using Co and Ni precursors and terephthalic acid exhibited all the peaks obtained in Co@BDC and Ni@BDC. Co@NH_2BDC and Ni@NH_2BDC exhibit the same diffraction pattern as that of Co@BDC and Ni@BDC. The peaks of CoNi@NH_2BDC were similar to those obtained in CoNi@BDC. The amino functionalised MOFs with different metal ratios exhibited similar XRD results. This revealed that the change in ratio of metals in amino functionalised MOF photocatalysts retained the crystallinity of MOFs.

1.5. Surface state and electronic level interaction studies

XPS spectra can be used to determine the presence of different elements, their oxidation states and electronic environment in the MOFs. The formation of metal organic framework with cobalt, nickel and effect of introduction of amino group in the organic linker of the framework can also be studied. The results of the XPS spectra of Co@BDC, Ni@BDC, CoNi@BDC, Co@NH_2BDC, Ni@NH_2BDC and 1Ni0.5Co@NH_2BDC are shown in figure 5.

In the XPS spectra of Co@BDC binding energies observed at 797 eV, 781 eV, 531 eV and 284 eV correspond to cobalt, oxygen and carbon respectively. XPS spectra of Ni@BDC shows binding energies at 856 eV corresponding to nickel, 284 eV and 288 eV to carbon and 531 eV to oxygen. In the XPS spectra of CoNi@BDC, cobalt possess binding energies at 781.2 eV and 797 eV, binding energies at 856.3 eV and 532 eV corresponds to nickel while oxygen and carbon possess binding energies at 284eV and 288 eV. The XPS spectra of Co@NH_2BDC possess binding energies at 782 eV and 797.3 eV corresponding to cobalt, binding energies at 285.1 ev and 288.8 eV corresponding to carbon and 531.9 eV, 400.03 eV corresponds to oxygen and nitrogen respectively.

In the XPS spectra of Ni@NH_2BDC binding energies at 856.9 eV and 873.9 eV, 531.9 eV and 288.9 eV corresponds to nickel, oxygen and nitrogen and the binding energy at 285.1 eV and 288.8 eV corresponds to carbon. XPS spectra of 1Ni0.5Co@NH_2BDC shows peaks at 781.2 eV and 800.8 eV corresponding to cobalt, and binding energies at 856.3 eV and 873.9 eV, 399.6 eV and 531.9 eV corresponds to nickel, nitrogen and oxygen respectively. Peaks at 285.1 eV and 288.6 eV correspond to carbon.

The binding energies at 781 eV and 797 eV of cobalt in Co@BDC and CoNi@BDc correspond to $2P_{1/2}$ and $2P_{3/2}$ orbitals of cobalt revealing the coordinate bonding of the organic linker with cobalt in Co-O manner in metal organic framework with +2 oxidation state. The binding energy at 781 eV corresponds to the spin–orbit characteristic of Co^{2+} (Huicong Xia et al., 2017). A little increase in the binding energy was observed for amino functionalised Co@NH_2BDC. This may be due to the change in the electronic environment of the framework by amino functionalisation. The binding energies of nickel in Ni@BDC, CoNi@BDC, Ni@NH_2BDC and 1Ni0.5Co@NH_2BDC along with a shake up signal around 862 eV correspond to Ni $2P_{3/2}$ and 873.9

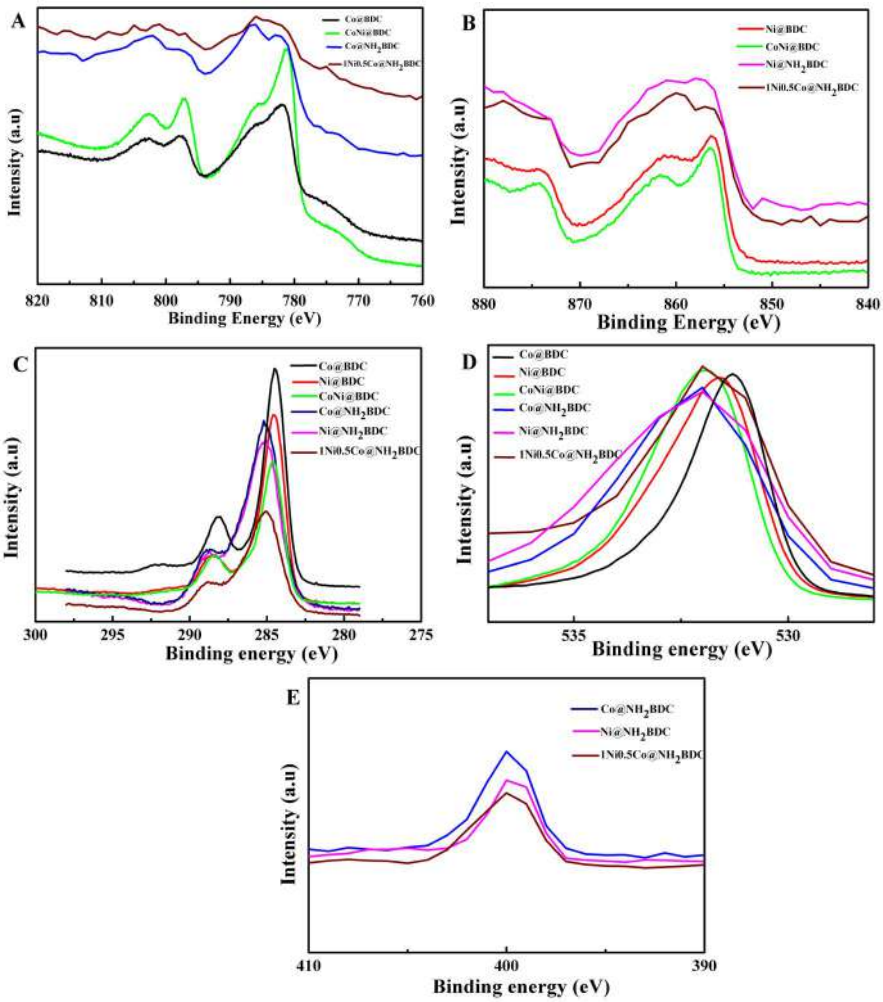

Fig 5. XPS of A) 2p scan of cobalt B) 2p scan of Nickel C) 1s scan of carbon D) 1s scan of oxygen E) 1s scan of nitrogen

eV correspond to Ni $2P_{1/2}$. This concludes that carbonyl bonded Ni is in +2 oxidation state. Since all other peaks were absent it can be confirmed that nickel is coordinated to organic linker in framework (Xin Liang et al., 2017). Carbon in Co@BDC, Ni@BDC and CoNi@BDC possess binding energies around 284 eV and 288 eV while in Co@NH_2BDC, Ni@NH_2BDC and CoNi@NH_2BDC possess binding energies around 285 eV and 288.8 eV. Binding energy between 284 eV and 285 eV can be assigned to C-C bond in the benzene ring of organic linker. The peak centered at 288 eV corresponds to carboxyl group of organic linker (COOH or HOC=O) (Nagy L. Torad et al., 2014). Binding energies of oxygen around 531 eV in Co@BDC, Ni@BDC correspond to carbonyl oxygen (C=O) in organic linker. In the amino functionalised metal organic framework Co@NH_2BDC, Ni@NH_2BDC, 1Ni0.5Co@NH_2BDC, the XPS spectra of nitrogen shows a binding energy around 400 eV corresponding to C-NH_2 bonding in the organic linker.

1.6. Evaluation of surface morphology of CoNi@BDC and CoNi @NH_2BDC

The surface morphology and phase purity of bimetallic CoNi@BDC and CoNi@NH_2BDC was characterized using HRTEM analysis. HRTEM and SAED pattern of CoNi@BDC and CoNi@NH_2BDC are shown in figures 6 and 7 respectively. CoNi@BDC and CoNi@NH_2BDC were comprised of large but thin stacked nano rods, aligned in layer by layer to form a 2-D layered structure which are connected to organic linker through –COOH group to form the final 3D structure. Metals Co and Ni were distributed on the bulk and the instantaneous degradation of the structure during imaging causes the metals to move into the bulk of the organic linker (Yihan Zhu et al., 2017).

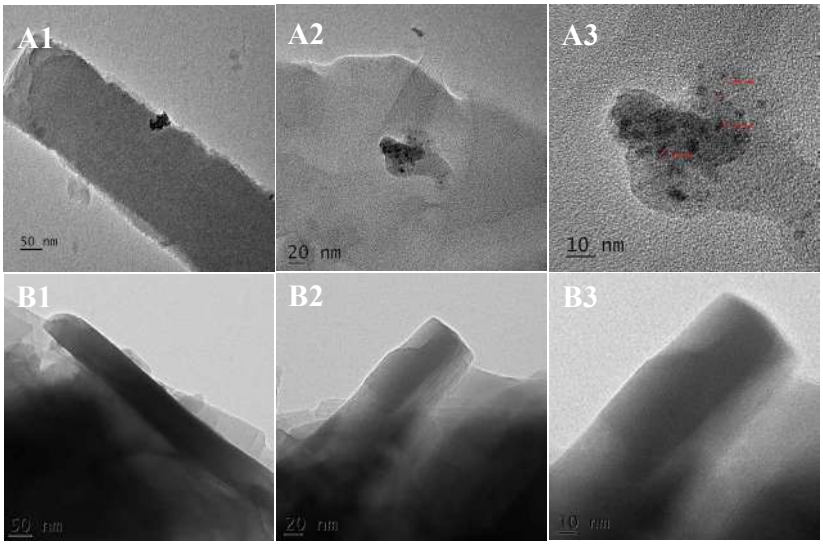

Fig 6. HRTEM of CoNi@BDC (A1, A2, A3) and CoNi@NH_2BDC (B1, B2, B3)

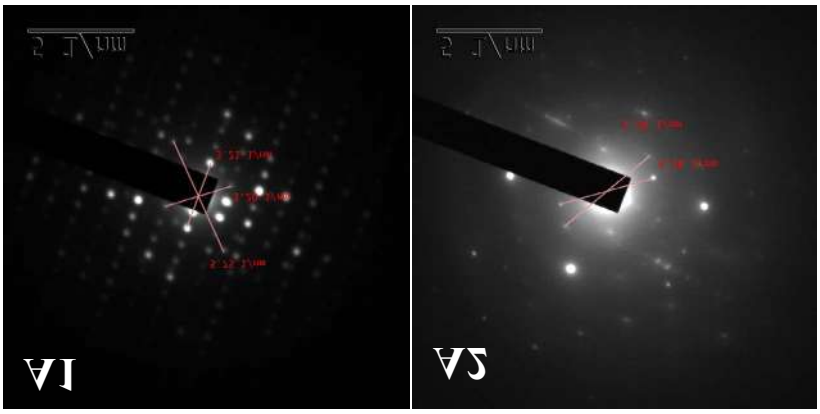

Fig 7. SAED pattern of CoNi@BDC (A1) and CoNi@NH_2BDC (A2)

The SAED analysis was carried out inorder to study the lattice parameters and crystalline nature of MOF photocatalyst. A bright spot of Co and Ni was generated revealing the crystalline nature of MOF photocatalayst with size 2-4 nm. The amino functionalised organic framework showed a decrease in the lattice distance when compared with original MOF photocatalyst. This shows that amino functionalised MOF was nano sized and shows an increase in the photocatalytic activity compared to MOFs containing BDC.

1.7. Evaluation of active surface area of MOF photocatalyst by BET

N_2 adsorption and desorption experiments were performed to obtain detailed information about the specific surface area of MOF photocatalysts. N_2 physisorption measurements were also used to study the porosity of the sample and its corresponding pore size distributions. Fig 8 shows the N_2 adsorption and desorption isotherms of CoNi@BDC and optimised 1Ni0.5Co@NH_2BDC photocatalysts.

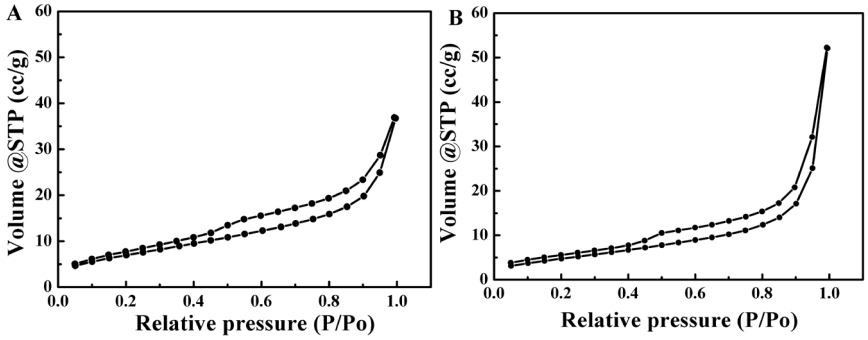

Fig 8. N_2 physisorption isotherms of A) CoNi@BDC B)1Ni.5Co@NH_2BDC

The isotherms shows type III hysterisis loop. This isotherm confirms the formation of a multilayer which is in good agreement with the morphological features analysed from TEM. The surface area was calculated from multi point method. Five points from 0.02 to 0.05 were taken to calculate the surface area. The calculated surface areas of CoNi@BDC and 1Ni0.5Co@NH_2BDC were 2621.9 m^2/g and 1810.3 m^2/g respectively.

2. Photocatalytic characteristics of MOF photocatalyst

2.1 Photoresponse and band gap determination

UV-visible spectroscopic analysis has been carried out in order to study the electronic interaction between the different components and band gap modification of the MOFs. Fig 9 show the UV visible spectra of MOF photocatalysts.

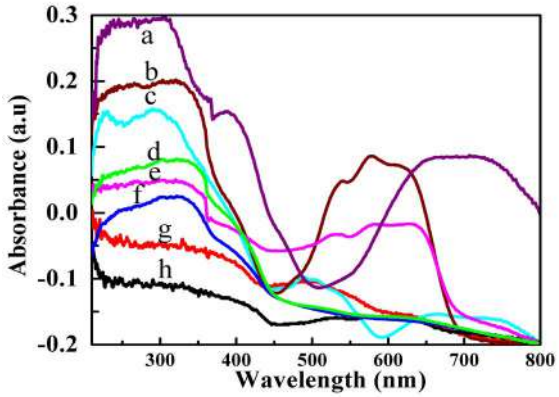

Fig 9. Diffuse reflectance UV-visible spectra of a) 1Ni0.5Co@NH_2BDC b) CoNi@NH_2BDC, c) 1Co0.5Ni@NH_2BDC d) CoNi@BDC e) Co@NH_2BDC f) Ni@NH_2BDC g) Co@BDC h) Ni@BDC

Photo response peaks were observed at about 415 nm and 416 nm for Co@BDC and Ni@BDC. In Co@NH_2BDC and Ni@NH_2BDC these peaks were observed at 500 nm. Bimetallic CoNi@BDC showed photo response at 578 nm. The amino functionalised bimetallic MOF photocatalyst 1Ni0.5Co@NH_2BDC showed a shift in the absorption to the visible region at 685 nm with a band gap of 2.2 eV as obtained from Kubelka-Munk plot (Tauc plot). This MOF showed the best photocatalytic performance, hence is the optimised MOF.

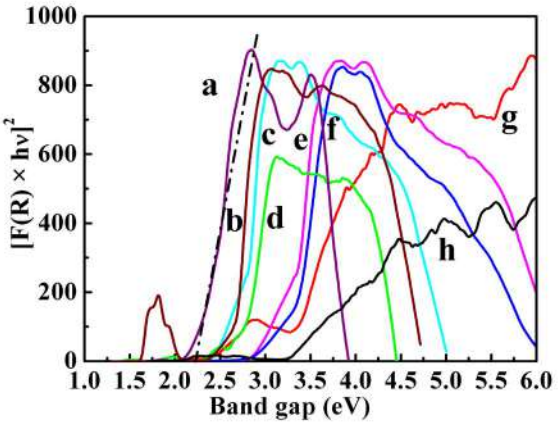

Fig 10. Tauc plot of a) 1Ni0.5Co@NH_2BDC b) CoNi@NH_2BDC, c) 1Co0.5Ni@NH_2BDC d) CoNi@BDC e) Co@NH_2BDC f) Ni@NH_2BDC g) Co@BDC h) Ni@BDC

The amino functionalisation of the bimetallic MOF photocatalysts increased its efficiency of hydrogen evolution because amino functionalisation increase its efficiency to act as antenna for the absorption of visible light in the visible region causing a red shift to the visible region and reducing the HOMO- LUMO bad gap (Yu Horiuchi et al., 2012).

2.2 Analysis of electron hole recombination by photoluminescence spectroscopy

A Photoluminescence spectrum gives information about the transition behavior of electrons in a photocatalyst by recording emission spectra on irradiation of light at particular wavelength. The electron hole recombination of MOF photocatalysts was understood from PL spectra. Figure 11 shows PL spectra of MOF photocatalysts excited at 380 nm.

The spectra show a well defined emission band at 460 nm. PL intensity of bimetallic MOF photocatalysts CoNi@BDC, CoNi@NH_2BDC, 1Co0.5Ni@NH_2BDC and 1Ni0.5Co@NH_2BDC is much lower than those of corresponding unimetallic MOF photocatalysts. Co@BDC, Ni@BDC, Co@NH_2BDC, Ni@NH_2BDC showed a comparatively high PL intensity. Among the unimetallic MOF photocatalysts, aminofunctionalised MOFs showed a slight decrease in its intensity. Aminofunctionalised bimetallic MOF photocatalyst showed a very low peak intensity in which 1Ni0.5Co@NH_2BDC was the most diminished one.

In case of unimetallic MOF photocatalysts, the amino functionalisation shifted absorbance to visible region as it had high absorption energy (E_{abs}) and negative E_{LMCT}, reducing the recombination of photogenerated charges. Introduction of two metals into the framework further decreased the intensity of PL spectra. The observations revealed that the incorporation of two metals reduced the rapid recombination of photogenerated electrons and increase the life time of excited electrons. The presence of two metals increases the rapid relay of electrons to the nodes of MOF photocatalysts for water splitting and thereby hydrogen generation. Amino functionalisation of bimetallic MOF photocatalysts further reduced the PL intensity due to less and negative E_{LMCT}. The result are in good agreement with those reported elsewhere (Xin-Ping Wu et al., 2018). The suitable band gap and least electron hole recombination were achieved by the amino functionalisation of optimised composition of cobalt and nickel in metal organic framework.

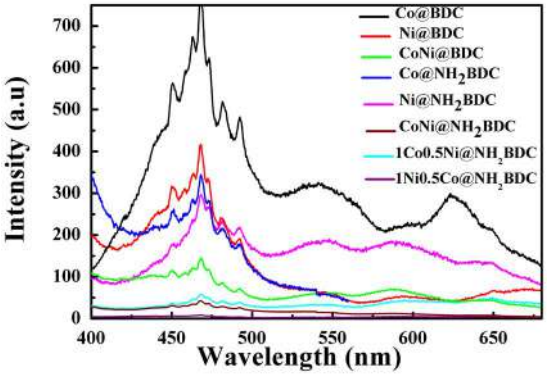

Fig 11. Photoluminescence spectra of MOF photocatalysts excited at 380 nm

2.3 Evaluation of photocatalytic activity of CoNi@NH_2BDC photocatalysts by hydrogen evolution by water splitting

The photocatalytic water splitting efficiency of unimetallic Co@BDC, Ni@BDC, Co@NH_2BDC, Ni@NH_2BDC and bimetallic CoNi@BDC, CoNi@NH_2BDC, 1Co0.5Ni@NH_2BDC, 1Ni0.5Co@NH_2BDC were studied using a solar simulator with irradiation of visible light.

MOFs were dissolved in water with continuous agitation. The reasonable water splitting with the catalyst began only after 30 min of light irradiation of visible light. The hydrogen generated by the catalytic water splitting reaction was collected and was allowed to analyse with GC.

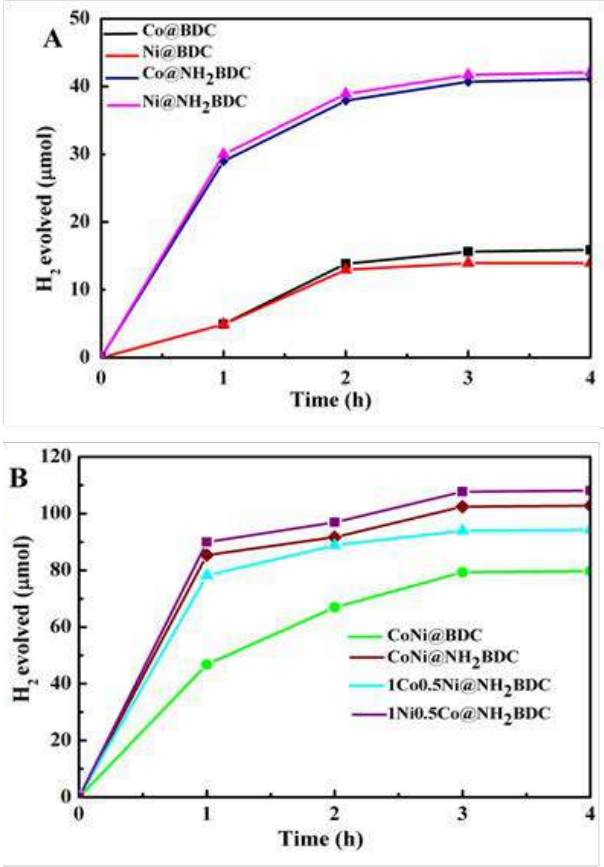

Fig 12. Photocatalytic hydrogen evolution from water splitting using the catalysts A)Unimetallic MOF photocatalysts B) bimetallic MOF photocatalysts during 4 hrs of light irradiation using solar simulator

Co@BDC and Ni@BDC generated 5 µmol of hydrogen after 1 h of light irradiation while amino functionalised Co@NH_2BDC and Ni@NH_2BDC generated 29 µmol of hydrogen. After 4 h of light irradiation Co@BDC and Ni@BDC generated 15 µmol of hydrogen while Co@NH_2BDC and Ni@NH_2BDC generated high rate of hydrogen production of about 42 µmol. The increased rate of hydrogen production in amino functionalised Co@NH_2BDC and Ni@NH_2BDC can be attributed by the presence of auxochrome NH_2 in the organic linker which has a negative E_{LMCT} and high E_{abs}.

In case of bimetallic MOF photocatalysts CoNi@BDC generated 50.7 µmol of hydrogen during first one hour of light irradiation. Amino functionalised bimetallic CoNi@NH_2BDC, 1Co0.5Ni@NH_2BDC, 1Ni0.5Co@

NH_2BDC generated 87.2 µmol, 88.2 µmol and 89.95 µmol of hydrogen respectively during first one hour of the reaction. After 4 h or light irradiaton, CoNi@BDC produced 86 µmol of hydrogen while CoNi@NH_2BDC, 1Co0.5Ni@ NH_2BDC, 1Ni0.5Co@NH_2BDC generated 106 µmol, 107.1 µmol and 108.1 µmol of hydrogen. The increased rate of hydrogen generation by using the bimetallic MOF photo catalysts is due to the presence of two metals in the nodes of framework thereby reducing rapid recombination of photogenerated electrons by fast relay of these electrons to the cluster by Ligand to cluster charge transfer mechanism (LCCT). This increase the lifetime of excited electrons for hydrogen evolution by water splitting.

2.4. Photocatalytic hydrogen evolution of CoNi@NH_2BDC with sacrificial agents

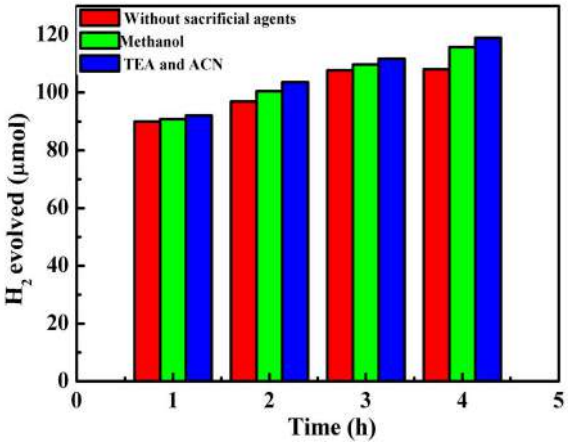

Fig 13. Photocatalytic hydrogen evolution from water splitting using optimised 1Ni0.5Co@NH_2BDC without any sacrificial agents, with methanol and with triethylamine - acetonitrile mixture during 4 hrs of light irradiation using solar simulator.

The hydrogen evolution reaction was reinforced with some selected sacrificial agents. The optimised 1Ni0.5Co@NH_2BDC MOF photocatalyst was studied for hydrogen evolution in the presence of sacrificial agents such as methanol, triethylamine and acetonitrile mixture. 1Ni0.5Co@NH_2BDC exhibited a hydrogen production activity of 89.95 µmol without any sacrificial agents, 90.8 µmol with methanol as sacrificial agents and 92.1 µmol with triethylamine and acetonitrile as sacrificial agents at first one hour of light irradiation. After 4 h of light irradiation 1Ni0.5Co@NH_2BDC generated 108.1 µmol of hydrogen without any sacrificial agents, 115 µmol with methanol and 119 µmol with triethyl amine acetonitrile mixture.

Optical results indicate that the introduction of amino group and optimisation of metal ratios in the clusters of MOF improved the electron transfer efficiency from the organic linker to the clusters of framework.

Conclusions

Heterobimetallic MOFs of Co and Ni have been successfully synthesized and characterized. Their photocatalytic activity has been analysed and confirmed. The crystal characteristics of the metals, Co and Ni were generated in CoNi@ NH_2BDC, more over the phases were confirmed to be similar as the collective phases of single metal organic frame works, Co@NH_2BDC and Ni@NH_2BDC. The symmetric and asymmetric modes of –COO group in the MOF was detected as separated due to the coordination of –COO to the Co and Ni through oxygen atom confirming the formation of the framework. An optimized elemental composition of 1Ni:0.5Co was tuned. The variation in electronic environment of each element by the effect of metals and organic linker was studied by XPS analysis. The change was as expected for the 1Ni0.5Co@NH_2BDC and was suitable for photocatalyzation process. Rod shaped morphology was manifested in CoNi@NH_2BDC by the effective incorporation of metals, Co and Ni in the nodes of organic linker having size between 2-4 nm. The amino functionalised catalyst had visible light response due to effect of linker and metal in the MOF. The band gap of the optimised MOF found to be as low as 2.2 eV with a reduced recombination rate as evidenced from the low intensity of PL spectrum. An amount of 119 µmol of hydrogen was yielded during the 4 h of 1Ni0.5Co@NH_2BDC catalysed photocatalytic water splitting in triethylamine acetonitrile mixture.

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RESEARCH ARTICLE

Evaluating Genetic Diversity Within Genus *Jasminum* L. (Oleaceae) Using Intersimple Sequence Repeats (ISSR) Marker

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Abstract Jasmines are an important group of plants extensively used in the perfumery industry, preparation of garland and for ornamental purposes. To analyze the genetic potential of the group, the authors evaluated its genetic diversity. Intersimple sequence repeats (ISSR) markers were used to evaluate genetic diversity among 40 accessions of 23 *Jasminum* species including six endemic species. The present study is the first report of the efficacy of 10 shortlisted ISSR markers out of 30 primers screened. Among 23 *Jasminum* species, *J. bignoniaceum* revealed maximum genetic diversity and Shannon's Index. Results revealed that the accessions of *Jasminum* species are grouped into three major clades, showing close agreement with the morphological relatedness. The diversity analysis also showed that the members of section *Alternifolia* are the ancestor to the rest of the *Jasminum* spp. The study provides direction for the future population study on *Jasminum* spp. This is the first report on the genetic diversity assessment of Indian *Jasminum* spp. incorporating approximately 50% species.

Keywords Genetic diversity · Intersimple sequence repeats (ISSR) · Jasmines

Significance Statement Genus *Jasminum* is an economically important oil-yielding taxa. Despite its high economic value, no molecular study was done to assess the genetic diversity within the genus in India. ISSR-PCR technique will help to estimate the genetic similarities and evaluate relationships between available accessions of *Jasminum* species in India.

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Introduction

The genus *Jasminum* L. (Oleaceae) includes approximately 200 species, distributed in the tropical and warm temperate regions of the Old World [1, 2]. In India, the genus is represented by 47 species, three subspecies and four varieties, of which 16 species are endemic. The majority of the endemic species have been reported from Eastern and Western Himalaya, Deccan Peninsula, and Andaman and Nicobar Islands [3]. The genus has been subdivided into five sections viz. *Unifoliolata*, *Alternifolia, Jasminum, Trifoliolata,* and *Primulina* [4, 5]. There is much ambiguity within the sectional classification owing to frequent overlapping of the species boundaries as well as phenotypic plasticity arising from the extensive reticulation of exomorphological features (Fig. 1). Jeyarani et al. [6] also concluded in their work on Indian Jasmines that the morphology-based sectional classification is not monophyletic. This in-turn calls for an in-depth systematic analysis of the group, employing different marker systems.

Jasmines are characterized by highly fragrant flowers, especially in *J. grandiflorum*, *J. sambac,* and *J. auriculatum* which are the three main species currently grown commercially for jasmine oil production. The genus *Jasminum* and other economically important genera within the family have been worked out by several workers who tried to trap the genetic diversity within the group. Of which Mukundan et al. [7] conducted a preliminary molecular

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Fig. 1 **a** *Jasminum grandiflorum* L.; **b** *Jasminum coarctatum* Roxb; **c** *Jasminum flexile* Vahl; **d** *Jasminum azoricum* L.; **e** *Jasminum angustifolium* (L.) Willd. var. *angustifolium*; **f** *Jasminum trichotomum* Heyne; **g** *Jasminum mesnyi* Hance; **h** *Jasminum bignoniaceum* Wall. ex A. DC

study involving 32 cultivars of *Jasminum* species using 140 RAPD primers. Taxa belonging to the family Oleaceae were examined by Besnard et al. [8] using the RFLP marker. Rosa et al. [9] constructed the first linkage map in the *Olea europaea* genome using random RAPD and AFLP markers and a few RFLP and SSR co-dominant markers. Carriero et al. [10] obtained a small insert genomic library of *Olea europaea*, highly enriched in (GA/CT)n repeats. Sensi et al. [11] produced a highly consistent AFLP banding pattern using automated ALF express II DNA sequences. Lopes et al. [12] studied genetic variability within intra-cultivar Iberian olive cultivars.

Jasminum species have been shown to have a large interspecific diversity. Morphologically, jasmines can be

Table 1 Taxa, collection sites and accession numbers of *Jasminum* used in the present study

S. no.	Botanical name	Accession no.	Collection sites	Latitude	Longitude	Morphological features (modified after Jeyrani et al. 2018)
1	*Jasminum grandiflorum* L.	JR012	Mundakkal, Kerala	8°51′57″	76°36′34″	White flowered species with pinnately compound, opposite leaves
2	*Jasminum grandiflorum* L.	JR028	Kamala Nehru Ridge, New Delhi	28°41′5″	77°12′57″	White flowered species with pinnately compound, opposite leaves
3	*Jasminum polyanthum* Franch.	JR026	Ooty, Tamil Nadu	11°25′8″	76°42′47″	White flowered species with pinnately compound, opposite leaves
4	*Jasminum bignoniaceum* Wall. & G. Don	JR023	Munnar, Kerala	10°2′15″	77°1′45″	Yellow flowered with compound, alternate leaves
5	*Jasminum bignoniaceum* Wall. & G. Don	JR041	Frog Hill View, Tamil Nadu	11°29′27″	76°31′49″	Yellow flowered with compound, alternate leaves
6	*Jasminum mesnyi* Hance	JR022	Munnar, Kerala	10°5′9″	77°4′5″	Yellow flowered with trifoliate leaves
7	*Jasminum mesnyi* Hance	JR033	University of Delhi North Campus, New Delhi	28°41′18″	77°12′40″	Yellow flowered with trifoliate leaves
8	*Jasminum affine* Wight	JR014	Chavara, Kerala	8°59′52″	76°31′42	White flowered with trifoliate opposite leaves
9	*Jasminum auriculatum* Vahl	JR013	Amaravila, Kerala	8°23′6″	77°5′52″	White flowered with trifoliate opposite leaves
10	*Jasminum auriculatum* Vahl	JR034	Banglore, Karnataka	12°56′55″	77°35′8″	White flowered with trifoliate opposite leaves
11	*Jasminum brevilobum* DC.	JR002	Chinnar WLS, Kerala	10°19′12″	77°12′20″	White flowered with trifoliate opposite leaves
12	*Jasminum brevilobum* DC.	JR035	Maruthumalai, Tamil Nadu	11°2′43″	76°51′26″	White flowered with trifoliate opposite leaves
13	*Jasminum azoricum* L.	JR019	Mundakkal, Kerala	8°52′48″	76°36′12″	White flowered with trifoliate opposite leaves
14	*Jasminum flexile* Vahl	JR003	Nilambur, Kerala	11°16′47″	76°14′53″	White flowered with trifoliate opposite leaves
15	*Jasminum flexile* Vahl	JR036	Palode, Kerala	8°43′18″	77°1′27″	White flowered with trifoliate opposite leaves
16	*Jasminum calophyllum* Wall. & G. Don	JR016	Agasthyamala Biosphere Reserve, Kerala	8°18′9″	76°53′77″	White flowered with trifoliate opposite leaves
17	*Jasminum calophyllum* Wall. & G. Don	JR037	Thenmala, Kerala	8°57′32″	77°3′48″	White flowered with trifoliate opposite leaves
18	*Jasminum caudatum* Wall. ex Lindl.	JR007	Valiyathannimoodu, Kerala	8°40′49″	77°1′20″	White flowered with trifoliate opposite leaves
19	*Jasminum caudatum* Wall. ex Lindl.	JR039	Rosemala, Kerala	8°55′14″	77°10′28″	White flowered with trifoliate opposite leaves
20	*Jasminum agastyamalayanum* Sabeena, Asmitha, Mulani, E.S.S. Kumar & Sibin	JR017	Agasthyamala Biosphere Reserve, Kerala	8°19′34″	76°50′57″	White flowered with trifoliate opposite leaves
21	*Jasminum sambac* (L.) Aiton	JR009	Chirayinkeezhu, Kerala	8°39′9″	76°47′7″	White flowered with unifoliate opposite leaves
22	*Jasminum sambac* (L.) Aiton	JR031	Morakkala, Kerala	10°1′12″	76°22′54″	White flowered with unifoliate opposite leaves
23	*Jasminum sambac* (L.) Aiton	JR008	Kollam, Kerala	8°52′32″	76°35′25″	White flowered with unifoliate opposite leaves
24	*Jasminum sambac* (L.) Aiton	JR032	University of Delhi North Campus, New Delhi	28°41′17″	77°12′40″	White flowered with unifoliate opposite leaves
25	*Jasminum sambac* (L.) Aiton	JR010	SL Puram, Kerala	9°37′15″	76°19′23″	White flowered with unifoliate opposite leaves
26	*Jasminum multiflorum* (Burm.f.) Andrews	JR018	Pallikkara, Kerala	10°1′23″	76°24′1″	White flowered with unifoliate opposite leaves
27	*Jasminum multiflorum* (Burm.f.) Andrews	JR040	Kozhikkode, Kerala	11°15′43″	75°46′4″	White flowered with unifoliate opposite leaves

Table 1 continued

S. no.	Botanical name	Accession no.	Collection sites	Latitude	Longitude	Morphological features (modified after Jeyrani et al. 2018)
28	*Jasminum multiflorum* (Burm.f.) Andrews (iruvachi)	JR020	Chittur, Kerala	10°40′47″	76°45′18″	White flowered with unifoliate opposite leaves
29	*Jasminum ritchiei* C.B. Clarke	JR006	Sulathan bathery, Kerala	11°39′56″	76°15′45″	White flowered with unifoliate opposite leaves
30	*Jasminum coarctatum* Roxb.	JR001	Palode, Kerala	8°43′11″	77°01′25″	White flowered with unifoliate opposite leaves
31	*Jasminum coarctatum* Roxb.	JR030	Munnar, Kerala	10°2′12″	77°01′5″	White flowered with unifoliate opposite leaves
32	*Jasminum angustifolium* (L.) Willd.	JR021	Morakkala, Kerala	10°1′45″	76°23′22″	White flowered with unifoliate opposite leaves
33	*Jasminum angustifolium* (L.) Willd.	JR029	Pallimukku, Kerala	8°52′33″	76°37′15″	White flowered with unifoliate opposite leaves
34	*Jasminum angustifolium* var. *sessiliflorum* (Vahl) P.S. Green	JR024	Wagamon, Kerala	9°41′2″	76°54′45″	White flowered with unifoliate opposite leaves
35	*Jasminum malabaricum* Wight	JR004	Madayippara, Kerala	12°1′56″	75°15′24″	White flowered with unifoliate opposite leaves
36	*Jasminum cordifolium* Wall. & G. Don	JR015	Agasthyamala Biosphere Reserve, Kerala	8°18′9″	76°53′77″	White flowered with unifoliate opposite leaves
37	*Jasminum cordifolium* Wall. & G. Don	JR038	Panamaram, Kerala	11°44′15″	76°4′29″	White flowered with unifoliate opposite leaves
38	*Jasminum laurifolium* Roxb. ex Hornem.	JR011	Thrippunithura, Kerala	9°57′13″	76°20′31″	White flowered with unifoliate opposite leaves
39	*Jasminum cuspidatum* Rottl. & Willd.	JR005	Muthanga WLS, Kerala	11°40′10″	76°21′46″	White flowered with unifoliate opposite leaves
40	*Jasminum trichotomum* B. Heyne ex Roth	JR025	Parambikkulam, Kerala	10°23′32″	76°46′32″	White flowered with unifoliate opposite leaves

either deciduous or evergreen and can be erect, spreading, or climbing shrubs or vines. Their leaves may be opposite or alternate and simple, trifoliate, or pinnate. The flowers are white or yellow [11]. Based on morphological characteristics, several groupings/sections have been proposed for jasmines [12–14]. Mahmood et al. [15] used Randomly Amplified Polymorphic DNA (RAPD) technology to find out the genetic relationship among the 21 accessions of *Jasminum* species. The resulting dendrogram divides the accessions into two distinct clusters. Shekhar et al. [16] applied RAPD PCR for fingerprinting of eight jasmine species. In their study, most of the bands were monomorphic with some polymorphic bands. They did not mention the name of the species studied but showed that this method can be useful for studying genetic diversity polymorphism, cultivar characterization, and genetic population conservation of Jasmine species. Ghehsareh et al. [17] conducted a study using ISSR markers to analyze genetic variations of the fifty-three accessions of Iranian Jasmines representing eight species. Their study demonstrated the efficacy of the ISSR to detect Jasmine genetic diversity and their relationships. Previous studies reveal that no report on the study of the molecular relationship between Indian Jasmine species is available using the ISSR marker. Hence, the objective of the present study was to analyze the genetic diversity and relationship among the *Jasminum* species collected from Peninsular India, by employing ISSR markers.

Material and Methods

Collection of Plant Materials

An extensive survey was carried out to collect Jasmines from different regions of peninsular India (viz. Karnataka, Kerala, Tamil Nadu, and Telangana). Forty accessions belonging to 23 species of *Jasminum,* collected from India mostly from Kerala and Tamil Nadu, corresponding to ca. 23 species, were included in the present study (Table 1). Among the studied accessions, six taxa are endemic to the Western Ghats and Peninsular India (*J. brevilobum*, *J. calophyllum*, *J. agastyamalayanum*, *J. malabaricum*, *J. cordifolium,* and *J. trichotomum*). The voucher specimens of the taxa collected have been deposited in Sree Narayana College Herbarium, Kollam (SNCH), and Tamil Nadu Agricultural University Campus at Coimbatore (MH) herbaria. The details of the plant material with information about collection sites and sample codes are provided in Table 1. The authors have included species from all the sections proposed by De Candolle [4] and Green [5].

Table 2 List of ISSR primers and results of experiments performed among 40 accessions of Indian Jasmine species using ISSR markers

S. no.	ISSR primer code	Primer sequence	Annealing temperature (°C)	Number of bands per primer	Number of polymorphic loci	P_p (%)
1.	UBC-ISSR-807	AGA GAG AGA GAG AGA GT	42	17	17	100
2.	UBC-ISSR-816	CAC ACA CAC ACA CAC AT	47	17	17	100
3.	UBC-ISSR-822	TCT CTC TCT CTC TCT CA	42	13	13	100
4.	UBC-ISSR-825	ACA CAC ACA CAC ACA CT	47	19	19	100
5.	UBC-ISSR-828	TGT GTG TGT GTG TGT GA	47	16	16	100
6.	UBC-ISSR-832	ATA TAT ATA TAT ATA TYC	46	20	20	100
7.	UBC-ISSR-835	AGA GAG AGA GAG AGA GYC	46	15	15	100
8.	UBC-ISSR-838	TAT ATA TAT ATA TAT ARC	49	16	16	100
9.	UBC-ISSR-844	CTC TCT CTC TCT CTC TRC	47	22	22	100
10.	UBC-ISSR-850	GTG TGT GTG TGT GTG TYC	47	20	20	100

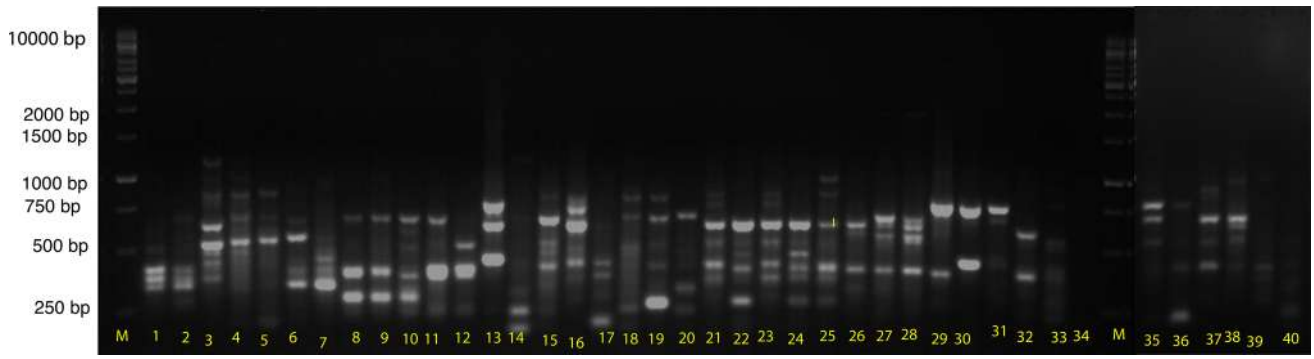

Fig. 2 Fingerprints of 40 *Jasminum* accessions with ISSR-825 primer with 1 kb DNA ladder. The numbers appearing in the gel image corresponds to the serial number of taxa in Table 1

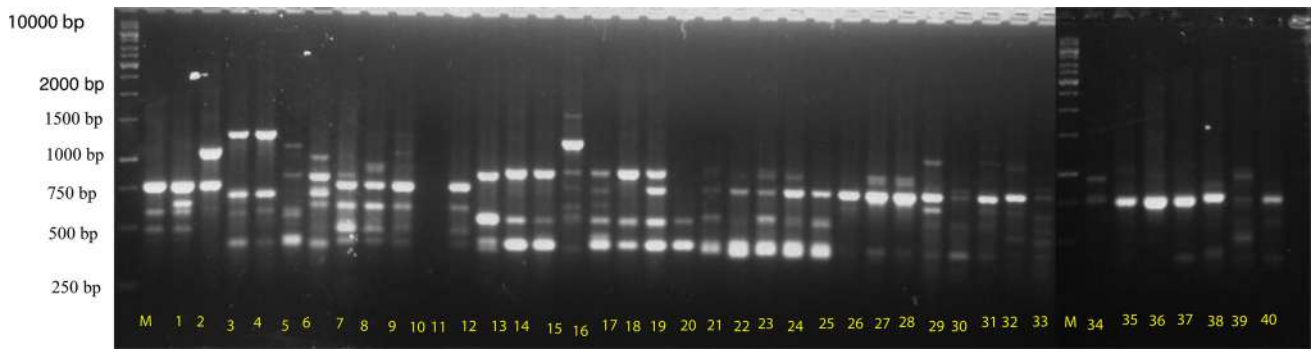

Fig. 3 Fingerprints of 40 *Jasminum* accessions with ISSR-828 primer with 1 kb DNA ladder. The numbers appearing in the gel image corresponds to the serial number of taxa in Table 1

DNA Extraction and Quality Evaluation

Young leaves were collected from wild populations and stored at − 80 °C for further analysis. Genomic DNA was isolated from the leaves following the modified protocol of Doyle and Doyle [18]. The DNA obtained was further purified with PEG (20%) to remove the impurities [19]. The DNA was quantified using a Nanodrop spectrophotometer, and the quality was checked on 1% agarose gel and eventually diluted to obtain a final concentration of 100 ng/µl. The obtained DNA was further analyzed for molecular relationships using ISSR markers.

ISSR-PCR

After preliminary screening with 30 ISSR primers, 10 primers were selected that showed reproducible amplification. PCR reactions were performed in a 25-µl reaction

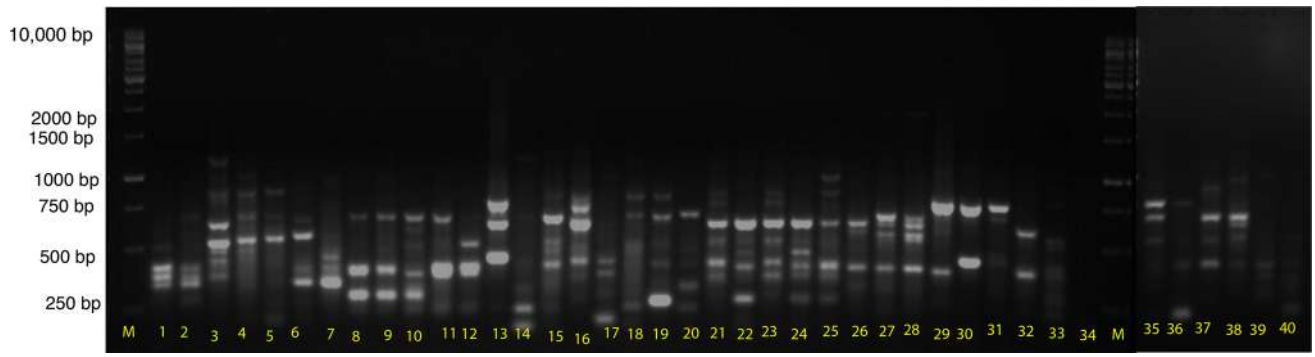

Fig. 4 Fingerprints of 40 *Jasminum* accessions with ISSR-835 primer with 1 kb DNA ladder. The numbers appearing in the gel image corresponds to the serial number of taxa in Table 1

mixture containing 2.5 µl of 10X PCR buffer, 15 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit Taq Polymerase (Sigma, USA), 20 ng of genomic DNA and 20 ng of ISSR Primer (Sigma-Aldrich, USA). PCR was performed using the following program: Initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 54 °C for 60 s and extension at 72 °C for 60 s with a final extension at 72 °C for 8 min. A 96-well applied Biosystems Veriti Thermal Cycler (USA) was used for amplification and repeated all the reactions twice. Amplified fragments were resolved on a 2.0% (w/v) agarose gel in 1X Trisacetate EDTA (TAE) buffer containing ethidium bromide (0.5 µg/ml). A 1 Kb ladder was used to estimate the size of unknown DNA fragments, and gels were photographed under UV using a UVP Gel Documentation system (Jena, Germany).

Data Analysis

Data analysis was carried out by scoring well-marked amplified fragments of ISSR markers. The amplified products were scored for the presence (1) or absence (0) to make a binary data matrix. Genetic diversity parameters including the percentage of polymorphic loci (P_p), the observed number of alleles (n_a), Nei's gene diversity (h) and Shannon index (I) using POPGENE software (Version 1.31) [20] were performed for the present study. The distance matrix was used to construct a dendrogram by the neighbor-joining (NJ) method with 1000 bootstrap replicates, and the principal coordinate analysis (PCoA) was carried out using DARwin version 6.0.155 [21, 22].

Results and Discussion

ISSR markers revealed abundant polymorphism at both interspecific and intraspecific levels, implying that all of them could be applied to germplasm identification and genetic diversity assessment in the genus *Jasminum*. Most informative primers identified from preliminary studies produced 1277 discrete amplified fragments. The amplified bands were in a size range of 250–2000 base pairs with an average of 17.5 fragments per primer combination. ISSR primer UBC-844 produced a maximum number of bands, i.e., 22 and UBC-822 produced a minimum number of bands, i.e., 13 (Table 2). All ISSR primers used in the present study produced 100% polymorphism (Figs. 2, 3, 4).

Genetic diversity parameters calculated based on POPGENE showed that *J. bignoniaceum* possessed maximum Nei's gene diversity (h = 0.2439) and Shannon's Index (I = 0.4061), whereas *J. trichotomum* showed minimum Nei's gene diversity (h = 0.0849) and Shannon's Index (I = 0.1680) (Table 3). The neighbor-joining dendrogram based on dissimilarity matrix indicated that 40 accessions of Jasmines were segregated into three major clusters (Fig. 5). To better understand the relationships among the accessions, PCoA was conducted using the genetic dissimilarities data set. PCoA was largely congruent with the assignments generated by NJ clustering (Fig. 6). The four quadrants accounted for 17.5%, 25%, 25%, and 32.5% of the total variation, respectively.

For the analysis of genetic diversity, it is important to know the type of markers, how many of them represent scorable variation in the entire genome and whether they should be used for diversity estimation [23]. There have been several efforts to transfer agro-economically important genes from wild to cultivated ones through conventional breeding practices [24]. However, knowledge of genetic relationships among various wild species is necessary for successful and efficient exploitation of genetic diversity present in the wild species and such information is not available in the genus *Jasminum*, especially using molecular markers. In this study, the authors used ISSR markers to determine genetic relationship within 40 accessions of 23 species and to determine whether the markers can be effectively used in assessing genetic diversity.

To obtain essential oils from different *Jasminum* species, it is essential to determine the genetic diversity and

Table 3 Genetic diversity indices (Nei's genetic diversity and Shannon's information index) for 40 accessions of *Jasminum* spp. (*SD* standard deviation)

S. no.	Morphological clustering according to sectional classification	Sample size	Observed number of alleles (n_a)	Effective number of alleles (n_e) ± SD	Nei's gene diversity (h) ± SD	Shannon's Information index (I) ± SD
1	*Jasminum grandiflorum* L.	20	2.00	1.23 ± 0.09	0.18 ± 0.11	0.32 ± 0.07
2	*Jasminum grandiflorum* L.	20	1.88	1.20 ± 0.08	0.16 ± 0.09	0.28 ± 0.09
3	*Jasminum polyanthum* Franch.	20	2.00	1.29 ± 0.11	0.21 ± 0.08	0.35 ± 0.09
4	*Jasminum bignoniaceum* Wall. & G. Don	20	2.00	1.33 ± 0.11	0.24 ± 0.08	0.41 ± 0.10
5	*Jasminum bignoniaceum* Wall. & G. Don	20	2.00	1.29 ± 0.09	0.22 ± 0.07	0.38 ± 0.09
6	*Jasminum mesnyi* Hance	20	1.88	1.23 ± 0.09	0.17 ± 0.05	0.30 ± 0.09
7	*Jasminum mesnyi* Hance	20	2.00	1.29 ± 0.11	0.22 ± 0.06	0.37 ± 0.07
8	*Jasminum affine* Wight	20	2.00	1.22 ± 0.08	0.18 ± 0.05	0.32 ± 0.07
9	*Jasminum auriculatum* Vahl	20	2.00	1.29 ± 0.11	0.22 ± 0.07	0.37 ± 0.09
10	*Jasminum auriculatum* Vahl	20	2.00	1.29 ± 0.09	0.22 ± 0.06	0.37 ± 0.07
11	*Jasminum brevilobum* DC.	20	1.88	1.25 ± 0.13	0.19 ± 0.09	0.33 ± 0.14
12	*Jasminum brevilobum* DC.	20	2.00	1.30 ± 0.13	0.22 ± 0.07	0.38 ± 0.10
13	*Jasminum azoricum* L.	20	1.88	1.15 ± 0.13	0.12 ± 0.09	0.23 ± 0.15
14	*Jasminum flexile* Vahl	20	2.00	1.19 ± 0.06	0.15 ± 0.09	0.28 ± 0.09
15	*Jasminum flexile* Vahl	20	2.00	1.24 ± 0.07	0.19 ± 0.04	0.34 ± 0.06
16	*Jasminum calophyllum* Wall. & G. Don	20	2.00	1.29 ± 0.14	0.22 ± 0.09	0.37 ± 0.12
17	*Jasminum calophyllum* Wall. & G. Don	20	2.00	1.22 ± 0.07	0.17 ± 0.11	0.31 ± 0.11
18	*Jasminum caudatum* Wall. ex Lindl.	20	2.00	1.22 ± 0.09	0.18 ± 0.06	0.32 ± 0.09
19	*Jasminum caudatum* Wall. ex Lindl.	20	1.88	1.23 ± 0.14	0.18 ± 0.10	0.31 ± 0.16
20	*Jasminum agastyamalayanum* Sabeena, Asmitha, Mulani, E.S.S. Kumar & Sibin	20	2.00	1.15 ± 0.14	0.12 ± 0.10	0.22 ± 0.14
21	*Jasminum sambac* (L.) Aiton	20	1.77	1.23 ± 0.16	0.17 ± 0.11	0.30 ± 0.19
22	*Jasminum sambac* (L.) Aiton	20	1.88	1.26 ± 0.11	0.20 ± 0.08	0.34 ± 0.14
23	*Jasminum sambac* (L.) Aiton	20	2.00	1.25 ± 0.12	0.19 ± 0.08	0.34 ± 0.12
24	*Jasminum sambac* (L.) Aiton	20	2.00	1.25 ± 0.10	0.20 ± 0.07	0.34 ± 0.10
25	*Jasminum sambac* (L.) Aiton	20	2.00	1.29 ± 0.10	0.22 ± 0.07	0.37 ± 0.09
26	*Jasminum multiflorum* (Burm.f.) Andrews	20	2.00	1.19 ± 0.14	0.15 ± 0.09	0.28 ± 0.13
27	*Jasminum multiflorum* (Burm.f.) Andrews	20	1.88	1.22 ± 0.13	0.17 ± 0.09	0.30 ± 0.14
28	*Jasminum multiflorum* (Burm.f.) Andrews	20	1.88	1.23 ± 0.13	0.18 ± 0.09	0.31 ± 0.14
29	*Jasminum ritchiei* C.B. Clarke	20	1.88	1.18 ± 0.12	0.15 ± 0.09	0.27 ± 0.14
30	*Jasminum coarctatum* Roxb.	20	2.00	1.16 ± 0.07	0.14 ± 0.05	0.26 ± 0.08
31	*Jasminum coarctatum* Roxb.	20	2.00	1.21 ± 0.11	0.17 ± 0.07	0.30 ± 0.10
32	*Jasminum angustifolium* (L.) Willd.	20	2.00	1.26 ± 0.09	0.20 ± 0.06	0.35 ± 0.08
33	*Jasminum angustifolium* (L.) Willd	20	1.77	1.15 ± 0.13	0.12 ± 0.10	0.22 ± 0.16
34	*Jasminum angustifolium* var. *sessiliflorum* (Vahl) P.S. Green	20	1.55	1.07 ± 0.10	0.06 ± 0.08	0.12 ± 0.14
35	*Jasminum malabaricum* Wight	20	1.88	1.14 ± 0.10	0.12 ± 0.08	0.22 ± 0.13
36	*Jasminum cordifolium* Wall. & G. Don	20	1.77	1.14 ± 0.11	0.11 ± 0.09	0.21 ± 0.15
37	*Jasminum cordifolium* Wall. & G. Don	20	1.88	1.17 ± 0.13	0.14 ± 0.09	0.25 ± 0.15
38	*Jasminum laurifolium* Roxb. ex Hornem.	20	2.00	1.22 ± 0.09	0.18 ± 0.06	0.32 ± 0.08
39	*Jasminum cuspidatum* Rottl. & Willd.	20	1.77	1.14 ± 0.11	0.11 ± 0.09	0.21 ± 0.15
40	*Jasminum trichotomum* B. Heyne ex Roth	20	1.77	1.10 ± 0.08	0.08 ± 0.07	0.17 ± 0.12

their relationships within the genus. By the use of DNA profiling using various markers such as RFLP, DAFs, RAPDs, microsatellites, genetic uniqueness can be determined and can be quantified [25]. However, PCR-based markers are more suitable for large-scale analysis [26]. To the best of the existing knowledge, this is the first

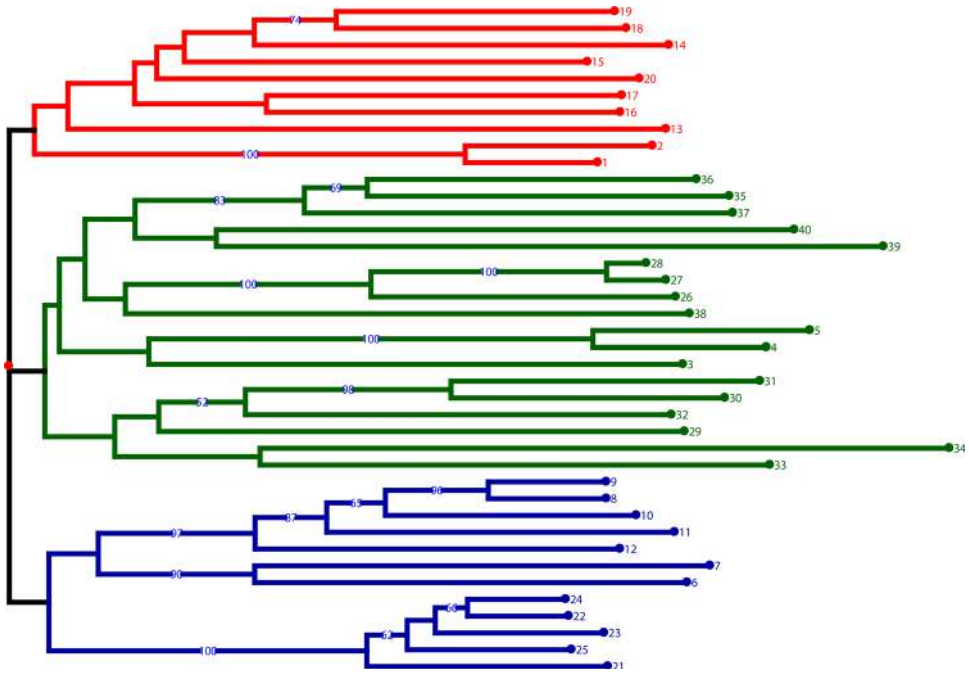

Fig. 5 Dendrogram of *Jasminum* accessions based on the dissimilarity matrix developed using ISSR markers (numbers at the node represent the bootstrap values; NJ tree generated using DARwin software). Respective nodes correspond to serial number of taxa in Table 1

report on the genetic diversity study within the genus *Jasminum* from India incorporating approximately 50% species of which six are endemic.

Morphological Implications Inferred Using ISSR Markers

Neighbor-joining analysis from ISSR markers resulted in the grouping of 23 species represented by 40 accessions into three major clusters. Previous studies from Iran on Jasmines also revealed that ISSR is an effective marker in evaluating genetic diversity [17]. The NJ tree obtained using ISSR analysis consists of three major clusters. The first major cluster (1) consists of 10 accessions of 10 species. These species are characterized by compound and opposite or subopposite leaves. This cluster is divided into two subclusters (A and B). Subcluster A consists of eight accessions of five species which belong to section *Trifoliata* (*J. caudatum* with two accessions, *J. flexile* with two accessions, *J. calophyllum* with two accessions, *J. agastyamalayanum* and *J. azoricum*). This subclade is characterized by trifoliate and opposite or subopposite leaves. The lateral leaflets are slightly reduced in size with odd leaflet. The members are less fragrant except in *J. azoricum*. It is an ornamental climber with equal size pinnae (both lateral and odd) and good fragrant flowers. In this subcluster, *J. calophyllum* and *J. agastyamalayanum* are endemic to the southern Western Ghats. Subcluster B consists of two accessions of *J. grandiflorum* which belongs to section *Jasminum*. The member is an ornamental climber characterized by pinnate, opposite leaves and good fragrance.

ISSR cluster 2 composed of two subclusters (C and D) with 18 accessions of 11 species. Subcluster C consists of 12 accessions of 8 species. *J. polyanthum* (pinnately compound and opposite leaves) and *J. bignoniaceum* (pinnate, alternate leaves and yellow flowers) form a separate group under the subclade. All other species of this subclade belong to section *Unifoliata*, characterized by unifoliate and opposite or subopposite leaves. The flowers of the subclade are less fragrant except *J. cuspidatum* and *J. polyanthum* with a good fragrance. In this subclade, *J. malabaricum* and *J. cordifolium* are endemic to the Western Ghats. Subcluster D composed of six accessions of three species which are characterized by unifoliate leaves.

ISSR cluster three consists of two subclusters (E and F) with 12 accessions of five species. Subcluster E consists of seven accessions of four species which are characterized by trifoliate leaves. In the first group of this subclade, the members consist of a highly reduced or even absence of lateral leaflets. Flowers are white and possess good fragrance. The second group (subcluster F) is represented by two accessions of *J. mesnyi* which belongs to section *primulina* which are characterized by trifoliate leaves and yellow flowers. *Jasminum brevilobum,* endemic to peninsular India, belongs to this subclade. Subcluster F composed of five accessions of *J. sambac* which were characterized by simple, opposite or subopposite leaves and widely cultivated for fragrant flowers.

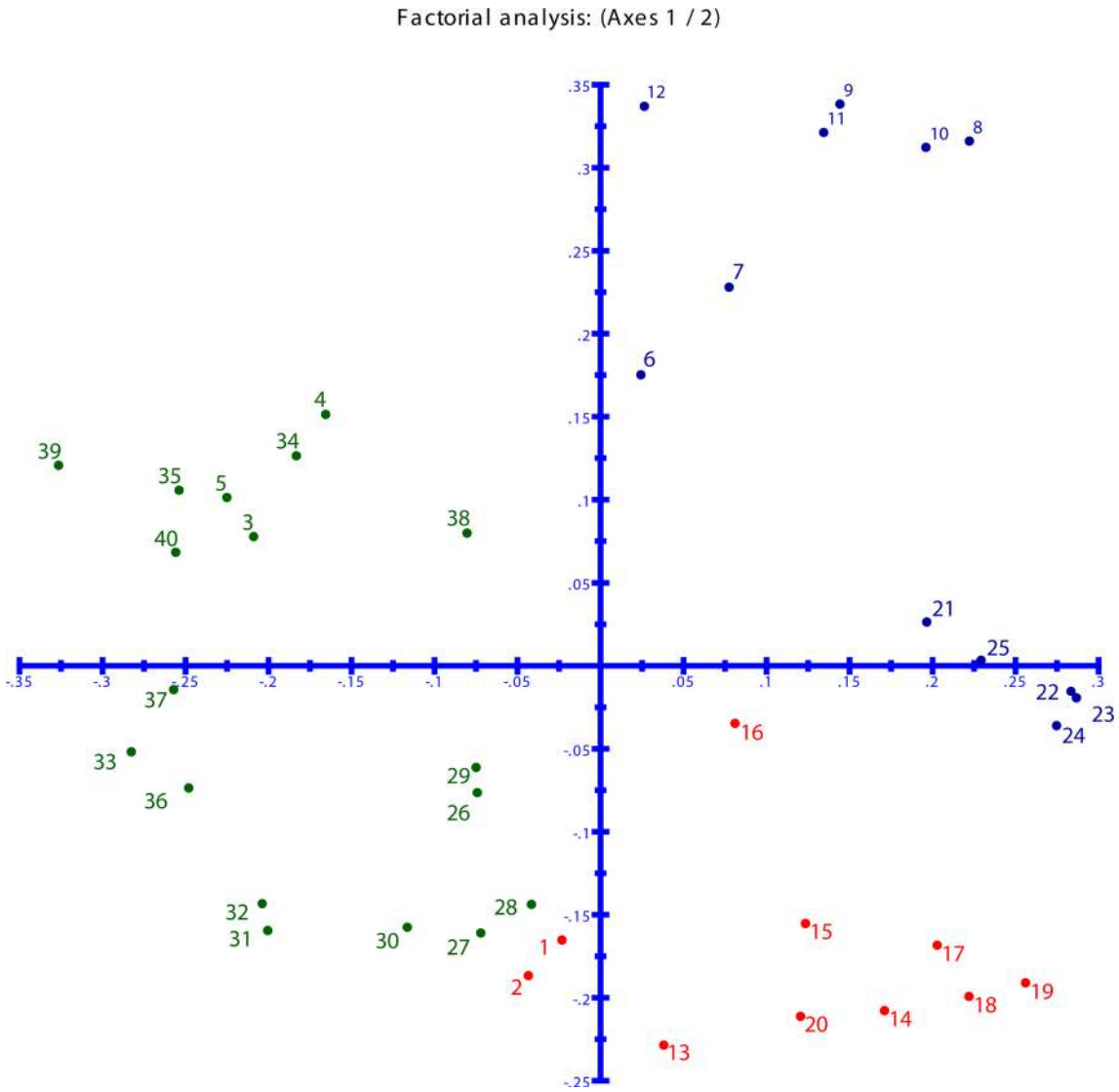

Fig. 6 Principal coordinate analysis of 40 *Jasminum* accessions based on ISSR marker data using DARwin software. Respective numbers in the axes correspond to serial number of taxa in Table 1

Conclusions

In the present study, the authors used ISSR markers to evaluate interspecies diversity in *Jasminum*. To the best of the existing knowledge, this is the first attempt to assess the genetic diversity of Indian *Jasminum* spp. using ISSR markers. All the species possessed high genetic diversity. The present work establishes the usefulness of the ISSR marker in studying genetic diversity and morphological relatedness among *Jasminum* species. The findings will be useful to discriminate closely related accessions.

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Author's contribution AKP conceived the idea and provided all the laboratory facilities at the Department of Botany, University of Delhi and also helped in designing the experiments and finalizing the manuscript. RY collected specimens for herbarium preparation and preserved leaf materials for DNA extraction and also helped in experimental work and manuscript preparation. NJJ helped in collection of materials and manuscript writing. DV helped in experimental design, manuscript preparation, and final discussion. SAR helped in experimental work. SK helped in analysis of data and manuscript writing. JT helped in experimental work, data analysis, and manuscript writing. MDD helped in experimental design, laboratory work, interpretation of data, and manuscript writing.

Compliance with Ethical Standards

Conflict of interest There is no conflict of interest among the authors to publish this manuscript.

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