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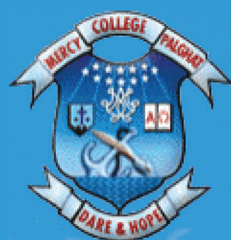

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Editorial

"Accept the challenges so that you can feel the exhilaration of victory".
George S. Patton

The impact of pandemic COVID-19 is observed in every sector around the world. The education sectors of India as well as world are badly affected by this. The outbreak of COVID-19 has advised us that change is inevitable. It has worked as a catalyst for the educational institutions to grow and opt for platforms and techniques, which have not been used before. The education sector has been fighting to survive the crises with a different approach and digitizing the challenges to wash away the threat of the pandemic. The COVID-19 outbreak is a sharp reminder that pandemics, like other rarely occurring catastrophes, have happened in the past and will continue to happen in the future.

The year 2019 marks the 15th year of publication of Scientia (ISSN: 0976-8289), an annual science journal from Mercy college, Palakkad. We are happy to bring out this issue of Scientia which features 14 articles from various areas of science. Increased competition for the publication of scientific research has led to an increased emphasis on determining the perceived "quality" or "status" of a specific journal. After all, scientists, like everyone else, want to publish papers in journals where their work is likely to have the highest impact. Scientia is interested in publishing a wide range of manuscripts presenting original research and reviews in all areas of science. For original research, the common thread is that the work should reveal novel concepts of broad importance to the scientific community.

Categories of papers in this volume include 6 reviews and 9 full papers. Interesting article like Persistent Organic Pollutants-its tale and fate in times of COVID -19, CRISPR/Cas9-mediated gene editing techniques are included in this volume. Scientia congratulates all our contributors and readers for your achievement of 2019 and wishes all of you a Happy New Year.

With warm personal regards

Dr. Jayasree S.
Chief Editor

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A Review of Myctophids in the World Oceans with special emphasis on Indian Ocean

Meera K.M.[1]* and V.N. Sanjeevan[2]
[1]Vimala College (Autonomous) Thrissur, Kerala,India 680009
[2]Kerala University of Fisheries and Ocean Studies, Panangad Road, Madavana, Junction, Kochi, Kerala 682506

Abstract

Mesopelagic fishes are one of the largest underexploited marine resources with wide range of distribution in the world oceans. Myctophids are the key members of mesopelagic communities and their total resource in the world oceans is estimated to be 660 million tons. Family Myctophidae comprises of about 249 species in 33 genera which account for about 65% of total global catch of small mesopelagic fishes. In this paper, an attempt is made to review the existing information on the occurrence and distribution of myctophid resources in the world Oceans.

Key words: Mesopelagic, Myctophids, Lantern fishes, Indian Ocean, Migrations

Introduction

The mesopelagic is the daytime twilight zone in the world oceans between 100 and 1,000 m depth[1]. The mesopelagic fishes constitute 1000 million tonnes of biomass in the world oceans[2] in mesopelagic zones.Myctophids are the most species-rich family of mesopelagic communities in the world's oceans[3]. The family myctophidae commonly known as lantern fishes makes up about 65% of all mesopelagic fishes and has a global biomass estimated at 660 million tons[4]. They are an ancient family of fish present on earth since at least the early Eocene period (≤ 55.8 million years ago)[4,5] now comprising 230-250 species [7,8,9] distributed in all of the world's oceans[3,10,11]. Thus, fishes of the family Myctophidae are an integral part of the trophodynamics of oceanic ecosystems around the world [12].

Habitat and Ecology

Individuals of myctophids are commonly smaller than 10 cm[13,14,15,16,17] but species >15 cm exist [8,17,18]. They have a life span from one year [9] to more than five years [19]. The characteristic large eyes of myctophids are adapted to visual detection of prey and predators, and communication through bioluminescent flashes in dark waters at several hundred meters depth[20]. The fish abdomen is covered in bioluminescent photophores used for counter illumination[21] intraspecific communication such as sexual signaling[22,23]illuminating their surroundings and inducing bioluminescent signals by their prey [23].These photophores are important species/ genus-specific characteristics of the myctophids[7]. They are characterized by rapid growth, early maturity, short life span and high mortality rates [24,3,25.]

During the day myctophids live at great depth, but at night they migrate to surface waters where they feed. They are capable of crossing density gradients such as thermocline and halocline that generally inhibit mixing by physical process and thus invade epipelagic zone during night. Some species show size stratification with depth and some with adults and juveniles are non-migratory. But many myctophids exhibit strong diel vertical migrations [26,27,28,29,30]. During which they are subjected to wide environmental changes in temperature, salinity, Dissolved oxygen etc and these are believed to be feeding migrations.

Distribution of Lantern fish ranges from Arctic to Antarctic waters and surface layers of water at

* *Corresponding author, E-mail: meeravimala2019@gmail.com*

night to depths exceeding 2000 m [31] during day time (Fig.1). The family also includes species known as pseud oceanic, associated with continental shelf and slope regions and in the neighborhood of oceanic islands [32]. Continental slopes encompass a wider set of physical niches, and provide an environment for the development of a recognizable and trophically dependent community of benthic and benthopelagic fish[33]. The down-slope zonation of lantern fish may result from the combined effects of depth and water column structure [34].

Fig.1: Distribution of Myctophid in World Ocean

Distribution in Atlantic Waters

The majority of current knowledge on Atlantic myctophids resulted from the study of the collections of the Woods Hole Oceanographic Institution (WHOI) [31]and InstitutfürSeefischerei[32]. In the south western Atlantic (0º-60ºS), 79 myctophid species under 22 genera were recorded[35,36]. Konstantinova[37] and Figueroa [38] described the distribution of 40 myctophid species, with respect to the water masses between 40º30'- 47º00'S. Off the coasts of Suriname and French Guiana, 15 species from 7 genera were reported [39].In the Eastern Central Atlantic, Wienerroither[40] reported 52 species from the Canarian archipelago. Figueiredo[41]and Santos [42] reported 37 species from off south eastern and southern Brazil (22º-34ºS), with sampling effort concentrated from 100 to 500 m., although this number is lower than that recorded off south eastern and southern Brazil between 22-34ºS (41 species)[41, 43]. Eastern and south-south eastern Brazilian waters share 12 of 16 myctophid genera. Regarding the four genera exclusive within each area, broad or tropical genera (*Centrobranchus, Diogenichthys*, *Lampadena*, *Notolychnus*) occur between 11-22º S, while cold water genera associated with the subtropical currents (*Electrona*, *Gymnoscopelus, Lampichthys, Scopelopsis*) occur between 22-34ºS. Clarke, 1973 reported 47 species under 18 genera from Hawaii, Gartner [44], 49 specie under, 17 genera from eastern Gulf of Mexico (GOM) and Ross[45], 38 species under 17 genera from north-central GOM. Collectively, Brazilian waters have a high diversity of myctophids (79 species, 23 genera[46]) comparable to that registered in the North Atlantic (82 species, 20 genera [31]. The occurrence of larger myctophids from Atlantic with increasing depth has been documented for myctophid fishes caught in trawls [47,48].

Distribution in Pacific Waters

Fishes of the family Myctophidae, are often the dominant component of micronektonic

communities in the North Pacific, achieving very high abundances. They dominate the fish biomass in oceanic waters of the Northeast Pacific [49,3,50], and their transport on to continental shelves represents an important flux of energy into these systems, as represented in food web models of the California Current [51]and biomass estimations[50, 52]. Three lanternfish species (*Tarletonbeania crenularis, Stenobrachius leucopsarus, and Diaphus theta*) form the bulk of micronekton fishes found in the North Californian Current. These 3 species were reported to account for two thirds of all fishes collected in Isaac-Kidd midwater trawl tows in the upper 200 m off Oregon, USA [49,53,54]. The three species of mesopelagic fishes viz., *Lampanyctus leucopsarus, Diaphus theta, Tarletonbeania crenularis* dominated in the Pacific Ocean. Barnett[55]studied species structure and temporal stability of mesopelagic fish assemblages in the Central Gyres of the North and South Pacific Ocean in 1983 and identified 9 myctophids viz*Ceratoscopelus warmingii, Triphoturus nigrescens, Lampanyctus sp., Notolychnus valdiviae, Benthosema suborbitale, Bolinichthys longipes, Lampanyctus steinbecki, Diaphus mollis, Lobianchia gemellarii* in North gyre and 8 myctophids *Notolychnus valdiviae, Ceratoscopelus warmingii, Lampanyctus steinbecki, Diogenichthys atlanticus, Lampanyctus niger, Scopelopsis multipunctatus , Lampadena urophaos, Bolinichthysphotothorax* in the south gyre. Sassa[56] studied assemblages of vertical migratory mesopelagic fish in the transitional region of the western North Pacific and found that myctophidae family was the most speciose representing 17 species in their study. In subarctic and mixed waters of the northern part of the Pacific Ocean, myctophids comprise 80 to 90% of the total catch of micronekton[3]. Mesopelagic fish, *Stenobrachius leucopsarus* collected from this area comprises both migratory and non-migratory populations[49]. *Diaphus theta* is abundantly distributed in the subarctic and transition water of the North Pacific[57]. Wang and Chen[58] reported 40 species of myctophids from the Taiwan and the Tungsha Islands, out of which 17 species were first records from this area.

In the Arctic region myctophid species were unexploited and relatively unperturbed areas. In the southern Newfoundland and Norwegian fjords, *Benthosema glaciale* and the mesopelagic fish, *Maurolicusmuelleri*, are important fishes. These cold-water fish, in places very abundant on the high seas and move from the sub-Arctic to the full Arctic as ice retreats [59.]

Distribution in Antarctic Waters

Sabourenkov[60] reported 20 species of myctophids in the sub-Antarctic and the Antarctic area. The most abundant species were *Electrona carlsbergi, E. antarctica, Protomyctophum anderssoni*, and *Gymnoscopelus nicholsi*. These species predominate over other myctophids both in the sub-Antarctic and Antarctic, and in some places around the Southern Ocean form dense concentrations. In the Antarctic waters to the south of the Antarctic Convergence, 35 species of myctophids are found, i.e. within the CCAMLR Convention Area [61]. Of these 35 species, 11 have circumpolar distributions and are mainly widespread from the Antarctic Polar Front zone (APF) to the edge of the Antarctic continental slope. Other species have more restricted distribution and are found in localized areas in APF waters (eight species in the Atlantic sector of the Southern Ocean, 13 species in the Indian Ocean sector and four species in the Pacific sector). The total biomass of myctophids in Antarctic waters is estimated to be 70-200 million tonnes[62]. Myctophids apparently represent the second largest (after krill) and most widely distributed biological resource in Antarctic waters. Four species of *myctophids Krefftichthys anderssoni, Electrona antarctica, Electrona carlsbergi and Gymnoscopelus nicholsi* having circumpolar distribution, contribute the bulk of the biomass.

Iwami and Kubodra[63] recorded the distribution patterns of 15 species of myctophids from Western Indian Ocean and related areas of Antarctic Ocean (30°S-69°S and 54°E-30°E) and classified them into four types based on their distribution; (1) endemic to the Antarctic water (2) distributed in the northern part of the Antarctic water

and the Sub-Antarctic water (3) distributed in the Sub-Antarctic water; (4) distributed in the Sub-tropical waters. Electrona antarctica and Gymnoscopelus opisthopterus show the pattern of Type 1. Species representing the Type 2 distribution pattern were *Krefftichthys anderssoni, Protomyctophum bolini and Gymnoscopelus braueri. Protomyctophum parallelum, Protomyctophum tenisoni and Lampanyctus achirus* were found only in the Sub-Antarctic water and belong to Type 3. The rest 7 species, *Benthosema suborbitale, Bolinichthys indicus, Ceratoscopelus warmingii, Gonichthys barnesi, Hygophum hygomii, Lampanyctus pusillus and Lobianchia dofleini*, have never been recorded south of the Antarctic Convergence and represented the pattern of Type 4.

Distribution in Indian Ocean

Distribution and abundance of myctophids in the Indian Ocean region have been studied by several authors and they have reported that the myctophids form a major component in the mesopelagic fishes[64,65,66,9,67]. Myctophids form an important component of the acoustically dense Deep Scattering Layers (DSL) [68,69,8]. The abundance of myctophids in the Indian Ocean, mentioned in the International Indian Ocean Expedition (IIOE; 1959-1965) was confirmed by acoustic and trawl survey's by R/V DR. FRIDTJOF NANSEN during 1975-1976[70,71]. These studies estimated a total biomass between 8-20 million tons in the whole Gulf of Oman. The Arabian Sea has one of the world's largest myctophid resource dominated by a single species, *Benthosema pterotum*. The US GLOBEC [65]reported high concentrations of this species along the Western and Central Arabian Sea and estimated its biomass to be around 100 million tonnes. Valinassab[72] reported the life span of this species as less than one year and concluded that 100 million tonnes of *B. pterotum* perish and sink downward yearly. Though the biomass of this species in Arabian Sea was later (2001) revalidated to 48 million tons, it is now recognized that *Benthosema pterotum* is the largest single species stock of fish in the world [64,65,73,66,74]. Other myctophid species like *Benthosema fibulatum, Diaphus spp., Myctophum spinosum and Symbolophorus evermanni* were occasional in number, more common than *B. pterotum* in the Gulf of Aden [75,64,73]and Eastern Arabian Sea [74]. Along the southern Omani and north-eastern Somali coast, *Benthosema fibulatum* dominated trawl collections and acoustic survey records. The Oman fish diversity was studied by Jufaili[76] and reported 9 species of myctophid fishes from Oman waters. In the eastern Arabian Sea, *Diaphus arabicus and Hygophum proximum* are common forms[64,77,73].Along the coast of Pakistan, myctophid concentrations consist almost exclusively of *B. pterotum* with densities decreasing towards the west.[73]

Survey in the western Indian Ocean estimated the presence of 97 species of myctophids belonging to 23 genera [79].*Benthosema pterotum* is the dominant species in the Western and Northern Arabian Sea, followed by *Benthosema fibulatum and Diaphus spp*. In the Gulf of Oman, the acoustic measurements indicated a density of 25-63 *B. pterotum* per m2 surface area [78]. Gjosaeter [78] reported a catch rate of 20 t h-1 of myctophids from the seas off Oman (20°-24°N Latitude (lat) and 57°-67°E Longitude (long)) at a depth of 130 m during day time using a pelagic trawl. Myctophid catches exceeding 400 $kg.h^{-1}$ were obtained from several stations located in north-western Arabian Sea (0°- 26°N; 43°-67° E long). Dalpadado and Gjosaeter [80]reported the presence of 16 species of myctophids in the area 07°06'-08°27'N lat; 79°29'- 81°59'E long, off Sri Lanka, during the cruises with R.V. "Dr. Fridtjof Nansen". Kinzer-[77]reported the presence of 11 species of myctophids from 18°- 24°30'N lat; 62°- 67°E in the Arabian Sea. *Diaphus arabicus* was the dominant species between18° and 24°N in the Arabian Sea, contributing 66-73% of the myctophid samples, in terms of numbers [77]. Observations on the mesopelagic fishes taken by mid water trawl in the equatorial region (03°S-03°N lat; 76°-86°E long) of Indian Ocean shows that the average catch of myctophids was higher in the southern side of the equator when compared with the northern side [81].

Distribution and Ecology in Arabian Sea

The ecology of the mesopelagic fauna in the eastern Indian Ocean was studied by Legand and Rivaton[82, 83]. Similar ecological studies covering the more southerly parts of the western Indian Ocean were carried out by [84,85]. Aspects on the distribution and ecology of the myctophidae from the Western and Northern Arabian Sea and abundance of lanternfish (myctophidae) in the Western and Northern Arabian Sea were carried out by [70,78]. Potential exploitable micronektons from the Deep Scattering Layers (DSL) of the Indian EEZ was studied by [85]. He found that myctophids appear in large shoals / swarms in the North West part of Indian EEZ with a decreasing trend from north to south.Echo sounder records show that many myctophids aggregate in compact layers, especially during daytime when they are relatively quiescent in depths below 200 - 400 m. Jayaprakash[81] studied mesopelagic fishes from equatorial waters contiguous to Indian EEZ and recorded 12 myctophid species from the area. Karuppasamy[85] reported 27 species of myctophids from Indian EEZ. Somvanshi[86] reported five species of myctophids from south-west coast of India. Vipin[67] reviewed myctophid resources of Indian Ocean and reported 137 species in the Indian Ocean, and Karuppusamy[87] reported 13 species of which five are from the eastern Arabian Sea. Sebastine[88] studied myctophid fishery along the Kerala coast with emphasis on population characteristics and biology of the headlight fish, *Diaphus watasei*. Only limited information is available on the commercial exploitation of lantern fishes. Local people of Suruga Bay, Central Japan exploited *Diaphus spp*[89].

Commercial Exploitation

Commercial fishery for *Diaphus coeruleus and-Gymnoscopelus nicholsi* (edible species) in the southwest Indian Ocean and southern Atlantic began in 1977 and catch by former USSR reached 51,680 t in 1992, after which the fishery ceased due to decline in catch. The Commission for Conservation of Antarctic Marine Living Resources (CCAMLR) estimated 200,000 t TAC (Total Allowable Catch) for this resource in its jurisdiction area (CCAMLR Convention Area). Industrial purse seine fishery for *Lampanyctodes hectoris* was developed in South African waters and closed in the mid-1980s due to processing difficulties associated with the high oil content in the fish [73]. Lantern fishes are harvested commercially only along off South Africa and in the sub-Antarctic waters[31,4]. Oman started trial fishing of myctophids in 1996, 1998 and stopped it as the running cost was too high for viable returns from the fishery. Shaviklo[90] reported the initiation of commercial fishery for the myctophid fishes in the Persian side of the Oman Sea.

Biochemical Studies

Biochemical analysis of myctophids have been attempted by several workers. Myctophids are high in proteins and mineral content, variably lower in lipids and uniformly low in carbohydrates[79,91,92,93,73,94,96] which indicates its nutritional importance. A number of studies have evaluated the lipid content of vertically migrating myctophids and found that they include triglycerides, believed to serve primarily as an energy store and wax esters, mainly used for buoyancy. Gopakumar[95] reported that lantern fishes are a good source of potassium, sodium and calcium.

Post Processing and Utilization

Gopakumar[95] and Nair[96] have conducted studies on processing and utilization of lantern fish (Benthosema pterotum) collected from the Gulf of Oman. The main products developed from the lantern fish were dried products, fishmeal and fish hydrolysate. Haque[97] described a method for fish meal production from myctophids Benthosema pterotum from Gulf of Oman. Noguchi[98] reported that, based on the bio- chemical character of each lantern fish species, they can be utilized for production of (i) feed for aquaculture (ii) surimi from minced meat and (iii) cosmetics and lubricating oil from body fats. The quality of the highly refined wax was evaluated as equal to the quality of commercial purified wax from Orange Roughy. The

quality of the sulphurised lantern fish wax was equal to the quality of commercial lubricating and cutting oils[98]. Shaviklo[90] reported commercial fishing of myctophids in the Persian side of the Oman Sea, which began in recent years, exclusively for fish meal production in an onshore fish processing company located in Qheshm Island, south of Iran. Studies show that both fish meal and hydrolysate from lantern fish can be used for fish, poultry and animal feed and as an excellent protein supplement with beneficial effects. Wax esters comprised 86.2-90.5% of the total lipid. Globally, several attempts have been made to utilize lantern fishes for human food, but no successful product development has been reported. Myctophid is a good source of protein and fat, hence it could well be a potential source of alternative protein and fat. At present myctophid is not commercially exploited in India, although myctophid by-catch is used for preparing fish meal by some local populations[99].

Conclusion

Increasing customer demand for fish coupled with high humanpopulation growth have led to the search cheap source of proteinthereby intensifying the exploitation of marine ecosystem. World per-capita fish consumption increased from an average of 9.9 kg in the 1960sto 14.4 kg in the 1990s and 19.7 kg in 2013[100]. Preliminary estimates point towards an increase in per capita fish consumption in the coming decades. In addition to the population growth, the other factors that have contributed to rising consumption include reductions in wastage, better utilization, improved distribution channels, growing demand linked to population growth, rising incomes, urbanization and International trade. Since, the human activities are more concentrated on the coastal waters the coastal resources are over exploited and therefore to meet the increasing nutritional demands, alternate sources from deep Sea and open Oceans need to be searched. Myctophid fishes in the mesopelagic realm are most promising potential resources at present to resolve this issue. Available information in abundance of myctophids and their utilization indicate that there is excellent scope for development of myctophid fisheries in the world Ocean and utilization of these resource for fish meal and oil for the expanding aquaculture industry, surimi and cosmetic, nutraceutical and industrial products.

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Diabetes mellitus: Type1, Type2, and Gestational Diabetes

Nihala Naseefa C H, Sheeba P* and Honey Sebastian
Vimala College (Autonomous), Thrissur, Kerala, India 680009

Abstract

Diabetes mellitus an endocrinological or metabolic disorder in which the blood sugar level goes high when the body does not produce enough insulin to meet the needs or due to lack of insulin-directed mobilization of glucose by target cells. Diabetes is also a common lifestyle disease that affects a large population. TYPE 1, TYPE 2, and gestational diabetes being the different types of diabetes that are seen in people belonging to different age groups, if not given much attention might increase the complexity of the disease and have a rooted impact on diabetic patients.

Keywords: Diabetes Mellitus, TYPE1 DM, TYPE2 DM, GDM

Introduction

Diabetes mellitus is a heterogeneous disease with multiple etiologies[1].It is a common chronic disease in children[2].Hyperglycemia being one of the defining features of this disease[3], Death from this crisis is significantly high in older adults. According to the international diabetes federation 'Diabetes is one of the largest global health emergencies of the 21st century, moreover occurrence of depression is two to three times higher in people with diabetes mellitus[5].

As per the reports published by The WHO around 1.6million people die from diabetes every year and the number of cases has been increasing over the past few years. Diabetes mellitus causes destruction and damages the heart, blood vessels, eyes, kidneys and nerves[6]. Both genetic and environmental factors contribute to the pathogenesis of this disease[7]. It is characterized by increased levels of blood sugar as a result of insufficient insulin in the body[8]. Due to the action of insulin on target tissue, there occur abnormalities in carbohydrate, fat and protein metabolism. The inadequate insulin secretion or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action results in insulin deficiency[9].

Severity of this disease increases, depending on type and duration. Some even remain asymptomatic. In unrestrained conditions, it might even lead to stupor, coma and even death due to ketoacidosis or from non ketotic hyperosmolar syndrome[10]. If much attention is not given it can lead to both micro as well as macro vascular complications, including kidney failure amputation blindness and cardiovascular disease[11]. This disease includes a range of hyperglycemic conditions which is categorized by American Diabetes Association (ADA). The first type1 Diabetes mellitus (T1DM) is an autoimmune disease with progressive β cell destruction, leading to polyuria, polydipsia, weight loss, and hyperglycemia. Those affected with this become completely dependent on non-endogenous insulin. Type2 diabetes mellitus (T2DM) is caused by the combination of insulin resistance and impairment of insulin secretion. The third category encompasses forms with specific known, genetic and non-genetic etiologies, these monogenic diabetes are highly penetrant moreover have similar clinical presentations to that of T1DM or T2DM. The

* *Corresponding author, E-mail: rksheeba@gmail.com*

final and fourth category of diabetes is gestational diabetes[12].

Diabetes mellitus can affect the brain; the term diabetic encephalopathy itself expresses an intimate relationship between DM and brain dysfunction, especially for aging, Alzheimer's disease, and depression[13]. Those patients with uncontrolled BP despite antihypertensive therapy are at increased risk of developing Diabetes mellitus[14]. Diabetes-associated with cardiovascular autonomic neuropathy, can damage the autonomic nerve fibers that innervate the heart and blood vessels, in turn causing abnormalities in heart rate and vascular dynamics. Moreover it affects the multiple organ systems. It is also a major cause of morbidity as well as mortality in diabetic patients[15].Changing lifestyles such as unhealthy diet and physical inactivity etc. accelerate the growth of this disease[16]. Epigenetic modifications considered as the byproducts of environmental stimuli can influence the genetic susceptibility to diabetes[17].

Acupoint therapy has proved to have specific curative effects and notable advantages in the treatment of diabetes and its common chronic complications. It has superior efficacy and minimal side effects than western medicine alone[18]. According to the available literature, ChromograninA,contributes to the pathogenesis of DM[19].

Precision medicine incorporates information about the genetic makeup of an individual; it can also improve the diagnosis and treatment of diabetes. Because of all these reasons, it becomes a part of personalized or individualized medicine. This focuses on the psychosocial and dietary components of management[20]. Diabetes mellitus most commonly occurs after the neonatal period, resulting from complex interactions between both environmental and incompletely-penetrant genetic factors. Advances in molecular genetics over the past decade hastened the realization that the diabetes that occurs in very early life is most often due to the underlying monogenic defects - disorders caused by mutation(s) in a single gene[21]. The percentage of types of diabetes diagnosed[22] is shown in Figure 1 (Source:- https://diabetesmellitusucd.wordpress.com/what-exactly-is-diabetes/)

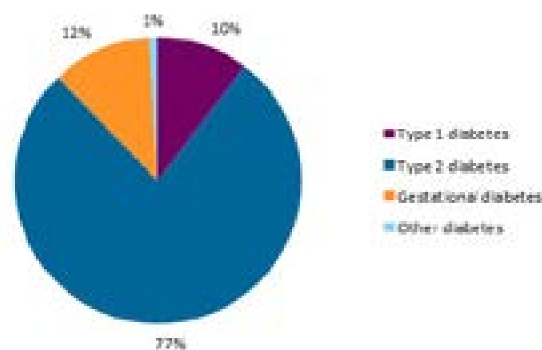

Fig.1 Pie chart showing the percentage of types of diabetes

Type1 Diabetes Mellitus

Type1 diabetes also called juvenile diabetes or insulin dependent diabetes, where the body is unable to make insulin as a result Blood sugar level goes high and makes theperson ill[23]. Type 1 DM is the result of autoimmune destruction of the insulin-producing pancreatic β-cells[24], due to which the body fails to produce insulin; hence the patients with T1DM remain insulin-dependent for their lifetime[25]. This autoimmune disease represents 5-10%of all cases of diabetes[26]. Constant hunger, blurred vision[27], polyuria, polydipsia, weight loss, fatigue etc. are the symptoms of T1DM[28], which are shown during childhood or adolescence and can sometimes even develop even in the later stages[29]. Type 1 diabetes can be more variable in adults[30]. The development of T1DM varies with seasons and birth month where more cases are diagnosed in autumn and winter; moreover those born in the spring have a higher chance of being affected with Type 1 diabetes[31]. This chronic multifactorial disease[32] can result from both environmental and genetic factors[33].

Both genetic and non-genetic factors are involved to the same extent in the pathogenesis of this disease[34]. Innate immunity and inflammatory mediators have a role in T1DM; they inhibit or stimulate the β-cell regeneration and cause peripheral insulin resistance[35]. The world health organization initiated "The DIAMOND project"; in order to describe the occurrence of T1DM in children[36]. Early diagnosis can improve the therapeutic strategies and overall health span[37]. Therapy

concept consists of insulin therapy, gene therapy, nutritional knowledge, training, glucose self-monitoring, psychosocial care etc.[38] Immunomodulatory properties of vitamin D, is beneficial for the prevention and treatment of T1DM[39]. Isolated islet cell transplantation is an effective treatment of type 1 diabetes in those who suffer from hypoglycemia[40]. Monitoring carbohydrate intake and balancing carbohydrate intake and insulin levels might help in controlling T1DM[41].

Type2 Diabetes mellitus

Type 2 Diabetes mellitus which is a non-insulin dependent DM[42] characterized by hyperglycemia, insulin resistance, and relative insulin deficiency that results from the interaction between genetic, environmental and behavioral factors[43]. There has been a rapid increase in T2DM over the past three decades, especially in developing countries[44]. It is increasingly seen in children, adolescents, younger adults (due to the increase in obesity, physical inactivity, and energy-lacking diets) and in persons older than 45 years[45].Smokers are at increased risk of developing T2DM[47]. Moreover High alcohol consumption increases the risk of abnormal glucose regulation in men[48]. Vitamin K2 has a significant effect on T2DM[49]. High intake of red meat, sweets etc. contribute to the increase of this disease[50]. Intake of foods with high glycemic index and glycemic load, especially rice, increases the risk of type 2 diabetes mellitus especially in Chinese women[51].

Vitamin D is a potential and inexpensive therapy that decreases the risk and improves the glycemic parameters in patients[52]. Thus, vitamin D is a possible therapeutic agent in the prevention and treatment of this disease where the Glucose tolerance is restored when the level of vitamin D is normalized[53]. Consumption of fruits and vegetables might prevent the development of T2DM[54].

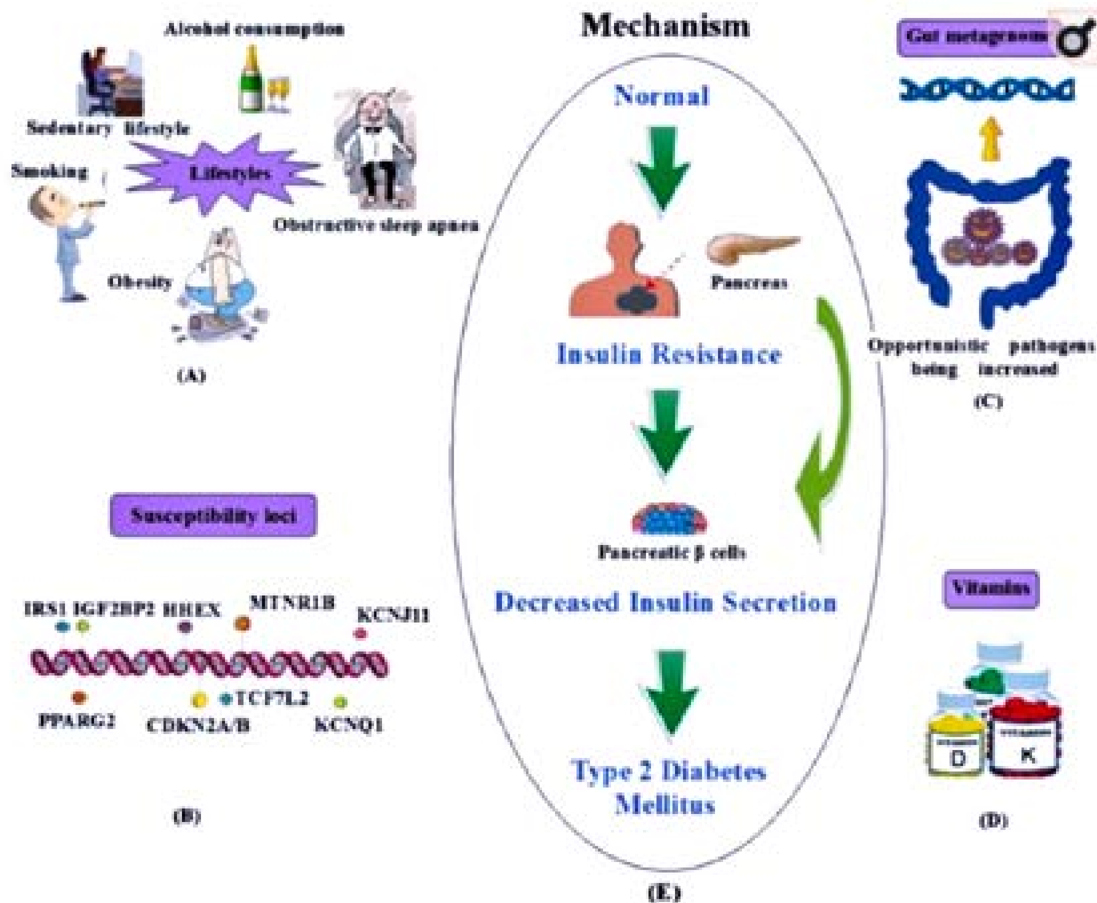

Fig. 2. Summary of the influencing factors and mechanisms of T2DM

Compared with brown rice, white rice is protective against type 2 diabetes[55]. Moreover, Diet and exercise may be an initial adequate treatment to prevent the further growth of this disease[56]. As shown in Figure 2 (Source: https://www.semanticscholar.org/paper/COMPARITIVE-EFFECT-OF-SYZGIUMCUMINI-AND-Thasneem-Rajasulochana/7cd3d0cdc7bb937010ac279956a910cb86660449/figure/1),

lifestyle factors like sedentary lifestyle, physical inactivity etc. contribute to T2DM that cause macro vascular as well as micro vascular diseases and cancers[46].

Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is any degree of hyperglycemia, recognized for the first time during pregnancy[57]. That occurs when the pregnant woman is unable to produce an adequate insulin response to compensate for the normal insulin resistance[58]. GDM being the most common medical complication of pregnancy[59] is restricted to those women where there is a development of glucose intolerance or is discovered during pregnancy[60].

Management without medication or nutritional therapy is called diet-controlled gestational diabetes (A1GDM) and that managed with medication to achieve adequate glycemic control is known as A2GDM[61]. Risk factors of GDM include insulin resistance diseases (like PCOS) genetic polymorphisms, intrauterine environment[62], obesity etc.[63]. Since the disease can be transmitted to the next generation[64], offspring of mothers with GDM are at a high risk of developing GDM, diabetes, obesity, cardiovascular disease and structural hypothalamic changes [65]. The initial treatments include nutrition therapy[66], which includes treatments that restrict the intake of carbohydrate[67], Glucose monitoring, Pharmacotherapy, Obstetrical management of GDM (that concentrate on foetal surveillance, evaluation of foetal growth, timing and mode of delivery, and care during labour and postpartum etc.)[68], physical activities etc.[69]. Above all, insulin therapy becomes a necessity in 40% of cases to obtain glycemic control[70].

Conclusion

Diabetes mellitus is a chronic metabolic disease that can be controlled on keeping the blood sugar level normalized. Hyperglycaemia, a common effect of uncontrolled diabetes, over time leads to serious damage to many of the body’s systems, especially the nerves and blood vessels.The prevalence of diabetes has been rapidly increased. As per the statistics of World Health Organization, 8.5% of adults aged 18 years and older had diabetes in 2014, whereas diabetes was the direct cause of 1.6 million deaths in 2016. Between 2000 and 2016, there was a 5% increase in premature mortality from diabetes. People with type 1 diabetes require insulin, people with type 2 diabetes can be treated with oral medication, but may also require insulin. The International Federation Diabetes Atlas (9th Edition), reported that the global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%). Unhealthy eating habits, lack of exercise; accelerate the growth of this disease as well as many other diseases. Healthy lifestyle, weight control, exercise, dietary changes, avoiding tobacco use, proper medications and regular screening and treatment for complications are the steps that can be adopted to manage this disease. We are the one who have the control over this and our activities can have an effect on this metabolic disease, as a result of which the disease can be brought to its normalcy.

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Persistent organic pollutants-its tale and fate in times of COVID -19

Navya Antony [1] and Rosily A.V.[2]
[1&2]Department of Chemistry, Morning Star Home Science College Angamaly -683573, India

Abstract

Preserving the nature in its fullest beauty and purity is the ever faced challenges faced by the world. The developments which facilitate the improved life and betterment of society resulted in bringing up adverse effects on biota. This paper focuses on the Persistent Organic pollutants (POPs), its sources and adverse effect imposed by them on humans, plants and animals. In the context of COVID-19, the lock down also could not help in controlling the POPs released to the environment.

Key words: Pandemic, organic pollutants, Environment, Contaminants

Introduction

COVID -19 Pandemic which has turned out to be the greatest global health crisis of our time is now the major challenge facing by the whole mankind all over the world. The World Health Organization, Government and health authorities has suggested to strictly follow precautionary measures to control the widespread of this pandemic. One among the major control measures that was implemented is lockdown so that social distancing is well achieved and worked out which helped to control the community transmission of the disease. In association with lock down humans get confined themselves within their homes, industries remained closed for long time, travel ban restricted vehicles over road and air, reduced waste disposal in water, landfills etc pollution in air, land and water were also drastically reduced. Thus pandemic has resulted in contrasting consequence on mankind, in the sense that, on one side it has executed world wide destruction of human lives and adversely affected the industry and economy but at the same time created a positive impact by providing signs of healing the nature [1]. But a still major concern is the use of Persistent organic pollutants as disinfectants.

Persistent Organic Pollutants (POPs) are a group of toxic chemicals that are of global concern due to their potential for long range transport, persistence in the environment and possibility to bioaccumulate in fat tissues of humans and get biomagnified in food chains thus creating harm to the environment. As per mentioned in Tox Town reports "The Stockholm Convention on Persistent Organic Pollutants is an international treaty to protect human health and the environment from POPs. In 2001, it originally covered the 12 POPs of greatest concern, called the "dirty dozen or legacy POPs," aldrin, chlordane, DDT, dieldrin, dioxins, endrin, furans, heptachlor, hexachlorobenzene, mirex, PCBs, and toxaphene. Along with that 10 more organic pollutants were added in 2009 and 2012[2]. POPs are highly toxic even at low concentration as they resist photolytic, chemical and biological degradation [3,4]. Another 16 additional chemicals were added to the treaty in 2017". In this paper the main objective is to discuss about sources, types and impact of POPs in humans and environment in context of COVID -19.

* *Corresponding author, E-mail: navyasanu2020@gmail.com*

2. Sources of POPs

As reported by World Health Organization[5] "The most commonly encountered POPs are organochlorine pesticides, such as DDT, industrial chemicals, most notably polychlorinated biphenyls (PCB), as well as unintentional by-products of many industrial processes, especially polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF), commonly known as 'dioxins'". Persistent Organic Pollutants may enter the food-chain in variety of ways as intentional and un-intentional products which are the consequences of human careless acts on disposing wastes (Fig 1).

But some POPs have been added to improve product characteristics or even to disinfecting agents to irradicate microorganisms. Although most developed nations have put restrictions upon the use of POPs in industrial products or in places of humanitarian concern, a great number of developing nations are still using it for agricultural purposes etc and have only fairly begun the initiatives to control its production and usage. POPs are generated during the incineration at industrial plants, household furnaces and combustion process such as forest fires, municipal waste incineration, the burning of animal remains, coal ignition etc[6,7]. It is common practice nowadays to use waste incinerators in most institutions especially in context of COVID -19 pandemic. As the wastes include mix of hazardous substances including plastics, the incineration will evolve annex A POPs to a certain extent. Hence recycling of materials is in general to be preferred over incineration with energy recovery.

3. Intentionally produced POPs

Intentionally produced POPs include Chemicals produced in industries and Chlorine containing pesticides. The core 10 intentionally produced pollutants identified are: aldrin, endrin, chlordane, DDT, dieldrin, heptachlor, mirex, toxaphene, hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs). Diary products and animal meat serves as the major source of Aldrin. Studies in India indicate that a person intake daily an average of 19 micrograms of Aldrin. In the case of Chlordane which is carcinogenic in nature humans were affected normally when exposed to air and may affect the human immune system. DDT was widely used against diseases spread by insects. DDT continued to be used to control disease, and it was sprayed on a variety of agricultural crops. Dieldrin another intentionally produced pollutant is highly toxic to fish and other aquatic animals, particularly frogs even with exposure to low levels. These POP residues have been found in air, water, soil, fish, birds, and mammals, including humans.

The primary source of exposure to the general population happens through intake of food and other diary and agricultural products. "For example, dieldrin was the second most common pesticide detected in a US survey of pasteurized milk." This insecticide is sprayed on the leaves of crops

Fig 1: Waste Disposal –a major source of POPs

to prevent pests. Heptachlor is also considered as a possible human carcinogen. Most other POPs are toxic to plants, aquatic animals and humans[8].

4. Unintensionally produced POPs

Polycyclic aromatic hydrocarbons (PAHs), part of polychlorinated biphenyls, polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs) are categorized as unintentionally generated persistent organic pollutants[9]. Along with that combustion and burning of organic contaminants, plastics etc creates dioxins unintentionally. Their existence is associated with man-made activities and is common in high-density industrial zones. These POPs accumulate in the food chain due to their high lipophilicity, high stability and lower vapor pressure.They are not easily metabolized and hence cause bioaccumulation in humans and animals [10]. It will cause cancer, birth defects, induce genetic damage, cause miscarriage, or otherwise interfere with the reproductive process. These groups of highly hazardous chemicals persist for long time when released into the environmental media and may cause a real threat to man and the environment. These persistent organic pollutants (POPs) are controlled by international conventions and agreements such as Stockholm, Basil, and Rotterdam.

4.1 Polychlorinated dibenzo-p-dioxins (PCDD)

These chemicals are released accidentally because of incomplete combustion and thermal method involving Chlorine and organic matter furthermore during the manufacture of pesticides and chlorinated substances for terminating pests in agricultural fields. PCDD are also released as a results of bound activities that involves burning of hospital, municipal and waste material waste that are currently at peak level within the context of dumping materials used by people infected by COVID-19 at hospitals and during quarantine period. In addition to that industrial activities like cement kilns firing dangerous waste, production of chemicals generating elemental Chlorine, thermal process in metallurgical industry etc additionally causes hefty environmental pollution by the emission of POPs collectively in between the processes. In addition to the emission from vehicles and automobiles, burning of landfill sites, firing of fossil fuels ,wood, other biomass and industrial boilers[11] also contribute in generating POPs.

4.2 Polychlorinated dibenzofurans (PCDF)

Furans being structurally similar to dioxins possess many toxic features in common. Like dioxins they are also carcinogenic and get retained in environment for prolonged period without any degradation causing harmful effects in biota. In most cases these compounds are produced unintentionally conjointly from many of the same processes that produce dioxins, and also during the production of PCBs. Furans have also been detected in infants born to women who had been exposed to PCBs. Low birth weight was also reported for infants of POP affected mothers[12].

5. Impact of POPs on health and environment

POPs have stable structure and hence can persist in the environment for decades. It is highly toxic to animals and humans and causes illness by inhalation, contact through skin and intake of food contaminated by those disinfectants, drinking water where waste from industries has been polluted by POPs. Long term exposure of these contaminants may impart harmful effects on respiratory system, immune system, neurological system, reproductive system etc[13,14]. Thus the POPs are really silent killers which imposes adverse affects on society, wild life and plants. Similar to the case with many environmental pollutants, it is very difficult to establish that a specific symptoms or illness is attributed to any specific POPs. They enter into cyclic in nature, accumulating in the bigger animals as they consume smaller ones and get into the food chain[15].

Marine mammals are greatly affected and increased mortality is reported to be due to immune dysfunction. In plants effects include disruption of photosynthesis in microscopic plants. “The toddler spends much time on the floor may

be exposed to contaminated soil thorough skin or because of ingestion"[16]. Epidemiologic studies and reports show a trend towards increased risk of hormone-related cancers (e.g. breast, prostate, testicular). Some POPs are considered as Endocrine disrupting chemicals which interfere with the endocrine system. Thus it is difficult to distinguish between the direct and indirect effects of POPs and its impact on living and non–living things [17,18]. Because of the increasing risk of community spread due to corona virus ,infectious clinical waste are disposed after one time use and are incinerated. The studies showed that it is better to avoid deposition of persistant organic pollutants in landfills and measures have to be taken to reduce the evolution of POPs from incinerators[19] by gas cleaning technologies.

6. Conclusion

Persistent organic pollutants put their footprints in air, water, soil and biota in the industrialized and non industrialized regions. Due to the adverse effects posed by POPs, their production and usage has been banned by various nations. But due to the lack of alternative substitutes some farmers are forced to use it even nowadays to prevent their crops from pests. Due to lock down even though the nature get purified the hospital waste and urban waste got dumped ever increasingly than before which proportionally boosted up POP concentration in air soil and water. COVID-19 resulted in creating negative impact by increasing the percentage of POPs generation. Thus POPs continues to contaminate the environment and threaten human health. Let us hope that studies will pave way to irradicate use of POPs and could replace materials that generate POPs with other harmless substitutes through the process of bioremediation. This can offer a green technology solution to the problem of environmental pollution.

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Seed Certification Methods in Soil Testing Laboratory at Department of Agriculture, Villupuram

Sridevi[*1], R. Damodaran[2] and P. Balamurugan[1]

[1] Bharathidasan Institute of Technology, Bharathidasan University, Trichy- 620024

[2] Office of Joint Director of Agriculture, Department of Agriculture, Villupuram-605602

Abstract

This review discusses seed certification methods adopted in Seed Testing Laboratory, Villupuram. Different processes are carried out to test the quality of seeds like purity, drying, moisture content and seed germination. This helps us to understand in detail how the seeds are subjected to testing in various aspects and certified for planting for further agricultural production or consumption.

Keywords: Pollen shelters, Shedding tassels, Boerner divider, riffle divider, seed certification.

Introduction

Through sustainable development goals, the global community is committed to arrive at a world that is free of hunger by the year 2030 by sustained food production than the current scenario. Apart from land and water resources need for the increased agricultural production, crop varieties are the major factor to support the farmers [1].

The International seed Testing Association (ISTA) is a nonprofit association that provide certain methods and seed testing services for seeds of international trading standards. The purpose of ISTA is to develop standard methods for sampling and testing seeds of all types as a seed's quality is especially important for the farmer as well as the company (ISTA) for better agricultural farming, seeds, soil, and climate are basic ingredients. When the seeds are tested, they are studied for what purpose they can be used, to pass legal standards ex. trading. 'seed quality', 'crop variety' and 'quality seed are different in their aspects. There are few attributes where seeds are being tested which are then selected for planting.(1) Physical purity (2) Incidence of noxious weed seed (3) Germination or viability (4) Provenance or origin (5) Density (weight per volume or number) (6) Moisture content (7) Varietal purity (8) Vigor (9) Incidence of seed-borne diseases (10) Efficacy of various seed treatments (11) Homogeneity. Among these, only three attributes, Physical purity, weed seeds presence and germinations are routinely tested.

Seed certification can also be defined as a legal sanction system of seed production for specific requirements produced under the supervision of seed certification officials[2]. The purpose of the seed testing laboratory was to certify the seeds that

Table 1.The Inseparable Crop Plant

Crops	Designated inseparable Crop Plant
Wheat	Barley, Oats, Gram and Triticale
Barley	Oats, Gram, Wheat and Triticale
Oats	Barley, Wheat, Gram and Triticale
Triticale	Wheat, Barley, Oats, Gram and Rye

* *Corresponding author, E-mail: sridevi.kothandapani@gmail.com*

Table 2. The Common Weed in Crops

Crop	Name of The Weed Species	Botanical Name Of The Weed Species
Paddy	Wild rice	*Oryza sativa* L. Var.*Fatua prain*
Wheat	Wild morning glory	*Convolvulus arvensis* L. *Phalaris minor Retz*
Rape Mustard	Mexicana prickly Poppy (Satyanashi)	*Argemone mexicana* L
Taramira	Poppy (Satyanashi)	*Argemone mexicana L*
Sunflower	Wild sunflower Orabanche	*Helianthus spp. Orabanchecumana*
Safflower	Wild safflower	*Carthamusoxyacantha*

were being marketed in the shops[3]. To maintain and make high-quality seeds available to the public. Seed Certification is also designed to achieve prescribed standards. Inseparable crop plants and lists of common weeds in crops are listed in tables 1 and 2.

Materials and Methods Adopted

The following seed testing methods were carried out at Seed Testing Laboratory, Joint Agricultural Office, Biocontrol lab, Villupuram. The seed testing process is done through several methods that are involved in seed testing and they are discussed below.

Types of Seeds

There are different types of seeds like Breeder Seed, Foundation Seed Stage I and II, Registered seeds and Certified Seeds.

Types of seed sample

There are different types of seed sample type that come to the seed testing laboratory.

- Service sample is the Sample received from the farmers.
- Certified sample is the Sample received from certification agencies or officers.
- Official sample is the Sample received from the seed inspectors.

Seed sampling

Seed lots are not completely homogeneous due to

Fig .1 Equipment for Purity Analysis
A. Seedblower B. Hand Lens and Spatula

several reasons. A representative sample of seed lot should be subjected to testing. If the sample is a good representative of the whole lot the analysis will reflect the true quality of the seed.

Sources of contamination

Contaminants were responsible for poor quality seeds. Contaminants might be as genetic or physical. Genetic contaminations may be Off-types, Pollen shelters, Shedding tassels. Physical/Genetic contaminations be Inseparable other crops plants, objectionable weed plants, diseased plants

Purity analysis

A purity test consists of separating the sample into four components: pure seed, other crop seeds, weed seeds, and inert matters. The sample is divided into a sub-sample consisting of roughly 2,500 seeds. We use a divider to indiscriminately select the seeds that we will test. Equipment used for Analysis Purity are work board, Seed blower, Spatula, Hand lens, Stereomicroscope. Some of the equipments are shown in Fig.1

Dust testing

Dust testing is also a method of seed testing where it is done by studying its characteristics like distinctness, uniformity, stability

Mixing and dividing of seeds

The main objective was to obtain the representative homogenous seed sample for analysis by reducing the submitted sample.

Types of Dividers

Boerner divider, mechanical dividing, riffle divider, centrifugal or Gamet divider, random cup method, modified halving method, spoon method, hand halving method. The equipment used for dividing are shown in the Fig.2.

Seed drying

Process of elimination of moisture from seed to a safe level through evaporation.

Different methods of drying

The process of seed drying is done either Physically or Mechanically.

A. Riffle Divider

B. Boerner Divider

Fig.2 Equipment for Dividing

Fig.3 Determination of moisture content in crops by moisture meter

Fig. 4 Prefabricated germination chamber

(i) Physical drying (or) natural drying (or) sun drying

(ii) Mechanical (or) artificial drying

a) Drying with forced natural air

b) Drying with forced artificially heated air

c) Drying with desiccants

d) Drying with infrared

Moisture content determination

The quantity of water present in the seed was referred to as seed moisture content and is expressed in percentage on a wet weight basis using moisture meter. The moisture content depends on varied with crop species, kind of seed, seed size & environmental condition particularly RH & temperature. The instrument used for the determination of moisture is shown in Fig.3.

Seed germination

It is defined as the emergence and development of the seed embryo, of those essential structures, indicates its ability to produce a normal plant under favorable conditions[4,5]. Germination tests shall be conducted with a pure seed fraction in a prefabricated seed germinator. Seeds are Counted and arranged equally on the substratum by a hand/vacuum counter/counting board. The substrates are placed in the germination room/germinator (Fig. 4). A minimum of 400 seeds are required in 4 replicates of 100 seeds each or 8 replicates of 50 seeds each depending on the size of the seed and size of containers of a substrate. The test is conducted under favorable conditions of moisture, temperature, suitable substratum, and light if necessary. No pre-treatment to seeds is given except for those recommended by ISTA. The Substratum serves as a moisture reservoir and provides a surface or medium for which the seeds can germinate, and the seedlings grow. Steps are illustrated in Fig.5.

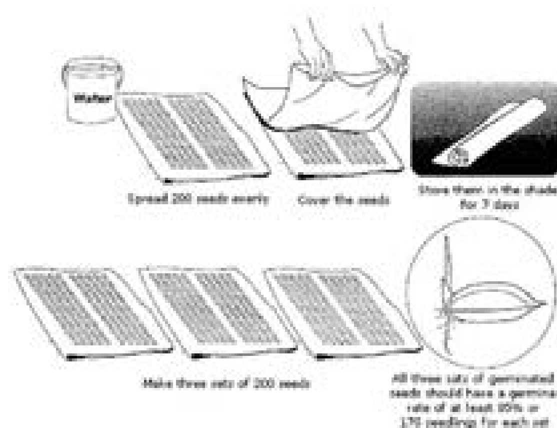

Fig. 5 Steps in seed germination

Fig.6 Seed germination in paper& sand substrates

Substratum

Sand/soil and paper are commonly used substratum. The seed germination in sand and paper will show in Fig.6. Paper substrates for germination used are Filter, blotter, or paper towel are generally used as substrates. The method of sowing is been followed in germination steps. The seeds are sowed in sand/soil substrates with certain dimensions. Seeds in sand (s) are sowed at 1 or 2cm depth by just pressing the topsoil.

Dust testing

The dusting of seeds is conducted to characterize the seeds that are being subjected to the following steps. They are characterized in Fig.7.

Distinctness

If a variety was distinguishable by at least an essential characteristic from any other variety in any country at the time of filing the application.

Uniformity

If subject to the variation that might be expected from

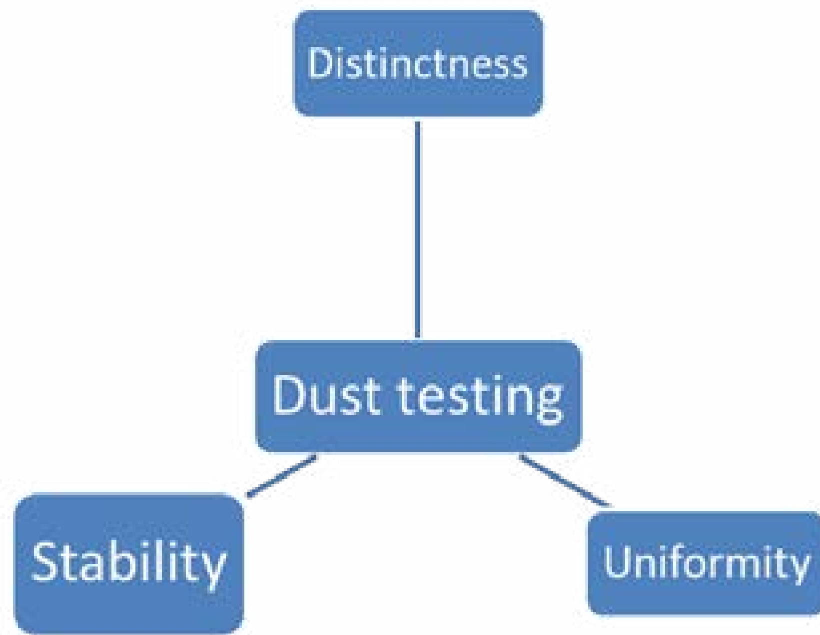

Fig.7 Seed dusting characteristics

the features of its propagation it was sufficiently uniform in its essential characteristics.

Stability

If, its essential characteristics remain unchanged after repeated propagation or, in the case of a cycle of propagation, at the end of each such cycle

Moisture content estimation

The sample should be weighed again, and the moisture content may be calculated by using the following formula, M **= (m2 – m3/ m2 – m1)** ×**100**

m1=weight of the container with lid (g)

m2=weight of the container with lid and sample before drying(g)

m3= weight of the container with lid and sample after drying (g)

Seed Germination

In paper substrates, Capillary movement of water takes place in the vertical direction at a velocity of 30mm/min. This type of substrates is free from pathogens or toxic substances. At 1st and 2nd counts, the seedlings which fulfill normal seedling conditions are removed, counted, and discarded. All hard seed, diseased and abnormal seedlings, non-germinated seeds are left until the final count when their number is recorded. Diseased seed and seedlings which may affect healthy seeds may be removed before the final count. ISTA classified the seedlings into different categories based on the development of essential structures. Seeds were collected randomly from pure seed fraction.

Categories of seedlings

Upon germination, the seedlings could be inferred as Normal seedlings, Abnormal seedlings, Hard seeds, Fresh ingeminated seeds, Hard seeds are included as germinated seed and it should be reported in the analyst report or certificate.

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CRISPR/Cas9-mediated gene editing: prospects and ethical concerns—an overview

Usha Bhagirathan*
Department of Zoology, Sree Kerala Varma College, Thrissur- 680 011, India

Abstract
The clustered regularly interspaced short palindromic repeats (CRISPR)-associated system (Cas), a simple and efficient tool for genome editing, have been used to modify genes in model systems including animal zygotes and human cells. CRISPR/Cas9 technology exploits the principles of bacterial immune function to target and remove specific sequences of mutated DNA. This review article discusses recent advances in CRISPR/Cas9 technology, current research, applications, ethical and biosafety concerns that it raises and prospects for gene therapy for genetic disorders in near future.

Keywords: CRISPR/Cas9; gene editing; gene therapy; ethics

Introduction

The CRISPR/Cas9 RNA-endonuclease complex, composed of the Cas9 protein and the guide RNA (gRNA) (~99 nt), is derived from the adaptive immune system of *Streptococcus pyogenes* SF370. It targets genomic sequences containing the tri-nucleotide protospacer adjacent motif (PAM) and complementary to the gRNA, and can be programmed to recognize virtually any genes through the manipulation of gRNA sequences[1,2]. Following Cas9 binding and subsequence target site cleavage, the double strand breaks (DSBs) generated are repaired by either non-homologous end joining (NHEJ) or homologous recombination directed repair (HDR), resulting in indels or precise repair respectively[2]. The ease and efficiency of the CRISPR/Cas9 system have rendered itself to many applications, including genome editing, gene function research, and gene therapy in animals and human cells[1,3,4].

The specificity of CRISPR/Cas9 is mostly guided by PAM and the 17–20 nt sequence at the 5′ end of gRNAs[4,5]. Unintended mutation in the genome can totally obstruct the application of CRISPR/Cas9, particularly in studies of development and gene therapy[6,7]. Three current studies discovered that off-target effects of CRISPR/Cas9 appeared rare in human pluripotent stem cells pointing out the risk that high frequencies of unintended targeting by CRISPR/Cas9 may be more common in cancer cell lines[8,9,10]. Lower rates of off-target effects (compared to human cell lines) have also been reported in mouse zygotes[11,12]. In spite of great strides in understanding the utilization of CRISPR/Cas9 in a variety of model organisms, much remains to be learned regarding the efficiency and specificity of CRISPR/Cas9-mediated gene editing in human cells, especially in embryos.

Background

Technologies for making and manipulating DNA have enabled progress in biology with the discovery of the DNA double helix. But introducing site-specific modifications in the genomes of cells and organisms remained obscure. Initial studies depended on the principle of site-specific recognition of DNA sequences by oligonucleotides, small molecules, or self-splicing introns. Later on, the site-directed zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs) using the

* *Corresponding author, E-mail: ushasreenath@gmail.com*

principles of DNA protein recognition were developed. But, difficulties of protein design, synthesis and validation remained an obstacle to widespread adoption of these engineered nucleases[13].

Prokaryotes have CRISPR segments of DNA containing short repetitions of base sequences that allow bacterial cells to record DNA sequences of viruses that it has been exposed to. This enables cells to boost the immune function of progeny. The Cas9 enzyme then functions to seek out those bits of viral DNA and cut them out of sequence. The complex is programmable, allowing scientists to target and remove specific bits of DNA with great precision. The cutting-edge discovery of the CRISPR/Cas9 technology has motivated scientists forward in attempts to treat or cure diseases caused by single-gene mutations[14].

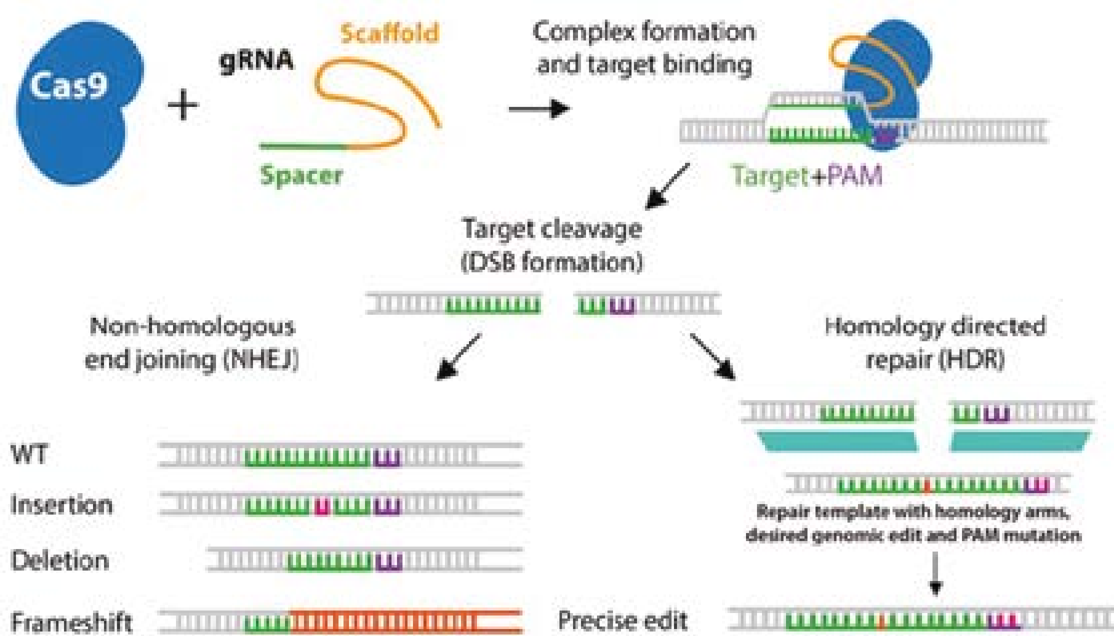

Fig. 1. Mechanism of CRISPR-Cas 9 gene editing (Source: www.addgene.org)

In genome editing by crispr/cas 9 mechanism, the genome of a target cell can be modified by expression of the double-stranded DNA endonuclease Cas9 and a guide RNA (gRNA). Expression of these components can be achieved by transfection with plasmids carrying genes for Cas9 and g RNA or by direct injection of Cas9 mRNA and gRNA. The gRNA (DNA-binding-domain) binds the target sequence (PAM) within the genome. This gRNA-DNA complex is specifically recognised by the cas9 protein, which induces a double-stranded break (DSB) in the DNA. Genome modification occurs, mainly through activating one of the two DNA repair mechanisms: non-homologous end joining (NHEJ) or homology directed repair (HDR). NHEJ introduces insertions or deletions within a sequence, whereas HDR requires delivery of a donor sequence through recombination with the targeting sequence can lead to point mutations or insertions.

Applications

CRISPR/Cas9 provides immense capacity to make remarkable progress in biotechnological, basic biological and medical research fields. Its application in genome studies will enable large-scale screening for drug targets and other phenotypes and will aid the production of engineered animal models that will promote pharmacological studies and the understanding of human diseases. CRISPR-Cas9 applications in plants and fungi also guarantee to alter the pace and course of agricultural research. CRISPR/Cas9 finds its application in generation of genetically modified (GM) mouse model of

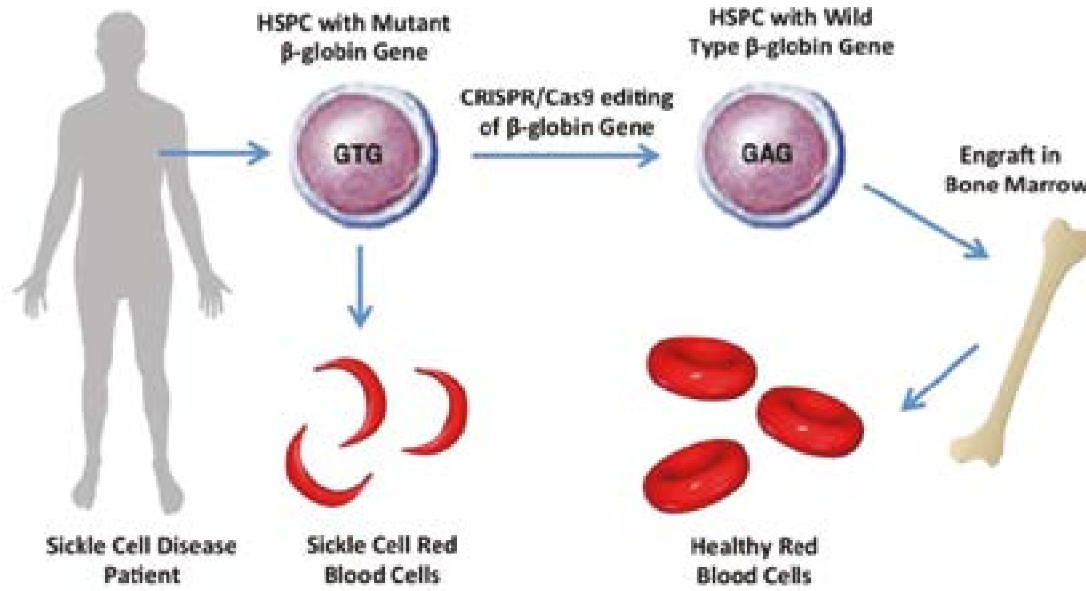

Fig.2 CRISPR/Cas9 technology in the study of sickle cell anaemia. (Source:cen.acs.org/articles/94/i41/CRISPR-edits-sickle-cell-mutation.html)

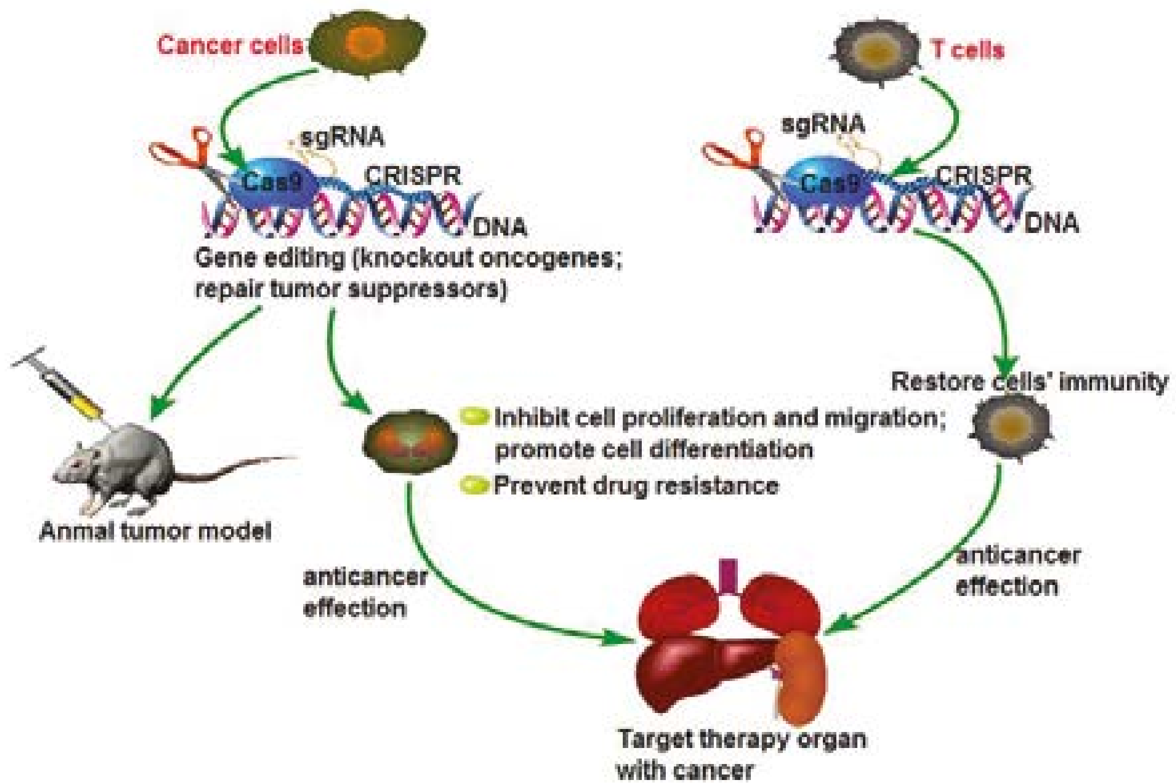

Fig.3. CRISPR/Cas9 technology in the treatment of Tumour. (Source: onlinelibrary.wiley.com/doi/pdf/10.1111/cge.13589)

human diseases, genome editing in specific tissues such as liver and brain, simultaneous generation of multiple gene mutations, flexible manipulation in epigenome for regulating the expression of specific genes and novel potentiality in RNA editing[15]. The therapeutic application of CRISPR-Cas

9 system has been reported to be successful in preclinical studies. This targeted gene therapy has shown therapeutic potential to tackle latent HIV infection, for treating cancer and for the prevention of cardiovascular diseases. Eye disorders like cataract, Leber congenital amaurosis and retinitis pigmentosa can be corrected by CRISPR-Cas 9 system. Haematological diseases like sickle cell anaemia, Haemophilia and thalassemia can be prevented by this gene therapy. Point mutations leading to SCID (Severe Combined immunodeficiency) can be corrected. This gene therapy system has the potential for treatment/ prevention of inborn errors of metabolism (eg: Ornithine transcarbamylase deficiency), muscular dystrophy, neurological disorders (cystic fibrosis) and skin diseases (dystrophic epidermolysis bullosa)[16].

Areas to be explored

Although the CRISPR/Cas9 technology has immense prospective in treating genetic disease, more research is needed before the science community begins human clinical trials. In a 2015 study by Liang et al. that was the first of its kind, CRISPR/Cas9 was used on tripronuclear (3PN) zygotes to better understand its effects in preimplantation embryos[17]. While targeting the β-globin gene (HBB), the CRISPR/ Cas9 complex also produced off-target effects. The efficacy of homologous recombination directed repair of HBB was low and produced mosaic embryos. In several of the 3PN zygotes, there were high rates of DNA repair using endogenous sequences instead of the therapeutic template. This is a major obstacle that must be overcome if the CRISPR/ Cas9 system is to be used therapeutically. Researchers must overcome a number of obstacles, such as the reaction of the human immune system, efficient modes of delivery, determining how to ensure that a corrected copy of DNA is inserted into the sequence, safeguarding against Cas9 proteins cutting at incorrect loci, and understanding and controlling off-target effects[18].

Ethical concerns

There are grave concerns regarding the ethical and safety implications of CRISPR/Cas9 research. Recently, a paper reporting gene editing in human embryos was published in the journal Protein & Cell, which raised concerns about the ethics of employing the CRISPR/Cas9 system[17]. Subsequently, both the editorial team of Nature and Science announced that although the CRISPR/ Cas9 system shows huge potential for genome editing, its use for modifying human germline cells should be considered very seriously, and progressive policy on this issue should be developed[19]. At the same time, Emilie Marcus, the editor-in-chief of Cell, stated that the journal would consider publication of manuscripts describing human germline modification, if they met high technical and ethical standards. The acceptance of this article should not be considered as endorsement or encouragement of modifying human germline cells, but should be viewed as a point to start the discussion about the human germline editing. Thus, embryo editing or engineering of human fetuses is becoming increasingly controversial among scientists. Some countries have already restricted CRISPR/Cas9 technology, by completely banning its use in humans. To address such a complicated debate, positive and negative aspects of germline editing should be weighed by an authoritative national agency, and both the scientific and social ethical concerns should be taken into consideration simultaneously[19]. If CRISPR/ Cas9 happens to be a common practice to edit genes for genetic improvement, the apprehension is that eventually those that choose not to or cannot afford the technology would be stigmatized. Once the precision of CRISPR/Cas9 comes close to that of other techniques approved for use on human embryos, it would become acceptable from a safety standpoint to move forward with human embryo studies.

Future prospects

The CRISPR/Cas9 technology is very popular because of its affordability and potential applications in curing genetic disease. In the last few

years, several animal studies have been published demonstrating its powerful gene editing capabilities. CRISPR/Cas9 mediated gene-editing was confirmed to be possible in adult animals, and was curative for the hereditary, single-gene mutation condition tyrosinemia[20]. Other studies have examined the effectiveness of CRISPR/Cas9 in treating single gene disorders such as Duchenne muscular dystrophy, as well as eye conditions like retinitis pigmentosa and Leber congenital amaurosis (LCA)[18,21]. A number of companies have invested in commercialising CRISPR technology. Corporate research companies such as Editas advocates that the first human clinical trials using CRISPR/Cas9 will aim to treat LCA. The eye presents an ideal testing location, as it is contained, immunologically isolated from the rest of the body, easily monitored externally, and can be measured using established standards of function. CRISPR/Cas9 technology has the potential for use in diseased individuals, as well as IVF embryos prior to implantation. In the case of diseased individuals, CRISPR/Cas9 can reduce the mutation load, which may subsequently decrease symptoms and the burden of disease. Application of CRISPR/Cas9 therapy with adult patients is less controversial than its use with embryos, as adults are able to provide informed consent. Furthermore, many patients with incurable diseases and compromised quality of life are eager to participate in clinical trials of new therapies, as there are no viable alternative treatments and it offers hope for a cure[14]. After solving the current challenges, the CRISPR-Cas9 system can be applied for clinical applications in patients. This system has been approved by an advisory committee at the US National Institute of Health as the first clinical trial to attack cancer cells[22].

Conclusion

There is a need to further improve the reliability and specificity of the CRISPR/Cas9 platform, a prerequisite for any clinical applications of CRSIPR/Cas9-mediated editing. The CRISPR/Cas9 genome editing system, with its accelerated development and expanded applications, is an indispensable tool for precise and efficient genome editing, but some related problems need more attention. First, the current knowledge of the CRISPR/Cas9 system at the biochemical and crystal structural levels is insufficient and requires additional research, including a deep analysis of the Cas9 protein, one of the main components in the CRISPR/ Cas9 system. The natural variation in Cas9 proteins isolated from different species might provide new Cas9 proteins with higher efficiency and thereby broaden the choices available for precise genome editing[23]. Moreover, specific modes for delivering Cas9, gRNA, and donor oligos to cells and tissues have been developed in numerous species, such as mice, Drosophila, zebrafish, worms, and humans. For example, the CRISPR/Cas9 system being used to create transgenic mice could be fused with other proteins or effectors to control or stimulate the expression or initiation of the CRISPR/Cas9 system in vivo. The off-target mutation rates of diverse CRISPR/Cas9systems, nevertheless, remain a challenge. Last, but certainly not least, the direct and precise genome editing raises ethical concerns, such as gene modification of human germline cells using the CRISPR/Cas9 system to create 'designer babies', which initiates arguments and queries among scientists and the public[17]. It is urgent that the government and related social organizations formulate and enact a series of laws and regulations to enable the safe and ethical application of the CRISPR/Cas9 system in basic research and clinics. There should be a clear distinction between genome editing in somatic cells and in germ cells. A voluntary moratorium in the scientific community could be an effective way to discourage human germline modification and raise public awareness of the difference between these two techniques. Legal concerns regarding the safety and ethical impacts of germline editing must not hinder the significant progress being made in the clinical development of approaches to potentially cure serious diseases[24]. A bright future is foreseen in which the CRISPR/Cas9 system will facilitate revolution and improvement of genome, RNA, and epigenome editing.

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Review on degradation of Textile Dyes using Algae

M. Vaishnavi[1], Roshnara Mohanty[2] B. Karpanai Selvan[2],
Soni Das[3] and N Jaya[*3]

[1]Coimbatore Institute of Technology, Coimbatore -641014
[2]CSIR NEERI Chennai Center, CMC, Taramani, Chennai-600113
[3]Anna University- BIT Campus, Trichy -620024

Abstract

Wastewater is a major environmental impediment for the growth of the textile industry besides the other minor issues like solid waste and resource waste management. Textile industry uses many kinds of synthetic dyes and discharge large amounts of highly coloured wastewater as the uptake of these dyes by fabrics is very poor. This highly colored textile wastewater severely affects photosynthetic function in plant. It also has an impact on aquatic life due to low light penetration and oxygen consumption. It may also be lethal to certain forms of marine life due to the occurrence of component metals and chlorine present in the synthetic dyes. So, this textile wastewater must be treated before their discharge. In this article, different treatment methods to treat the textile wastewater have been presented along with cost per unit volume of treated water. solid waste and resource waste management. Textile industry uses many kinds of synthetic dyes and discharge large amounts of highly coloured wastewater as the uptake of these dyes by fabrics is very poor. This highly colored textile wastewater severely affects photosynthetic function in plant. It also has an impact on aquatic life due to low light penetration and oxygen consumption. It may also be lethal to certain forms of marine life due to the occurrence of component metals and chlorine present in the synthetic dyes. So, this textile wastewater must be treated before their discharge. In this article, different treatment methods to treat the textile wastewater have been presented along with cost per unit volume of treated water. The removal process is the need of hour to remove contaminants from wastewater which are obtained from industries, residential or commercial buildings or sludge and recycle it into an effluent in an environmentally friendly manner which cause minimum impact to the environment. The textile waste water from the industries can be considered as one of the major threat to the environment Modern textile effluent treatment involves costly process to meet the pollution control board standards. This article is focused the utilization of the algae for the treatment of the textile dyes. The algae species like *Chlorella vulgaris, Scenedesmus* sp., *Sphaerocystis schroeteri, Cosmarium species, Scendesmus quadricauda, Lyngbya lagerlerimi, Nostoc lincki, Oscillatoria rubescens, Elkatothrix viridis and Volvox aureus* were discussed in this this review in three main topics as bioremediation , biodegradation and bioadsorption.

Keywords: Bioremediation, biodegradation, bio adsorption, waste water treatment, Algae.

Introduction

Textile wastewater carries large variety of dyes, chemicals and bleaching powder which is hazardous to the environment. Mainly dyeing and finishing process are responsible to produce large amount of toxicity to the environment. In order to treat these textile effluent microalgae can be used to degrade the dyes. Owing to growth of environ-tech and huge creative production in biotechnology field, microalgae has always been favoured. Since microalgae have been used in multiple technologies and due to its inexpensive growth requirements, it has become suitable for eco-friendly technologies. For managing the excessive toxic characteristics of metals, a fully-fledged

* *Corresponding author, jayaprabakaran@gmail.com*

array must be developed using microalgae. They are made as suitable in practical applications of wastewater bioremediation due to their extensive amount and their potential to develop and focus on heavy metals. Prevalent to the physiochemical processes which worked out to be in the eliminating the toxicity in heavy metals, microalgae are affirmed to be superior in uptake of heavy metals also. It is necessary to develop a reproving analysis to fill in the loopholes and for estimating their ability[1].

The toxicity of wastewater upon the variety of the fibre used in the textile and hence the wastewater characteristics may vary. Manufacturing process involves both dry and wet processes. Sizing, de-sizing, sourcing, bleaching, mercerising, dyeing, printing and the finishing techniques are involved in the textile manufacturing process[4 &29]. Many chemicals are added to the fibres and can be of two types such as natural and synthetic dyes. They can be categorized based upon their molecular structure and their mode of application. The discharged dye effluents from the textile wastewater is high in pH, chemical oxygen demand (COD), Biochemical oxygen demand (BOD), heavy metals (HM), temperature and total suspended solids (TSS). The textile wastewater constituents depend upon the type of the manufacturing process, the fibre used and the season[41].

The cost of textile waste treatment has been considered a serious problem for long time. Technologies are being developed so that products recovered from the wastewater areconverted to socially acceptable and economically viable products. Microalgae can be used for wastewater treatment because it has potential to remove the nutrients effectively from wastewater and to reduce the electrical energy used in the treatment systems. The great advantage of using microalgae is that they are available free of cost in nature and they can easily remove the existing organic matters in the wastewater which further leads to eutrophication. The main challenges in applications of algae in wastewater are process of culturing algae, since they require an optimal condition and control in their biomass composition. The need of the desired species in the wastewater management process with transition from laboratory to the pilot scale is a bit tedious one. But the favouring point is algae can easily grow with the help of solar energy which is abundant in nature followed by a small investment. Due to the positive characteristics of algae-based treatment, it has become an advantageous method especially in places where there is a lack of infrastructure for wastewater treatment systems. Algae can be cultivated in open air systems which permits for low cost investment, no cost solar energy and minimal amount of energy providing no control in temperature and lightning[1]. Algae can also be grown in a closed photobioreactor system where the temperature and lightning can be systematically controlled[36]. Algae are also grown in a sustainable bio film system but there is insufficient data regarding the performance of the system, its sustainable development and cost effectiveness[11]. Algae growth medium can be BG 11 medium[2] and can be maintained in Bold's Basal medium under the same conditions[7&9]. The textile wastewater contributes a major portion of pollutants to the environment and in order to reduce the pollutant there must be a suitable treatment.

Bioremediation

Bioremediation is a method to treat the polluted sites with microorganisms or with their enzymes. There are several processes where algae can be utilized for bioremediation of dyes and the process includes the usage of large algal biomass[32]. It has been suggested that some species of algae have the capacity to break down the compounds of dyes into simpler compounds[39]. For the removal of reactive yellow 22 which is in an azo dye in textile wastewater, *Spirogyra* sp. is the commonly used green algae especially for the discoloration of this dye by breaking down the dye molecules into simpler compounds[31&27]. *Chlorella Vulgaris* and *Oscillatoria tenuis* also can degrade azo dye using azoreductase enzyme[39]. For the treatment of Triphenylmethane dye and malachite green dyes a green microalgae *Cosmarium* sp., has been suggested[12]. Under thermophiles, *Phomidium* sp. can

decolorize 50 to 80 % of textile dyes[17]. By stimulating algal growth algal dye removal ability can be enhanced. The plant regulator triacontanol hormone was added to the *Synechocystis* sp. and *Phormidium* sp. to increase their dye removal activity[22]. It is reported that about 91% of malachite green has been decolourised by *Oscillatoria sp.*, and it has also decolourised about 75% of congo red and 23% of Nigrosin dyes at 0.05% of dye concentration[20].

An experiment conducted by using central composite design for the treatment textile effluent with *Chlorella vulgaris*[14]. Bold's Basal medium were used as the growth medium for *C. vulgaris*. The concentration of the wastewater and sodium bi-carbonate was taken as two variables for the central composite design where the polynomial equation was obtained to find the maximum cellular concentration in eight commercial dyes. A cell concentration C_{max} (270,009 cells per ml), specific growth rate l_{max} (0.53 per density) were obtained in less concentrations. The wastewater concentration was moderately added to the culture media used for cultivation of the microalgae in about 5% and 8.5% which showed both COD removal (69.25 and 69.90%) and color removal (75.68%) at larger rates.

Golenkinia radiate was used to treat the non-sterile textile wastewater[40]. The textile wastewaters from raw wastewater collection effluent (CE), equalization tank effluent (SE), active sludge effluent (ASE) and the effluent (E) where subjected for the study. From the equalization tank with the trail of 1.23 ± 0.83 per day, the highest specific growth rate was obtained. The physiochemical parameters were found to be high in CE when it is compared with SE, ASE and E. The highest range of physiochemical parameters were temperature 32° C, pH was found to be at 9, DO 7 (mg/L), COD 7000 (mg/L). *G radiata* showed toleration in various conditions and was not affected by changing in temperature, DO and pH. Various algae and sludge were treated which was also having a similar structural relationship for treating the heavy load printing and dyeing wastewater under solar energy[28]. The effects of aeration rate (0.1-0.15,0.4-0.5 and 0.7-0.8 L/min) and hydraulic retention time (HRT) (12h,16h and 20h) on conventional activated sludge (CAS) and algal-bacterial symbiosis (ABS). When compared with the treatment of printing and dyeing, waste water results of the experiment from the ABS system showed the highest performance in the removal of total phosphorus (TP) at higher rate of 10.5%, ammonia nitrogen (NH_4 + -N) at the incremental rate of 23.1% and chemical oxygen demand (COD), has increased by its removal rate at 12.5%, and also the colour has reduced by 80 times. Since the growth of the algae can be increased in low amount of dissolved oxygen it can also provide more dissolved oxygen for ABS. The ABS system has a stable particle size distribution so a stable treatment can be guaranteed from this system. Further decrement of colour and COD can be made sure by having longer HRT and a condition of having no outer source of carbon. Higher amount of dissolved oxygen was produced by adding algae which in turn provided a stable and larger amount of removal rate of nutrients by the ABS system.

Biodiesel from bioremediation of textile wastewater using microalgae were produced successfully[18]. One of the disadvantages in large quantity of micro algal cultivation is the nutrient and water requirement extensive amounts. Textile wastewater itself has many requirable nutrients as nitrates, phosphates, micronutrients etc. It also includes the organic dyes which is the potential carbon source for the cultivation of algae. This process can be used potentially for improvising the production of biodiesel and treatment of wastewater. An environmentally friendly approach, for higher effective products has been designed from *Chlorella variabilis* and also enhancing a maximum utilization of effluents from textile wastewater as a nutrient medium.As a source of nutrient, the productivity of biomass was at the rate of 74.96 ± 2.62 gram per m^2 per day and lipid production at the rate of 20.1 ± 2.2% with respect to the dry biomass. In order to transform the microalgal biomass carbohydrates into reducing sugars for the fermentation of microbes an integrated process was developed

based on the type of detergents. The data obtained from the experimental research showed an amount of about 109.4 grams of total lipids was obtained from a microalgal of biomass 495grams. The lipids were extracted from the mass of 34.65 grams of γ-linolenic acid and about 1.3 grams of pure ε-polylysine was obtained from 36.68 g of reducing sugars. With 74% recovery a bi-step effective eco friendly process was build with ethyl ammonium nitrate for obtaining ε-polylysine. A remediation of about 100% was obtained in aluminium and cobalt, and a highest range of about 82.72% boron, 45.6% of calcium, 42.1% of sodium, and 14.5% of potassium and of about 0.1% magnesium using *C. variabilis* was obtained. From the total phosphate the detrimental range of about 78.17% and with respect to this total phosphate inorganic phosphate showed a decrease of about 25.22% in the effluent which was identified as other valuable product. Unreacted dyes and high concentration of salts are one among the most critical issues concerning the environment with respect to the textile sector mainly in their effluent disposal aspects. The effluent from the textile wastewater consists of an important substrate which has higher amount of bicarbonate salt concentration which is useful for *Chlorella*.sp. growth. According to this study, 40% of textile wastewater effluent was used for growing the *Chlorella* species at a scale of 100 L in the open tanks, producing biomass which also includes nutraceutical ,γ-linolenic acid, which can be especially used in cooking oils. About an amount of 495 g of biomass was obtained from 34.65 g of γ-linolenic acid. Owing to the preparation of 1.3 g ε-polylysine, of about 36.68 grams of fermentable sugars were extracted from the de an oiled micro alga which has beneficial effects in the pharma industries for various biomedical applications[6].

Six microalgae were isolated for their ability to bio remediate the textile wastewater. Since the wastewater treatments are expensive, usage of algae has been increasing for textile wastewater cleaning purpose. For each algae strain biomass was generated in which deionised water was used for dilution purpose and the dry weight of algae was obtained at the rate of 0.4-1.65 gram per litre. An elemental analysis was carried out for the present phase and future phase of cultivation of algal strains and their amount of dye colour removal rate were also obtained. The supernatant was obtained after harvesting the biomass and with respect to that the removal /reduction of heavy metals like Se, Al, V and Cu were shown. At a lambda max of 558nm, in the textile wastewater chromogenic substances were present and they showed a decrement ranging about 47.10-70.03%. Hence with the addition of producing microalgae biomass and improvising the treated wastewater quality while coupling the treatment process of textile wastewater with algal farming it was a complete successful process. Ignoring the impacts which are negative, while using wastewater from textile industry it has paved a way to obtain biofuels from the biomass of the microalgae[34].

Biodegradation

Biodegradation is described as the biologically mediated breakdown of chemical compounds; it is an energy-dependent process and involves the breakdown of dye into various byproducts through the action of various enzymes[25]. Biodegradation of synthetic dyes not only results in decolorization of

Table 1 Efficiency of algae in biodegradation process (3)

Algae	Dye (conc.)	Decolorization (%)	Incubation period (days)	References
Chroococcus minutus	Amido Black 10B (100 mg L−1)	55	26	Parikh and Madamwar (2005)
Phormidium ceylanicum	FF Sky Blue (100 mg L−1)	80	26	Parikh and Madamwar (2005)
Gloeocapsa pleurocapsoides	FF Sky Blue (100 mg L−1) Acid Red 97	90 83	26	Parikh and Madamwar (2005)

the dyes but also in fragmentation of the dye molecules into smaller and simpler parts (breakdown products). Decolorization of the dye occurs when the chromophoric center of the dye is cleaved[25].

Usage of Algae for Biodegradation

C. vulgaris was used for the treatment of tectilon yellow 2G (TY2G), which is a mono azo dye. Efficiency of COD was increased at the rate of 88% in the concentration of 50 mg/L, at the rate of 87% with respect to the concentration of 200 mg/L and 89% for the concentration of about 400 mg/L by the acclimation of *C. vulgaris*, which had initial COD efficiency removal of about 69%, 66% and 63% at the same initial concentration as mentioned above. The absorbance spectral analysis profile were obtained for algae which is unacclimated, showed that the first stage of (initial) peak was produced at 45 nm vanished and the another peak at 220 nm showed a detrimental line and it showed another peak at 350 nm, which showed the transition of tectilon yellow 2G dye to an another product. It was verified as aniline using the analysis performed by HPLC. There was no end product aniline formation in acclimated algae. Degradation was the main mechanism in case of acclimated algae and bioconversion for unacclimated algae. Moreover, in shorter time higher COD removal efficiency, the higher initial algal concentration was achieved. Acclimatized *C. vulgaris* showed a higher efficiency in treatment with tectilon yellow 2G dye. In the initial stage of concentration TY2G never showed a significant removal range. The mechanism which was adopted by the TY2G dye was bioconversion or degradation, as concerned it did not show adsorption process on the biomass of algae. Degradation of the TY2G dye was observed in unacclimated algae due to their prolonged exposure, and aniline was obtained as an end product. However, aniline was not produced in acclimated algae. Comparing to untreated TY2G the toxicity levels has been decreased due to usage of non acclimated algae. A substrate was produced by the product aniline for the activated sludge microorganisms which also stimulates their rate of growth[1].

Batik effluent was carried out treatment using *Chlorella* sp. which was immobilized in alginate, starch and carboxyl methyl cellulose and growth was carried in batch culture mode. Using alginate as an immobilized matrix, higher range of decolorization was obtained in the batik effluent, at 77.9%. Box-Behnken design approach was used, for the optimizing the effects of microalgae bead concentration, light intensity, and pH, which were done by alginate- *Chlorella* sp immobilized for decolourization and removal of total nitrogen (TN). The decolourization of highest range 80% in the batik effluent was obtained by favouring the following conditions as at the concentration ranging of 150 micro algal beads, at the light intensity of 1000 and the pH of about 12. The bead concentration being 150 at the pH level being 7, the highest removal of total nitrogen 7% was being obtained at the light intensity 1000 lux. The higher range of potential is shown by the immobilised alginate *Chlorella* sp. for the treatment of batik effluent despite of its direct discharge into the environment. This method is also considered to be an effective and efficient method, both in terms of quantity and quality[23].

A methodology has been obtained for bio decolourization using microalgae from wastewater which was low-cost and effective treatment and also to be greenery approach. For the treatment two kinds of microalgae *Sphaerocystis schroeteri* and *C. vulgaris* was used. For this study purpose yellow colour dye was used at various concentrations of 1 mg/L, 5 mg/L, 10 mg/L and 20 mg/L. A 14 days experiment was conducted using this dye solution at the above mentioned concentrations and the absorbance was recorded. The highest range of decolourisation was obtained at the concentration of 10 mg/L by *S. schroeteri* and *C. vulgaris* at the percentage of about 43.12. By this study the decolourisation ranges, irrespective of their dye concentration and algae were obtained. The decolourisation ranges both the algae as *C. vulgaris* showed the maximum decolourisation of 43.12% and minimum decolourisation of 19.42%, while the *S. schroeteri* showed the maximum level of decolourisation of about 45.03% and minimum

decolourisation of 24.27%. Though, the dye concentration and the decolourisation were interdependent whereas comparatively, the percentage decolourization has no significant difference between the two microalgae. To the conventional treatment method an alternative measure was provided using algae as sorbent for the treatment of coloured wastewater. Based on economic and market analysis, effort is needed for the selection of suitable algae and commercialization of algae as sorbent. Therefore, an environmentally friendly and sustainable management can be achieved by using algae as sorbent for effluents colour removal before discharge into the water bodies[37].

Cosmarium species which is green algae is used for the treatment of Malachite Green (MG), which is a triphenylmethane dye. The concentration of the algae in various parameters like pH, dye concentration and temperature on the decolourisation of the dyes were analysed. The optimal level pH was 9. To understand the relationship between the concentration of the dye and its decolourisation kinetics of Michaelis-Menten was derived. V_{max} was in the range of 7.63 mg dye g/cell/hour and the K_m was in the range of 164.57ppm which was considered as the optimal kinetic parameters of this study. According to the study it has been revealed that the concentration of the dye, concentration of the algal species their temperature and pH was considered as the independent factors. According to the model of Michaelis-Menten the decolourisation of the specific dye depends upon the concentration of the dye. In case of the increase in the temperature of about 5- 45 °C the decolourisation rate can be increased[12].

The consequences of the indigo dye effluent were studied on the freshwater microalgae *Scendesmus quadricauda*. ABU12 which was found in freshwater but studied under controlled laboratory conditions. For about 100 to 175 times the dye effluent was diluted in the bold basal medium and the microalga was used in different concentrations. The effluent dye concentration and the microalgae growth rate showed were always inversely proportional. The rate of growth of the algae was decreased ($p<0.005$) as increase in the concentration of the dye. The factor of dilution was found to be 155 in EC_{50}. Dye effluent concentrations were significantly correlated with the changes in size of *Coenobia*. Due to the shift of the sizes of Coenobia (the size varied from large to small) there was an increase in the concentration of the dye effluents. The number of cells of the coenobium was reduced during the study. The structure of Coenobium of the genus *Scenedesmus* was affected due environmental stress; this also conveyed further for toxicity testing and was observed as an influential biomarker. Thus, the toxic characteristic of the effluent affecting the microalgae *S. quadicauda* at various concentrations were confirmed. Therefore, there is big risk of disturbing the food chain of the primary aquatic organisms by releasing the untreated effluents of indigo dye[10].

The reason for decrement in the decolourisation capacity of diatoms and cyanobacteria green algae were inspected, when there is a variation with respect to the molecular structure and functional groups of monoazo dye and diazo dye. The study proved that there is a decrease in the removal capacity of the green algae in azo dyes at the time rate of six days , but in turn has the rapid rate of decolourisation at the initial stages of study (for three days). The decolourisation capacity was found to be maximum at the rate of 5 ppm after the period of six days in incubation of Tartrazine dye along with the species *N. muscourm* and *S. bijugatus*. The structure of the dyes and the species used for the treatment were found to be similar. The culture of the diatom *Nitzschia perminuta* reached its death phase after the period of two days in incubation. From the study it was made sure that the conversion of the azo dyes into aromatic amine is made by breaking the azo dye linkage with the main enzyme azo reductase which is present in algae. In IR spectra the azo band reduction was found at the range of 1642 – 1631 per cm and a new peak formation at 3300 per cm. The Langmuir isotherm model was used for testing and to observe the algae behaviour regarding the sorption properties. The environmental issues are

raised due to the intense colour formation in the effluents. It can be said from these experimental studies that the dyes can be removed with greater potential, effectively and efficiently at lower concentrations of the micro algal culture. In addition to that it can also be said that this biosorption property of algae can be concluded as the cost effective approach for dye decolouration and it can also be taken as an eco-friendly treatment[33].

The discoloration of methyl red, G-Red (FN-3G), basic cationic orange II, and basic fuchsin dyes were reported using *C. vulgaris*, *Lyngbyala gerlerimi, Nostoclincki, Oscillatoria rubescens, Elkatothrix viridis and Volvox aureus*. According to the algae species the efficiency of the colour removal varies and even according to their properties such as molecular structure and its rate of growth it may vary from 4% to 95%. The decolourisation of the orange II and G-Red by *C. vulgaris* showed algal activity to remove 43.7 and 59.12, whereas about 5.02 and 3.25% of dyes were removed by *V. aurreus* respectively[15].

Bioadsorption

Bioadsorption is defined as the property of certain biomolecules to bind and concentrate selected ions/other molecules from aqueous solution[19].

Usage of Algae for Bioadsorption

The algae used for this biosorption process were selected in such a way that they could degrade the dyes used by the process of biosorption. Moghazy, (2019) The removal of methylene blue (MB) has been repored,which is an aqueous solution of the absorbents such as *Chlamydomonas variabilis* which is the activated biomass and raw biomass of green microalgae was used. In order to get the adequate algal biomass *C. variabilis* was obtained and cultivated. The biomass was oven dried and further H_2SO_4 added for activation of the algal biomass. The optimum adsorption was observed in methylene blue by using the *C. variabilis* algae at the conditions such as, 30 mins of contact time, pH at 7, 1.5 gram per litre for dried biomass as dosage of the biosorbent and 1 gram per litre for activated biomass. The zero discharge point was observed at the range of pH of about 6.8 for dried biomass and 6.9 was recorded for activated biomass, respectively. The highly active biosorbent was observed in activated biosorbent when compared to the dried bio sorbent. The highly active biosorbent where also confirmed by using Langmuir and Freundlich models in which a good fit was given by the activated biosorbent when compared to the dried biosorbent. According to the kinetic data model the pseudo-second-order equation was better than the pseudo-first-order one for the methylene blue adsorption. The experimental data also assured that the activated and the raw biosorbents are the efficient biosorbent of the green alga *C. variabilis* for methylene blue. Thus, the biosorbents of the *C. variabilis* has been considered as a promising tool for bioadsorption of methylene blue[30].

The adsorption of Astrazon red were evaluated by *Scenedesmus obliquus* and activated carbon. Activated carbon which was mainly used for this treatment was selected based upon its pore size and surface area distribution, as they determine the efficiency of the activated carbon. The treatment of the activated carbon where judged by comparing the results of before and after using activated carbon. The main reasons for the determination of the experimental results were due to the enlargement of the specific area surface and their total volume of the pore by their activation chemically. The higher range of adsorption was observed in activated carbon at the rate of 181.82 mg per gram at the temperature of about 25 ºC, these results were obtained from the Langmuir isotherm model[8].

The bioadsorption of the dye Basic Red (BR 46 by green)has been demostrated by macro algae *Enteromorpha sp.* For the biological decolourisation process the optimisation was carried out using the model central composite design. The variables which are used for the investigation are temperature, reaction time, initial dye concentration and algal biomass initial dye concentration. For carrying out the central composite model the value which is been predicted should be correlated

with the values obtained from the experiment (R^2 =0.988, Adj R^2 =0.978). Under optimal conditions about 83.45% of biological dye removal was achieved in the time of reaction of about 5 hours at the temperature of about 25 °C[24].

Bioadsorption has been carried out using the prokaryotic algae (*Phormidium animale*) and eukaryotic algae (*Scenedesmus* sp.) to treat the acid dye P-2BX(ARP-2BX) . Since this dye has been one of the extensively used and found in larger quantities in textile waste water, the constituents such as pH which may range from 2-10, type bioabsorbent used such as ash or dries, dosage level of the bio absorbent, dye concentration at their initial levels, the temperature and the contact time was obtained. *P. animale* removed the dye to the maximum concentration of about 99.70 ± 0.27 %. In the treatment pH range varied for both the species, *Scenedesmus* sp. had the optimal biosorption activity at the pH 8. For *P. animale* pH range was at 2 and the removal rates were four times higher in dried bioabsorbent. The biosorption concentrations of *P. animale* was 97.35 ± 0.65 % at 25 °C. *P. animale* has increased the biosorption rate of the dye ARP-2BX at the temperature of 45 °C to 99.70 ± 0.27 %. *P. animale* was declared as the favorable biosorbent by the FTIR elemental analysis for removal of P-2BX[21].

Desmodesmus sp. has been isolated from the textile dying unit for dye degradation and lipid production. Microorganisms uses light energy to carryout various activities of metabolism. The capability of microbes to associate with its environment is shown by Extracellular Electron Transfer (EET). To form a graphene oxide/ algae bio nanocomposite an efficient nano biotechnological approach was done in which *Desmodesmus sp.* with extracellular electrons was combined with graphene oxide nano sheets on its region where the electron rich draper is found to be present. *Desmodesmus sp.* was used for the suitable eco friendly and sustainable approach in which the azo dyes are reduced in addition to which the production of lipids is also done, which in turn can be helpful for the generation of biodiesel. These reductions and by products information were taken from the electrochemical tests which are carried using graphene a nano biocomposite material along with the algal species *Desmodesmus*. Hence to produce biofuel and remediation of the wastewater a reusable, eco-friendly, sustainable and economical approach was developed by the material which is to be a nanocomposite. Due to the over usage of the dyes which are in nature in the textile industries and releasing them without treating into the streams makes the water bodies to get affected at the high levels which in turn also affects the environment. Owing to this affects of dyes a coupling method was introduced in which the coupling takes place between the green algae *Desmodesmus sp.* which has an property of being electrogenic and the transporter of the electron graphene oxide is been used here for the decolourisation of the textile dye DR31 and it also helps in the generation of lipids which in turn is made as biofuel feedstock. Under aerobic conditions the isolated species of *Desmodesmus* algae shows the decolourisation at higher rate of about 36 % which also had the maximum capacity of being bioelectric. Further its decolourisation efficiency and electro genetic property was enhanced by successful amalgamation of graphene oxide sheen on to *Desmodesmus* sp. Thus, under visible light in 150 mins 90 % of DR31 dye was successfully removed in 150 mins by graphene oxide / algae bio nano composite. After decolourisation of the DR31 dye the lipid content was at 9% only from the algae which was increased by graphene oxide / algal bionanocomposite at the range of 11%. For $40 mgL^{-1}$ of aqueous solution of DR31 dye graphene oxide/algal bionanocomposite can be completely reused up to three times. Thus, for water treatment the usage of bionanocomposite is proved to be economic, eco-friendly, reusable and a sustainable solution[5].

Model organic cation: methylene blue sorption capacity has been tested using chemically modified *Sargassum muticum* biomass. The treatment was done in two stages as the carboxylic acid esterification process and the lipid extraction process. Due to the removal of lipid fraction from the

algal species, the biomass of the algae is modified chemically so there is an increase in the capacity of sorption process. The extractions of lipids were carried out using reflux treatment. The biomass which is remaining after the reflux treatment has the maximum range of decolourisation of dye which was confirmed using Langmuir isotherm in which the value of dye is obtained as 860 mg per gram. The pH range was 4 to 10 of maximum uptake. At the time period of 30 min to 60 min the equilibrium was achieved which depends upon the pre-treatment of the algae. The above mentioned complete process follows the empherical model of pseudo first-order. In aqueous solution, *S. muticum* showed a high adsorption capacity. After the removal of lipids from the algal species by which the biomass was modified, the capacity of the sorption was increased. The methylene Blue gets affected by the solution pH. The pH ranges were found to be 4 to 10 for maximum uptakes and the adsorption of the dye methylene blue becomes lower at the range of pH below 4 as the solution becomes more acidic. Langmuir isotherm model is used to explain the sorption equilibrium. Depending upon the algae pre-treatment the equilibrium was achieved, which shows that the methylene blue kinetics of the are relatively fast timing range being from 30min to 60 min. The estimation of the specific surface is strongly affected by the covered area which has an uncertainty in their existence and for comparing among the different absorbents the results of the specific area can be useful but not in absolute sense[38].

Biosorption process were carried out in which the capacity of the sorbent is estimated using the Kinetic and equilibrium models. Many researchers have proved that biosorbents can be used as treatment which can be conventionally used, and it can also be carried out as an alternative process of treatment. As the cell surfaces of the algae are naturally attributed by the chemical groups such as phosphate, carboxylate, amino and hydroxyl, these chemicals are mainly used for the separation of the toxic materials from the wastewater. So, it is believed that the algae cell wall which consists of these chemicals can be used for the biosorption process. In this process both the complexation and attraction due to electrostatic plays a vital role. Due to the adsorption capacity most of the cells in algae were coated with mucilaginous layers. According to the analysis of the markets, cost analysis, pilot scale studies with actual waste waters and demonstration of the large scale systems research must be done to make this treatment commercially successful[13].

Conclusion

In order to assist the textile wastewater treatment microalgae can be used as an alternative for retarding the impact of the environment which are caused by the pollutants. The textile wastewater has been tremendously becoming a great environmental impact, owing to the high demand in textile products which is also increasing proportionally due to its wastewater release during the period of treatment. Due to the colour which is produced in the textile dyes which not only causes environmental affects but also due to their toxicity characteristics it affects human being in mutagenic way in case of breakdown of their products. Bioremediation is considered to be as alternative method for physio-chemical methods of treating textile wastewater .Various methods showed that treating the textile wastewater with microalgae is considered to be effective and sustainable method. The above mentioned methods also showed the variations in biological parameters which has proven that treatment of textile wastewater using algae is considered to be effective in removal of dyes. Future studies should investigate the behaviour of algal cells in real textile wastewater, which usually contains more dyes and several auxiliaries such as inorganic salts, detergents and fixing agents.

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Study on bird diversity in Malayatoor and Iringole Kavu, Perumbavoor, Ernakulam District

Amrutha M. Nair and Teji K.T.*
Department of Zoology, Morning Star Home Science College, Angamaly-683572.

Abstract

The study on Bird Diversity in Malayatoor and Iringole Kavu, Perumbavoor was conducted from January 2018 to December 2018. 47 species of birds were observed at Iringole Kavu and 31 species were spotted at Malayatoor. Among these 23 species were found in both places. Commonly occurring birds at both places were White – Throated Barbet, Black-rumped flameback, Red Whiskered Bulbul, Common Myna, Shikre, Black Drongo, House crow, Racket Tailed Drongo, Asian Koel etc. Availability of rich food, favourable climatic condition and suitable breeding places etc… attract many birds to these areas. Habitat destruction is the most important threat of the birds living here.The uniqueness of the sacred grove, food availability and the presence of pond in Iringole Kavu are the reason for its rich bird fauna diversity. Sacred groves act as an abode for many rare, endemic, endangered species and economically important plants of fruit bearing and medicinal properties. Malayatoor was a place with large number of birds in the past, but due to the presence of quarries large number of trees were cut down which resulted in the decrease of birds in this area. Bird diversity in Iringole Kavu is greater than that at Malayatoor. Flood was also not affected the bird diversity of these two sampling sites.

Keywords: Malayatoor, Iringole Kavu, Perubavoor, Bird diversity, Monthly distribution, Bird species

Introduction

Birds are one of the major indicators of climatic change. They are highly migratory in behavior also. The impact of flood was less when compared to other faunal groups due to aerial behavior of the birds and preferred nesting places. There are altogether about 8650 species of living birds in the world today. In India about 1200 species of birds, representing 75 Families and 20 orders is present[1]. Bird Diversity in Western Ghats of Kerala have been conducted and significant result of these surveys was in identifying the relative conservation value of these forest areas in protecting endemic and threatened avian fauna[2].

The riparian zones of Meenachil River basin of Kerala were surveyed during January 2009 to December 2015. A total of 92 bird species belonging to 36 families in 15 orders were recorded. 85% of the birds recorded were resident forms and 15% were migrants[3].

Materials and Methods

The study was conducted from January 2018 to December 2018 at Malayatoor and Iringole Kavu. The observation of birds in the field was done with the help of Binocular and Photos were taken with a Canon DSLR Camera. Malayatoor is a fringe area to the idamalayar forest range and is rich bio-diverse hotspot. Malayatoor is a village in aluva taluk near angamaly in the north eastern corner of ernakulam district in the state of kerala in south india. Iringole Kavu is a miniature forest which is located in Perumbavoor of Ernakulum District, Kerala. It is a sacred grove. Varieties of birds present here make this place an attractive zone.

After spotting the bird, it was carefully observed without disturbing them. The time selected for bird watching was before 8am in the morning and after 5 pm in the evening as the birds are more active at these time. Bird watching was done for once in a month. The identification of birds was done by both sight and sound. Spot identification

* *Corresponding author, E-mail: tejishanil2003@rediffmail.com*

was carried out by the use of field guides.

Results and Discussion

During the study period 31 species of birds were identified from Malayatoor (Table, 1) and 47 species from Iringole Kavu (Table, 2). A total of 78 species of birds were identified from both the study areas among which 23 were common.

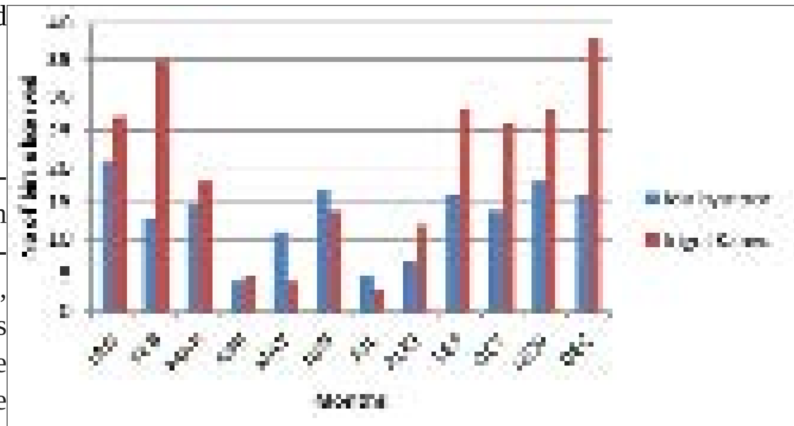

Fig.1. Graph showing monthly distribution of birds

Maximum number of species were observed during January, November and December at Malayatoor and minimum number in April, July and August. Maximum number of birds were spotted in the month of November, December and February at Iringol Kavu and minimum number in April, May, July and August (Fig. 1).

Among the identified species, Indian paradise flycatcher and brown breasted flycatcher is a winter visitors. Black hooded oriole shows migratory movement while White throated ground thrush, Crested serpent eagle, Brahminy kite shows local migration by monsoon and when food, water and habitat variations occur. During the study period birds belong to 25 families were identified and maximum bird diversity were identified in Cuculidae and Columbidae family. Bird diversity in Iringole Kavu was greater than Malayatoor and might be due to the richness of thick forest and might have facilitated the feeding and reproduction of birds. Uniqueness of the sacred grove, food availability and presence of pond may be the reason for its rich bird faunal diversity. Sacred groves act as an abode for many rare, endemic, endangered species and economically important plants of fruit bearing and medicinal properties. Apart from conserving biological diversity, this sacred grove that is situated in the middle of the human habitation are responsible for conserving water table of the soil. This is evident from the perennial nature of ponds, wells and tanks, which are situated in the nearby areas of the sacred groves[4].

Eyebrowed Thrush' (*Turdus Obscurus*), locally named 'Purikapullu' was spotted at Iringole Kavu, near Perumbavoor and the bird, which entered the Kerala checklist as 519th bird[5].

The seasonal variation, availability of food, water, suitable breeding places, favourable climatic conditions and undisturbed habitat affects the abundance and distribution of birds. Most of the birds are resident common birds and a very few ones show local migratory movements. Habitat destruction is the most important treat of the birds living here.

The study area falls under humid montane climate having rainfall during the long rainy season from June to October and the shorter rainy season occurring from March to April. The significant variation in the abundance of migratory bird species between the dry and wet seasons could be due to seasonal movement patterns, local and regional habitat changes, large–scale population changes and climatic conditions.

Human induced disturbance can have a significant negative effect on breeding success by causing nest abandonment and increased predation. Outside the breeding season, recreation (particularly power boating, sailing and coarse fishing on Wetlands) reduces the habitat choices of birds[6].

Commonly occurring birds in both of the places were White – throated kingfisher, White-cheeked barbet, Black rumped flameback, Red whiskered bulbul, Common myna, Shikre, Black drongo, House crow, Racket tailed drongo and Asian koel.

Table. 1 Birds identified from Malayatoor during the study period

SL. No	COMMON NAME	LOCAL NAME	SCIENTIFIC NAME
1	Little Cormorant	Cheria Neerkakka	*Microcarbo niger*
2	Cattle egret	Kalimundi	*Bubulcus ibis*
3	Purple heron	Chayamundi	*Ardea purpurea*
4	Great egret	Perumundi	*Ardea alba*
5	Indian pond heron	Kulakokku	*Ardeola grayii*
6	Crested serpent eagle	Chuttiparunthu	*Spilornis cheela*
7	Shikra	Shikra	*Accipiter badius*
8	Brahminy kite	Krishnaparunthu	*Haliastur indus*
9	White breasted waterhen	Kulakozhi	*Amaurornis phoenicurus*
10	Red wattled lapwing	ChekkanniThithirri	*Vanellus indicus*
11	Rock pigeon	Ambalapravu	*Columba livia*
12	Spotted dove	Aripravu	*Spilopelia chinensis*
13	Greater coucal	Chempothu	*Centropus sinensis*
14	Asian koel	Karingkuyil	*Eudynamys scolopaceus*
15	Common kingfisher	CheriaMeenkothi	*Alcedo atthis*
16	Green bee- eater	Naattuvelithatha	*Merops orientalis*
17	White cheeked Barbet	Chinnakutturavan	*Megalaima viridis*
18	Black drongo	Anaranchi	*Dicrurus macrocercus*
19	Rufous treepie	Olanjali	*Dendrocitta vagabunda*
20	House crow	Pena Kakka	*Corvus splendens*
21	Red vented bulbul	Naatubulbul	*Pycnonotus cafer*
22	Red-whiskered bulbul	Erattathalachi	*Pycnonotus jocosus*
23	Common tailorbird	Thunnaran	*Orthotomus sutorius*
24	Jungle babbler	Kariyilakili	*Turdoides striata*
25	Indian robin	Kalmannathi	*Saxicoloides fulicatus*
26	Common myna	Naatumyna	*Acridotheres tristis*
27	Jungle myna	Kinnarimyna	*Acridotheres fuscus*
28	House sparrow	Angadikuruvi	*Passer domesticus*
29	White-rumped munia	Aatakkarupan	*Lonchura striata*
30	Black –rumped flameback	Nattumaramkothi	*Dinopium benghalense*
31	Malabar Starling	Garudancharakili	*Sturnia blythii*

Seasonality affects food and cover availability of bird population, which in turn affects breeding success and ultimately survival of the bird species[7]. The seasonal variation in the amount of rainfall, temperature, spatial and temporal microhabitat conditions are known to affect the availability of various food items for birds.

Based on species sensitivity to their habitat, these could alter the diversity, abundance and distribution of birds in an area. Particularly it has been revealed that the processes acting in breeding and wintering grounds determine both the patterns of habitat occupancy and seasonal abundance in migratory bird species[8]. Tropical and subtropical countries witness a certain type of seasonal migration of birds. On the other hand, the spatial distributions of food and cover requirement of bird species is determined mainly by the vegetation

Table.2. Birds identified from Iringole Kavu during the study period

Sl. No	COMMON NAME	LOCAL NAME	SCIENTIFIC NAME
1	White-cheeked barbet	Chinnakutturavan	*Psilopogon viridis*
2	Coppersmith barbet	Chembukotti	*Megalaima haemacephala*
3	Plum-headed parakeet	Poonthatha	*Psittacula cyanocephala*
4	Black-hooded oriole	Manjakaruppan	*Oriolus xanthornus*
5	Orange headed thrush	Chenthalayankaatupullu	*Geokichla citrina*
6	Spotted dove	Aripravu	*Spilopelia chinensis*
7	Black –rumped flameback	Nattumaramkothi	*Dinopium benghalense*
8	Greater racket-tailed drongo	Kadumuzhakki	*Dicrurus paradiseus*
9	Vernal hanging parrot	Thathachinnan	*Loriculus vernalis*
10	Oriental magpie robin	Mannathipullu	*Copsychus saularis*
11	Bronzed drongo	Lalithakakka	*Dicrurus aeneus*
12	Indian-paradise flycatcher	Nagamohan	*Terpsiphone paradise*
13	Asian emerald dove	Omanapravu	*Chalcophaps indica*
14	Jungle owlet	Chembannathu	*Glaucidium radiatum*
15	White-throated kingfisher	Meenkothichathan	*Halcyon smyrnensis*
16	Indian pitta	Kavi	*Pitta brachyura*
17	Black drongo	Anaranchi	*Dicrurus macrocercus*
18	Rose-ringed parakeet	Mothirathatha	*Psittacula krameri*
19	Blue-tailed bee-eater	Veliyavelithathaa	*Merops philippinus*
20	Ashy woodswallow	Enakathevan	*Artamusfuscus*
21	Purple sunbird	Karuppanthenkili	*Cinnyris asiaticus*
22	Common cuckoo	Pekuyil	*Cuculus canorus*
23	Red spurfowl	Chembanmullankozhi	*Galloperdix spadicea*
24	Purple rumped sunbird	Manjathenkili	*Leptocoma zeylonica*
25	Greater coucal	Chempothu	*Centropus sinensis*
26	Great egret	Perumundi	*Ardea alba*
27	Common kingfisher	Cheriameenkothi	*Alcedo atthis*
28	Emerald Dove	Green dove	*Chalcophaps indica*
29	RufousTreepie	Olanjali	*Dendrocitta vagabunda*
30	Asian koel	Karingkuyil	*Eudynamys scolopaceus*
31	Green bee- eater	Naattuvelithatha	*Merops orientalis*
32	Rock pegion	Ambalapravu	*Columba livia*
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34	Indian robin	Kalmannathi	*Saxicoloides fulicatus*
35	Common myna	Naatu myna	*Acridotheres tristis*
36	Jungle Myna	Kinnarimyna	*Acridotheres fuscus*
37	House Crow	Pena Kakka	*Corvus splendens*
38	Red vented bulbul	Naatubulbul	*Pycnonotus cafer*
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Sl. No	COMMON NAME	LOCAL NAME	SCIENTIFIC NAME
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41	Crested serpent eagle	Chuttiparunthu	*Spilornis cheela*
42	Shikra	Shikra	*Accipiter badius*
43	Brahminy kite	Krishnaparunthu	*Haliastur indus*
44	Cattle egret	Kalimundi	*Bubulcus ibis*
45	Indian pond heron	Kulakokku	*Ardeola grayii*
46	Yellow billed babbler	Poothakiri	*Turdoides affinis*
47	Jungle Crow	Balikakka	*Corvus macrorhynchos*

structure and composition that is correlated with abundance and habitat use.

Conclusion

Iringole Kavu is noted for its rich bird diversity and should be considered as a sensitive ecosystem and there is an urgency to conduct periodic surveys which are needed to reveal the actual biodiversity of both the areas and action plan should be taken against habitat destruction.

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Study on Antimony Chalcogenide thin films

Anu Kuruvilla[1, 2], Melda Francis[1, 2], Lakshmi M[2*]
[1, 2] Christ College, Irinjalakkuda, University of Calicut, Kerala, India
[2]Mercy College, Palakkad, University of Calicut, Kerala, India.

Abstract

Antimony sulphides and selenides are two important chalcogenide compounds that find application in the field of photovoltaics.Sb_2Se_3 is one such binary chalcogenide with bandgap of 1–1.2 eV and absorption coefficient in the order of 10^5 cm^{-1} at short wavelength.Sb_2S_3 is another similar chalcogenide with a bandgap in the range 1.7-2.3 eV. In this work, antimony sulphide and antimony selenide films were prepared by Chemical Bath Deposition (CBD) method. The prepared samples were characterized using UV-Visible spectroscopy, X-ray Diffraction technique, XPS analysis, SEM and EDAX studies.

Key words- Antimony Selenide thin film; Antimony sulphide thin film

Introduction

Antimony chalcogenides (Sb_2Ch_3, Ch=O, S, Se, Te) with its high refractive index, photo-sensitivity, good electrical conductivity and transport properties, have wide optoelectronic applications. Among the various antimony selenides, Sb_2Se_3 has a bandgap of 1–1.2eV and absorption coefficient in the order of 10^5 cm^{-1} at short wavelength [1, 2].This material is a very promising absorber material for thin film photovoltaics. It finds applications as optical coatings in thermophotovoltaic devices and in fabrication of Hall Effect devices and cost-effective solar cells. Sb_2S_3 has a bandgap in the range 1.7-2.3 eV and it serves as potential absorber for photovoltaic applications. This material is known for its high refractive index and well-defined quantum size effects [3]. The constituent elements of the above two compounds viz. Sb, S and Se are earth-abundant and are low-cost.

In the present work antimony sulphide and antimony selenide films were prepared by Chemical Bath Deposition (CBD) method. This method of thin film preparation is presently gaining considerable attention as it has proved to be a less expensive low temperature process. It is also a non-pollutant method. It is the most convenient method for large area deposition. Bandgap determination of the prepared samples was done with the help of UV-Visible spectroscopy, structural characterization using the X-ray Diffraction technique, compositional analysis using XPS and morphological and chemical analysis were done using SEM and EDAX studies.

Experimental Technique

The substrate used for thin film deposition was glass slide washed with laboratory detergent and ultrasonicated in acetone. Antimony selenide was prepared by dissolving 1 g of $SbCl_3$ in 37 ml of 1M sodium citrate solution. 20 ml of ammonium hydroxide and 24 ml of 0.4 M $Na_2S_2O_3$ were then added simultaneously. This solution was made up to 100 ml by adding water [4, 5].The solution will be clear and devoid of any precipitate at the beginning. Uniform thin film was formed on glass slides vertically supported on the walls of the beaker. The deposition was carried out undisturbed for 1hr at room temperature. Obtained films were uniform, reflective and adherent.

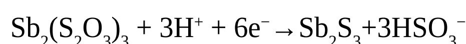
Antimony (III) chloride precipitates as oxochloride (SbOCl) in water. Strong ligands such as citrate, tartarate, triethanolamine and thio-sulfate

* *Corresponding author, E-mail: lakshmisethumadhavan@gmail.com*

form soluble complexes in $SbCl_3$ solution. This will prevent the precipitation of basic salts in aqueous solutions. Sodium selenosulphate is used as the selenium precursor [6].The formation of the Sb_2Se_3 film is based on the slow release of Sb^{3+} and Se^{2-} in an aqueous ammonia medium leading to the condensation on the substrate. Antimony Sb (III) complex reacts with Se^{2-} ions to give Sb_2Se_3 film.

The precursors used for the preparation of antimony sulphide thin film were antimony chloride and sodium thiosulfate. 0.5 M solution of antimony chloride was prepared in 10 ml acetone. 25 ml of 1 M solution of sodium thiosulfate was added to this solution under constant stirring. The resulting solution was made up to 100 ml by adding 65 ml of distilled water. In order to control the reaction rate, precooling of water and thiosulfate solution was done at 10˚C before mixing. Clean glass slides were placed vertically on the walls of the beaker containing this mixture. Room temperature deposition of the film for 1 hour resulted in the formation of orange-yellow Sb_2S_3 thin film. The substrate was removed from the bath and washed well with distilled water. The film was uniformly reflective, smooth and adherent to the substrate.

The reaction pathway for the formation of antimony sulfide film is as follows[3].The thiosulfate forms a very strong complex $Sb_2(S_2O_3)_3$ with antimony ion (Sb^{2+}), which hydrolyses to form Sb_2S_3.



$$Sb_2(S_2O_3)_3 + 3H^+ + 6e^- \rightarrow Sb_2S_3 + 3HSO_3^-$$

Results and discussions

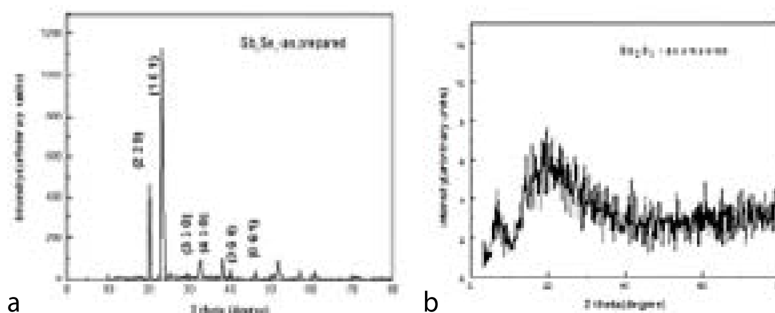
Structural CharacterisationXRD patterns were recorded using Cu Kα source of Rigaku D Max X-ray diffractometer. XRD pattern of as-prepared antimony selenide thin film is shown in figure 1(a). The obtained sample is polycrystalline with peak position matching with orthorhombic Sb_2Se_3 (PDF#651317).This pristine film shows peaks corresponding to (220), (101), (330), (430), (060) and (061) planes respectively. Figure1 (b) shows the XRD spectrum of pristine Sb_2S_3thin film and it suggests that the pristine sample is amorphous in nature.

Morphological Analysis

Morphology of the prepared samples was analyzed with SEM (Scanning Electron Microscope) model JEOL Model JSM - 6390LV. SEM images of pristine Sb_2Se_3 films are shown in figure 2(a). The films were found to be smooth, dense and without cracks. The image with a magnification of x5000 shows spherical structures uniformly distributed over the surface. The SEM image of Sb_2S_3 is shown in figure 2(b).The image shows that the grains are homogeneous and is spread throughout the surface. The grains are more or less uniform.

Chemical Characterisation

EDAX analysis as given in figure 3(a) of pristine Sb_2Se_3 film showed the presence of Sb and Se. The sensitivity of silicon and oxygen was found to be significant in the analysis. Sb to Se ratio was hence analyzed excluding the influence of the substrate. The expected ratio of antimony to selenium in Sb_2Se_3 is 2:3 or Se/Sb is 1.5. The Se/Sb the ratio obtained in the as-prepared thin film sample of Sb_2Se_3 is found to be only1.18.This suggests a



a b

Fig. 1. XRD pattern of pristine (a) Sb2Se3 thin film and (b) pristine Sb2S3 thin film.

Table 1. Elemental analysis of Sb2Se3.

Element	Atomic %
Se	45.89
Sb	54.11
Total:	100

Table 2. Elemental analysis of Sb2S3

Element	Atomic %
Sb	56.09
S	43.91
Total:	100

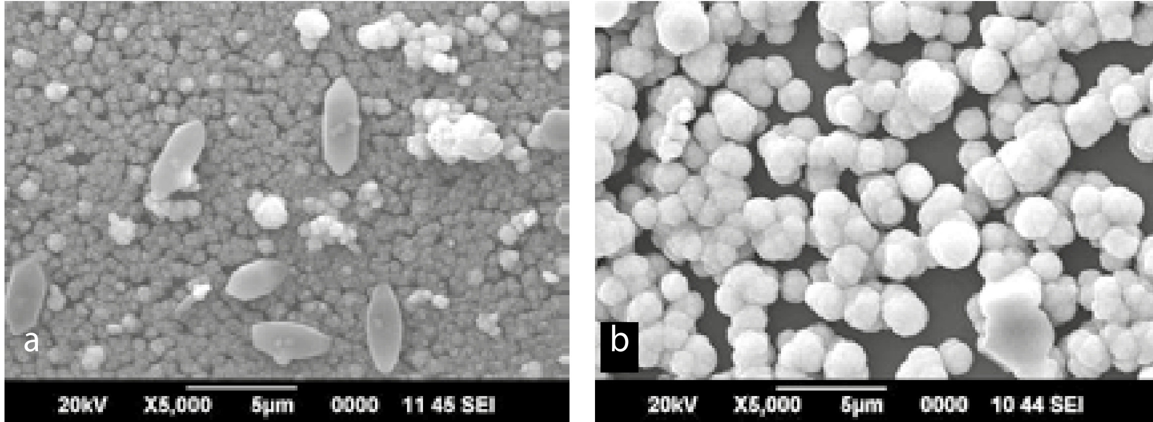


Fig. 2. SEM image of pristine (a) pristine Sb2Se3 thin film and (b) pristine Sb2S3 thin film.

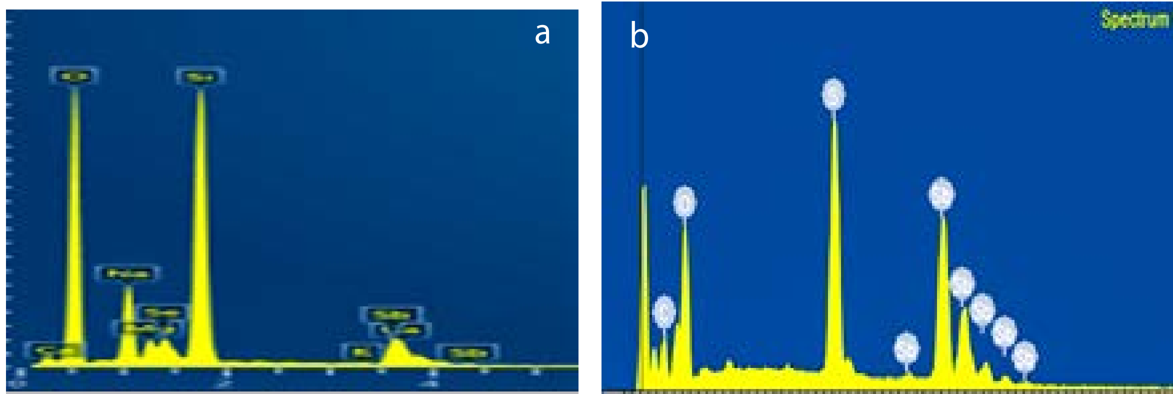


Fig. 3. EDAX image of (a) pristine Sb2Se3 film and (b) pristine Sb2S3

lesser concentration of Se than expected.

Elemental Characterisation of Sb_2S_3 thin film by EDAX analysis is illustrated in figure 3(b). The result shows the presence of Sb and S in the sample. While the expected stoichiometry of S/Sb in antimony sulphide film is 1.5, the obtained ratio was 1.28.

XPS Analysis

The XPS survey of pristine Sb_2Se_3 is shown in figure 4(a).The survey identifies the binding energies corresponding to Sb $3P_{3/2}$ orbital of antimony along with Sb $3d_{5/2}$ and Sb $3d_{3/2}$ orbitals of Sb_2Se_3. Selenium binding energy corresponding to Se $3d_{5/2}$ and Se $3P_{3/2}$ is also identified. The XPS survey of

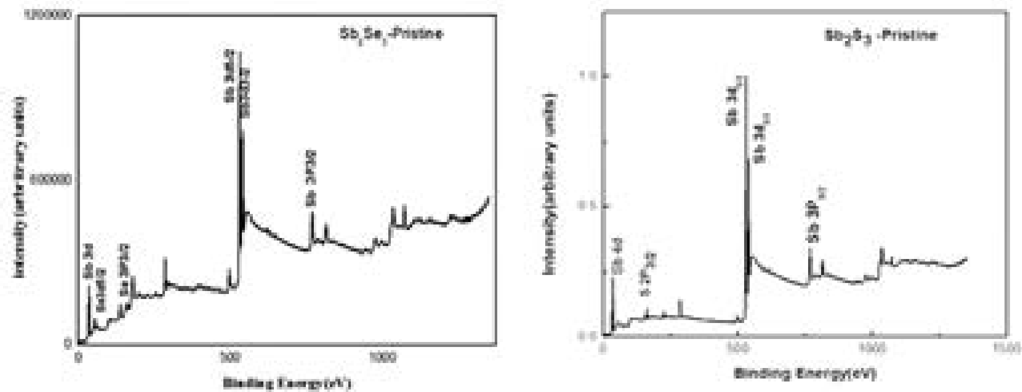


Fig. 4. The XPS survey of (a) pristine Sb_2Se_3 (b) pristine Sb_2S_3

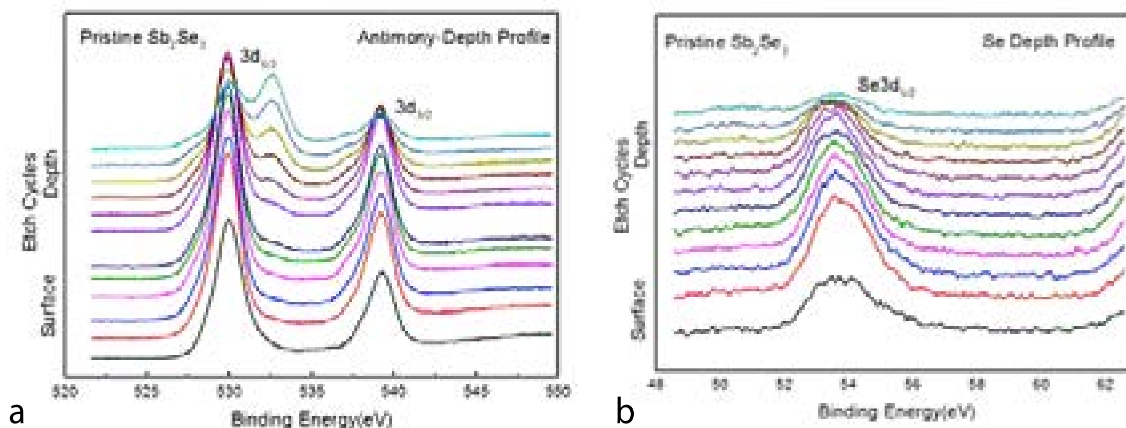


Fig. 5. (a) Depth profile of Sb in pristine Sb2Se3 (b) Depth profile of Se in pristine Sb2Se3.

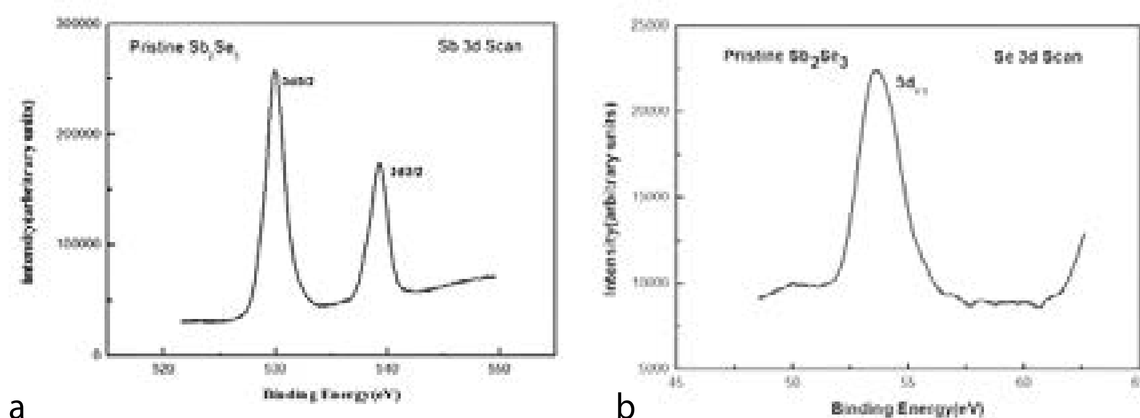


Fig. 6. (a) Sb 3d Scan of pristine Sb2Se3 (b) Se 3d Scan of pristine Sb2Se3.

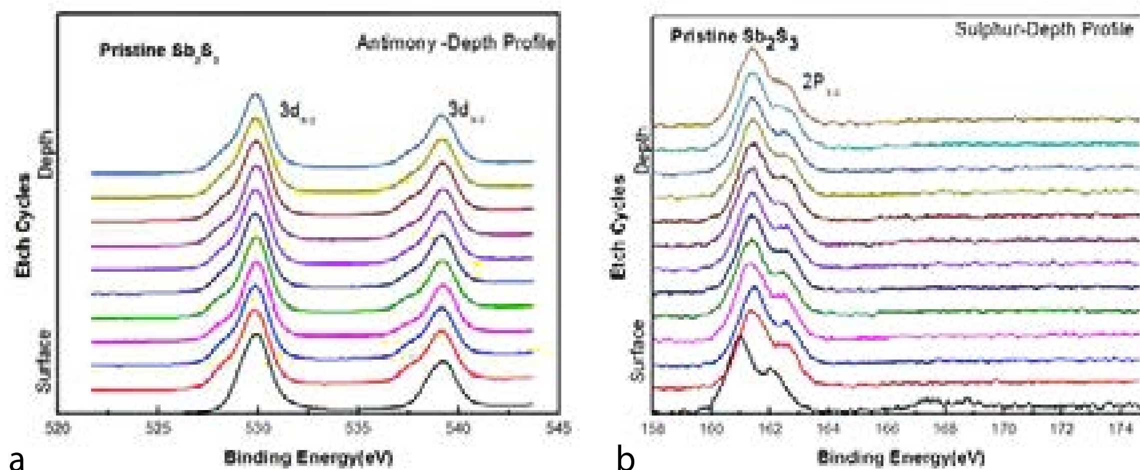


Fig. 7. (a) Depth profile of Sb, pristine Sb2S3film (b) Depth profile of S, pristine Sb2S3 film

pristine Sb2S3 is given in figure 4(b).The survey identifies the binding energies corresponding to Sb 3P3/2 orbital along with Sb 3d5/2 and Sb 3d3/2 orbitals of Sb2S3. The sulphur binding energy corresponding to 2P3/2 orbital is also identified for this compound.

Figure 5(a) and 5(b) shows the depth profile of Sb and Se in pristine Sb2Se3 thin film. Distribution of the elements is found to be uniform throughout the sample.

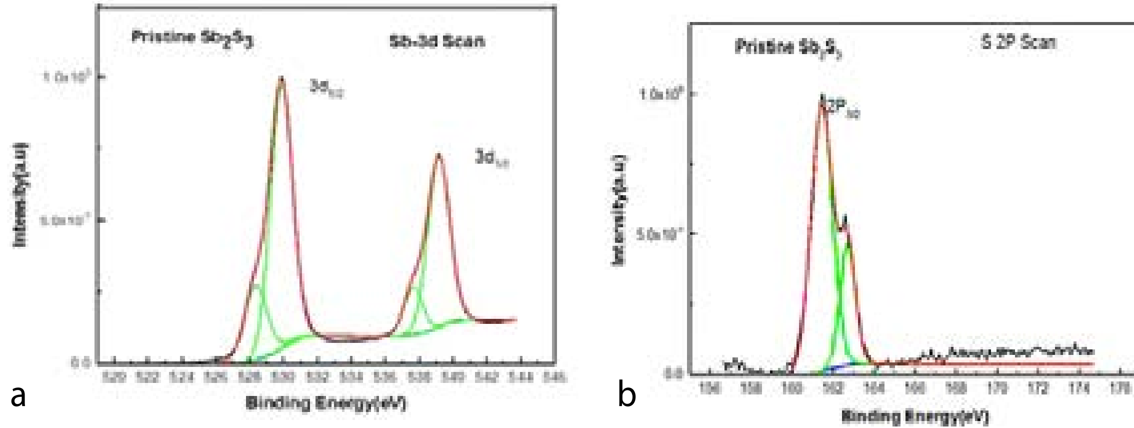


Fig. 8. (a) Sb 3d Scan of pristine Sb2S3 film (b) S 2P Scan of pristine Sb2S3 film

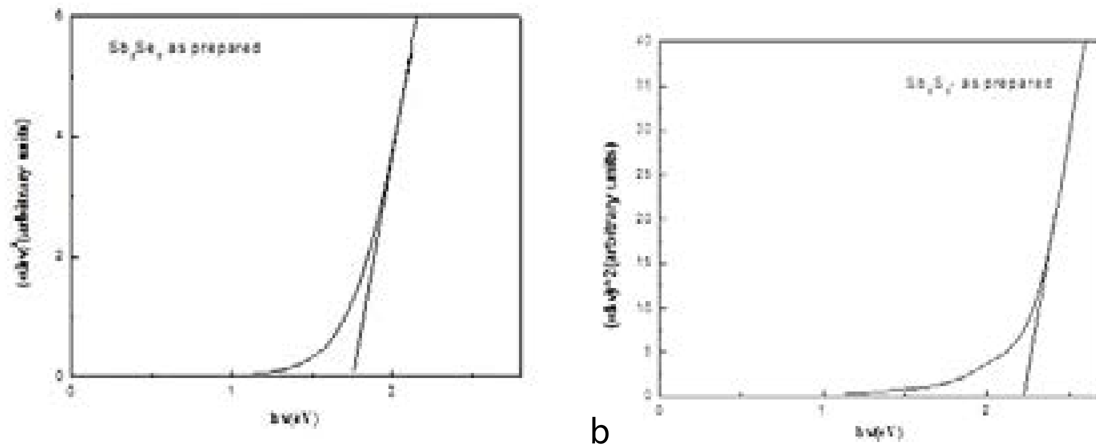


Fig. 9. Bandgap analysis of (a) pristine Sb2Se3 (b) pristine Sb2S3.

Analysis of Sb binding energy in Sb_2Se_3sample(figure 6(a)) gives binding energy values of 529.9eV and 539.2eV in the third etch cycle .This corresponds with the standard binding energy values of Sb $3d_{5/2}$(529.3eV)and Sb$3d_{3/2}$orbital of Sb_2Se_3(538.7eV). Se binding energy value identified at 53.7eV (figure 6(b)) matches with the Se $3d_{5/2}$ orbital of Sb_2Se_3.

Figure 7(a) and 7(b) shows the depth profile of Sb and S in pristine Sb_2S_3 thin film. Distribution of the elements is found to be uniform throughout the sample.

Analysis of the third etch cycle (figure 8 (a)) of Sb binding energy reveals that $3d_{5/2}$ and $3d_{3/2}$ peaks of antimony is a result of the superposition of two peaks. Deconvolution of $3d_{5/2}$ peak gives binding energies values at 528.31eV and 529.85eV respectively. In this 528.31eV matches with Sb $3d_{5/2}$of elemental antimony (standard value 528.25eV) while 529.85eV corresponds to Sb $3d_{5/2}$ orbital of Sb_2S_3 (standard value 529.7eV). Deconvolution of $3d_{3/2}$ peak gives binding energy values at 537.62eV and 539.13eV.The smaller peak at 537.62eV matches with Sb$3d_{3/2}$ corresponding to elemental antimony (standard value 538eV). Second peak at 539.13eV matches with Sb$3d_{3/2}$ of Sb_2S_3 (standard value 539.10eV).This result confirms the formation of antimony trisulphide phase along with traces of elemental antimony.

Deconvoluted peaks corresponding to sulphur binding energy in the third etch cycle (figure 8(b)) is obtained at 161.43eV and162.69eV.The first peak corresponds to $2P_{3/2}$ binding energy of Sb_2S_3 (standard value 161.20eV).The second peak corresponds to $2P_{3/2}$of elemental sulphur whose standard value is at 162.35eV.Thus the detailed analysis of

antimony and sulphur confirms the formation of Sb_2S_3 even though traces of the elemental form is also incorporated.

Optical Properties

The optical bandgap of the pristine sample is determined from the Tauc plot. (αhυ)^2 is plotted against energy value hυ and extrapolation of the linear portion of the graph to the x-axis gives the bandgap value [7, 8, and 9].Bandgap determination of the sample as in figure (9a) gives a direct bandgap of 1.853 eV for as-prepared Sb_2Se_3 samples. The pristine Sb_2S_3in figure (9 b) showed a direct bandgap of 2.23 eV.

Conclusion

Sb_2Se_3 and Sb_2S_3 thin films could be prepared by CBD method. The XRD analysis confirmed the polycrystalline nature of Sb_2Se_3 and amorphous nature of Sb_2S_3. EDX results revealed the overall stoichiometry of these films. Optical analysis showed a direct bandgap of 1.853eV and 2.23eV for Sb_2Se_3 and Sb_2S_3 films respectively. The binding energy analysis using XPS also confirmed the formation of antimony selenide and antimony sulphide thin film. Thus CBD proves to be a less expensive and less-pollutant method for the preparation of antimony chalcogenide films.

Acknowledgements

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Development of Bacterial Biosensor using gfp and lacz reporter genes for Arsenite detection in potable water samples from Gangetic Delta

R Balakrishnaraja[1], L Shadeesh kumar[1], V N Logesh[1], S John Vennison[2], D Immunal Gilwax Prabhu[2], P Thirumalaivasan[2] and B Karpanaiselvan[3]*

[1]Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam

[2]Department of Biotechnology, Anna University (BIT Campus), Tiruchirappalli

[3]CSIR-NEERI, Chennai

Abstract

Arsenic has long held a position of ambiguity with regard to its activity in biological systems. Arsenic contamination in deeper levels of groundwater, which became a high-profile problem in recent years due to the use of deep tube wells for water supply in the Ganges Delta. It causes serious arsenic poisoning to large numbers of people. Recent study found that millions of people in more than 70 countries are probably affected by arsenic poisoning of drinking water. Detection of arsenic levels in potable water can be done with development of whole cell bacterial biosensor using reliable and robust genetic engineering approaches. Isolated Pr-ABS-*arsR* gene was cloned in pUC18 and confirmed by agarose gel electrophoresis. Similarly, reporter gene *gfp* was also isolated and cloned in pUC18-Pr-ABS-*arsR* construct. To reduce the background expression second copy of ABS inserted into pUC18-Pr-ABS-*arsR-gfp* and pUC18-Pr-ABS-*arsR*-ABS-*gfp* was obtained. This modified construct was then transformed into *E.coli* DH5α. These transformed cells can be developed as whole cell biosensor and applied in field. A physical gadget for measuring arsenite was tested in both lac Z and *gfp* based whole cell biosensor.

Key words: Arsenite, ARS binding protein, whole cell biosensor, *gfp*, *lac Z*

Introduction

Industrialization and new technologies have not only made life more convenient for humans, but have also created various environmental problems, potentially poisoning living organisms, including people. Heavy metals from industries bioaccumulate in the aquatic food chain to the extent that the consumption of fish by birds and animals[8]. Organic compounds, originating from the widespread use of petroleum products, are highly toxic and causes concern about soil and drinking-water quality[1]. Conventional chromatographic methods for evaluations are both expensive and technically complicated. They tend to overestimate the bioavailability of the pollutants, since metals and xenobiotics often exist in an insoluble form in the environment[5,13]. The linking of biological component with a microelectronic device or system to enhance the rapid, low level accurate detection of various substances like body fluids, water and air[2]. In recent years, bacterial whole-cell biosensors have been developed as tools to detect and quantify the toxicity of samples from different environments. They are all living cells producing a measurable gene product in the presence or absence of the investigated compounds. In all cases, detection of various xenobiotic compounds by the bacterial biosensors described in all other review requires direct interaction between the compound measured and the biosensor, which therefore gives a direct measurement of the impact on living organisms. In

* *Corresponding author, E-mail: karpanai@gmail.com*

addition, we hope to show that the use of biosensors is not limited to risk assessment of pollutants, but also has evolved to constitute a valuable tool in studying microbial ecology and gene expression in complex environments.

Arsenic Forms and Toxicity

Arsenic in the atmosphere was from sources, such as volcanoes (gas fumes), microbes, and human activities alike burning fossil fuels.

The toxicity of arsenite is due to the formation of strong bonds with functional groups, such as the thiols of cysteine residues and the imidazolium nitrogens of histidine residues from cellular proteins. In the case of arsenate, its toxicity is the result of the mimetic effect of arsenate (AsO_4^{3}) and phosphate (PO^{3-}) which affects global cell metabolism[15]. In the present work, the fabrication of whole cell biosensor is carried out and used the green fluorescent protein as reporter protein (*gfp* gene).

Materials and Methods

Plasmid Isolation

The plasmid pBGD 23 was isolated from *E.coli* R773 by alkaline lysis method. Agarose gel electrophoresis was adopted for elution of the gene of interest.

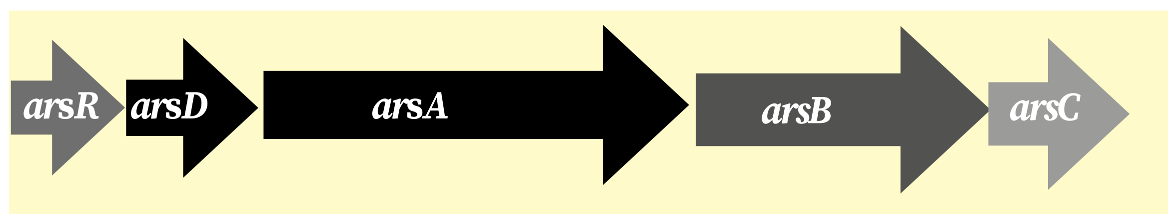

Fig.1 Ars operon of E.coli R773

Table 1. PCR specified conditions

Phase	Temperature (°C)	Time (seconds)
Denaturation	94	30
Annealing	55	30
Extension	72	30
Final extension	72	300
Final hold	4	-

Amplification of Repressor and Reporter Gene

Amplification of *arsR* gene with its promoter and *arsR* binding site with its primer's PCR. The primers for *ArsR*are**5` ccc tttcgtctt*caacgttt*ccaag 3`**; 129 bp upstream of the start of *arsR* and introducing a *Hin*dIII site and *ArsR* rev **5` aacata*tgaatt* cag gcaaattttttag 3`**, covering the stop codon of *arsR* and introducing a unique *Eco*RI site for amplifying *arsR* along with its promoter. The *gfp* gene present in the plasmid p*GFP*uv (from Clone tech. corporation, CA, USA) was amplified. The amplified reporter gene was cloned in downstream of sensing part (*arsR)* at appropriate restriction site in the MCS of pUC18-*arsR*. The resulting plasmid consists of *arsR* and *gfp* genes. Agarose (1%) gel electrophoresis was carried out for eluting repressor and reporter gene amplified through PCR.

Restriction digestion of PUC18 vector

Eluted *ArsR*andpUC18 vector was added with restriction enzyme buffer and one unit of the BamHI restriction enzyme. The reaction volume was made up to 40µl with sterile water. Then contents were centrifuged and incubated at 37°C for 2 hr. The reaction was terminated by adding phenol/EDTA and the sample was frozen immediately.

Ligation of Pr-ABS-ARSR gene into PUC18 vector

Restricted *Pr-ABS-arsR* and pUC18 vector were ligated by T_4 DNA ligase and the reaction volume

was made up to 20µl. It was mixed thoroughly and incubated at 12°C for overnight.

Transformation of PUC18-Pr-ABS-arsR construct into E.coli DH5α

E.coli competent cells and pUC18-Pr-ABS-*arsR* plasmid was added and mixed. It was then incubated in ice. A heat shock for 2 min was given at 42°C and again incubated in ice. The transformed cells (100 µl) were plated on selective plates (Luria agar containing 50 µg/ml of ampicillin) and incubated for 16 to 17 hr at 37°C.

Insertion of second ABS in constructed plasmid

Background expression can be reduced by the insertion of second copy of ABS to the constructed plasmid. The steps involved are a) Amplification of ABS with given primer sequence in polymerase chain reaction b) Restriction with Eco RI enzyme and ligation using T_4 DNA ligase into constructed plasmid c) Transformation into *E.coli* DH5α cells

Restriction with EcoRI enzyme and ligation using T_4 DNA ligase into constructed plasmid

The restriction pattern was followed same as 3.1.6, with restriction enzyme as EcoRI and its corresponding buffer. The amplified and eluted ABS sequence was then ligated with pUC18-Pr-ABS-*arsR-gfp* construct. Transformation of pUC18-ABS-*arsR*-ABS-*gfp* was done.

Amplification of response and reporter gene

The amplification of the repressor gene *arsR* and the reporter gene *gfp* was confirmed by agarose gel electrophoresis against marker DNA of 1kb size. The approximate size of insert gene was 0.7 kb. A Prominent band near 0.7 kb was observed under uv transilluminator (Genei, India). The amplified reporter gene was confirmed similarly which shows the size was approximately 1.1 kb.

Restriction digestion and ligation of Pr-ABS-arsR and gfp to PUC18

The restriction digestion of Pr-ABS-*arsR* and vector pUC18 using BamHI was confirmed by the presence of 0.7 kb and 2.9 kb linearized bands on the agarose gel. Similarly, *gfp* gene also restricted using Hind III enzyme and confirmed through 0.6 kb size band.

Confirmation of presence of second copy of ABS

The constructed plasmid was modified by inserting second copy of ABS to reduce background expression and confirmed through PCR. The ABS amplify the region between two ABS inserts and confirmed through agarose gel. The band obtained near 0.8 kb size as well as 0.3 kb showed the two copies of ABS in the modified construct pUC18-Pr-ABS-*arsR*-ABS-*gfp*.

Screening of recombinants

Through blue and white colonies growth was observed distinctly in 10^{-5} diluted plates. The white colonies were separated and patched in LB media with ampicillin (50 µg/ml). The transformants were isolated for modified plasmid and insertion of response and reporter gene was confirmed by restriction digestion by Hind III. Prominent band near 1.8 kb shows the presence of both genes in agarose gel electrophoresis under UV transilluminator.

Arsenite detection assay

The arsenite measurement assay procedure was carried out for various river water samples and found to detect higher concentration of arsenite than that of ICP-OES16. The ICP – OES found to quantify arsenite of 2 ppm concentration. The detection of arsenite was in the range of 7 µM to 50 µM for whole cell biosensor containing gfp as reporter gene. The fluorescence obtained for standard concentration of arsenite was shown in the figure below. Constructed pUC18 plasmid which can detect the arsenite in the potable water sample. Also, a physical gadget was fabricated to test the arsenite level of contamination.

RESULTS AND DISCUSSION

Arsenic has long held a position of ambiguity with regard to its activity in biological systems. Arsenic contamination in deeper levels of groundwater, which became a high-profile problem in recent years due to the use of deep tubewells for water supply in the Ganges Delta. It causes serious arsenic poisoning to large numbers of people. Recent study found that millions of people in more than 70 countries are probably affected by arsenic poisoning of drinking water. Detection of arsenic levels in potable water can be done with development of whole cell bacterial biosensor using reliable and robust genetic engineering approaches. Isolated Pr-ABS-arsR gene was cloned in pUC18 and confirmed by agarose gel electrophoresis. Similarly, reporter gene gfp was also isolated and cloned in pUC18-Pr-ABS-arsR construct. To reduce the background expression second copy of ABS inserted into pUC18-Pr-ABS-arsR-gfp and pUC18-Pr-ABS-arsR-ABS-gfp was obtained. This modified construct was then transformed into E.coli DH5α. These transformed cells were developed as whole cell biosensor and assayed for the presence of arsenite. The developed sensor emits fluorescence which is exploited to show presence of arsenite.

The developed paper strips were analyzed for the blue color development with the response to the arsenite concentration with the substrate addition of 10µl of 2mg/ml X-gal stock, the blue color development for minimum concentration of arsenite in standard arsenite stock solution was observed from 6.5µM.

The sensor was developed as portable, flexible physical gadget with transmitter, optic fiber and receiver elements for quantification of arsenite. The bridge board circuit was well worked out and shown as figure below. This was transferred into proto model with the help of PCP assistance which shows deflection in voltage of multimeter. The fabrication of the biosensor as given in the circuit was done. UV or Infrared transmitter is one type of LED which emits ultraviolet or infrared rays generally called as UV/IR Transmitter. Similarly, receiver is used to receive the rays transmitted by the transmitter. One important point is both transmitter and receiver should be placed straight line

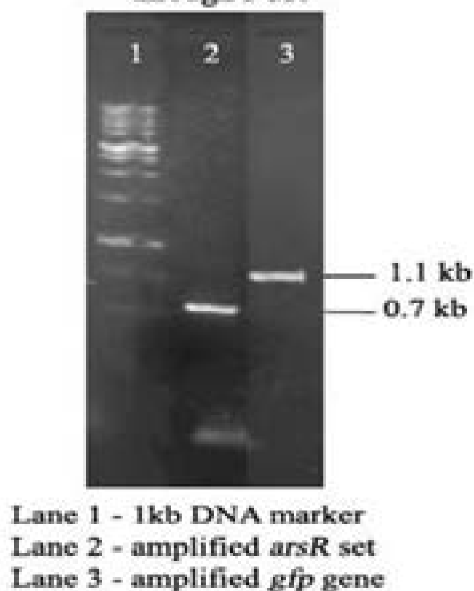

Fig.2 Agarose gel electrophoresis of amplified arsR gene and gfp gene.

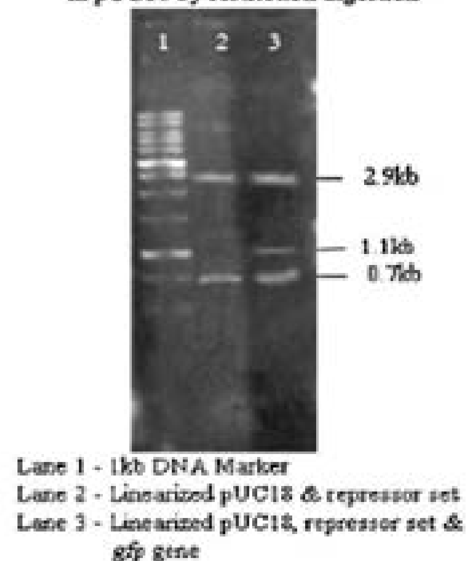

Fig.3 Agarose gel electrophoresis of repressor and reporter gene pUC18 by restriction digestion

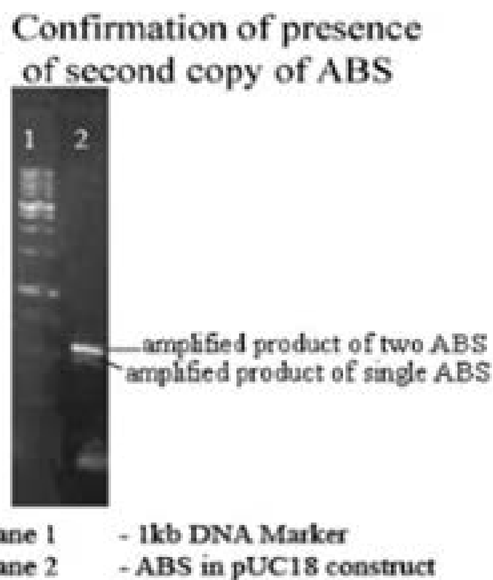

Fig.4 Agarose gel electrophoresis of second copy of ABS

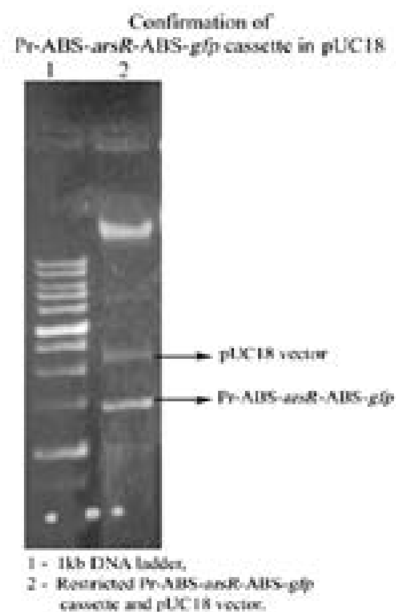

Fig.5 Agarose gel electrophoresis of Pr-ABS-arsR-ABS-gfp cassette

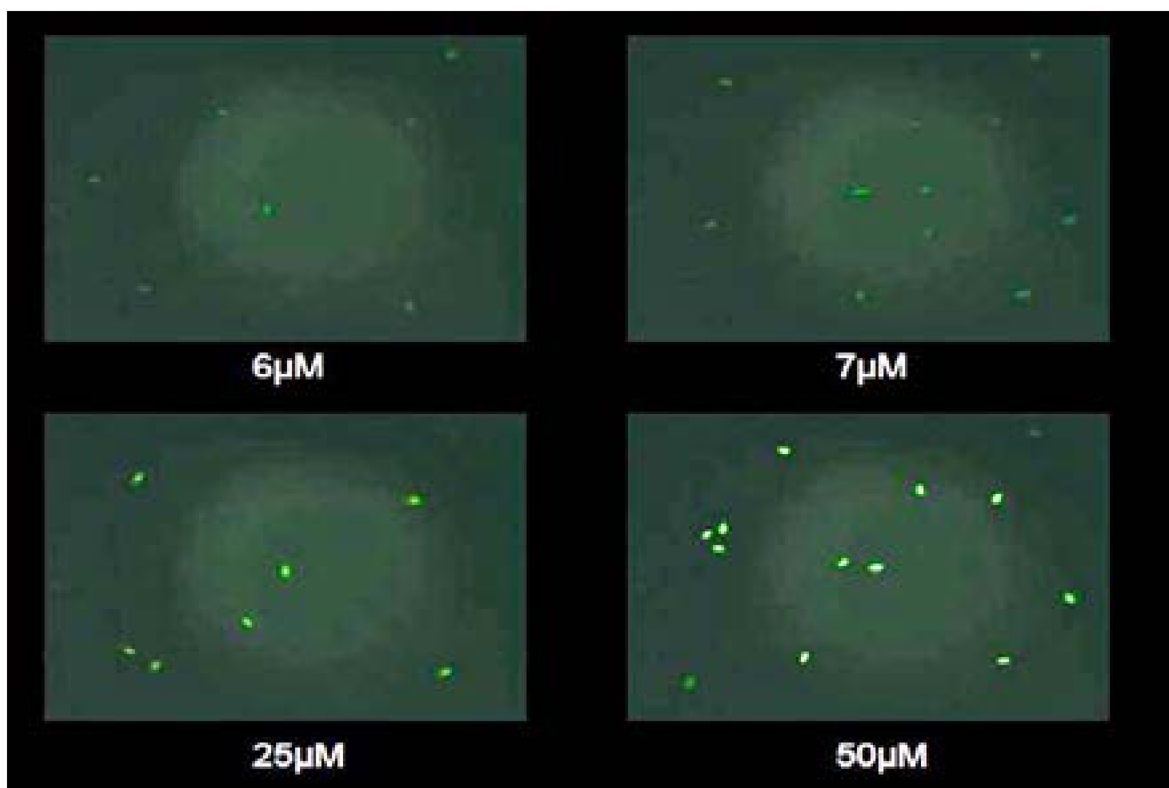

Fig.6 Detection of Arsenite at different ranges

to each other. Whenever the transmitted signal is high, the transmitter LED is conducting and it passes the rays to the receiver. The receiver in turn connected with comparator. The comparator is constructed with LM 358 operational amplifier. In the comparator circuit the reference voltage is given to inverting input terminal. The non-inverting input terminal is connected to receiver. When interrupt the emitted rays between the transmitter and receiver, the receiver is not conducting. So, the

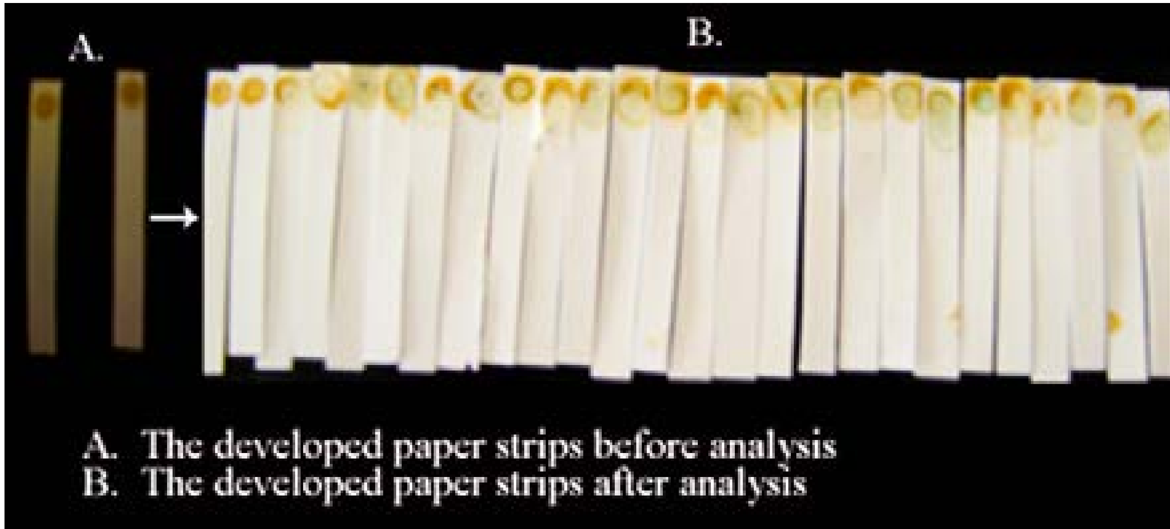

Fig. 7 Development of portable whole cell biosensor

comparator noninverting input terminal voltage is higher than inverting input. Now the comparator output is in the range of +5V. This voltage is given to microcontroller or PC and LED will glow. When transmitter rays pass to the receiver, the corresponding receiver is conducting due to that noninverting input voltage is lower than inverting input. Now the comparator output is GND so the output is given to microcontroller or PC. This circuit is mainly used to detect the disturbance in the form of light. Hence addition of suitable filter used to quantify the fluorescence or light intensity emitted by biosensor cells.

Contamination of drinking water with geogenic or anthropogenic arsenic is one of the most severe environmental and public health problems in developing countries, thus calling for reliable, yet simple and cost-effective methods for arsenic monitoring and removal from potable water[3]. Whereas commercially available, colorimetric field test kits have the advantage of being relatively cheap and simple to use for routine arsenic monitoring, they lack the sensitivity and accuracy required to measure arsenic at the toxicologically

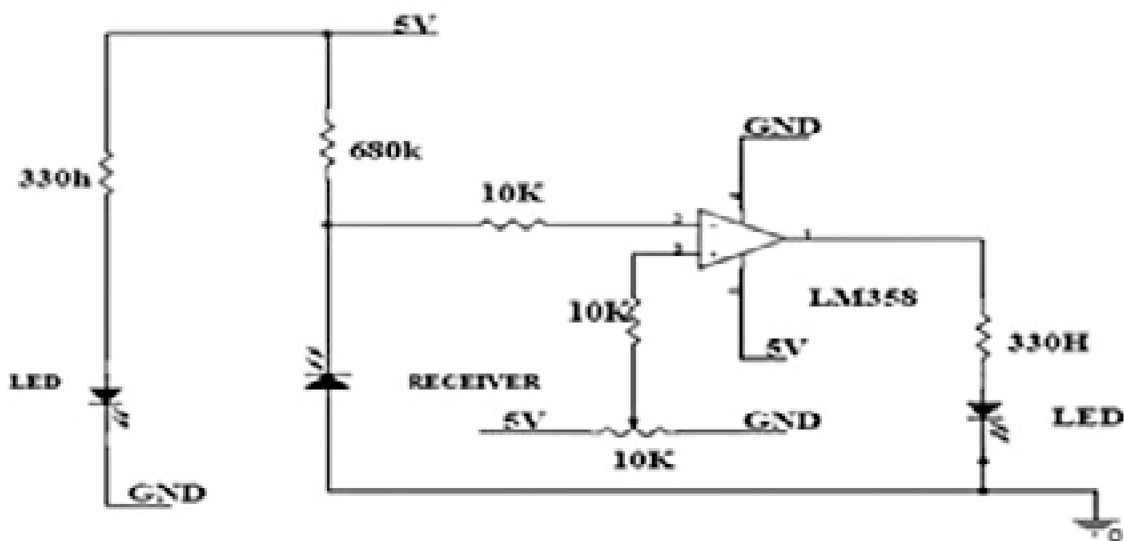

Fig 8 Schematic circuit diagram of fluorometer device

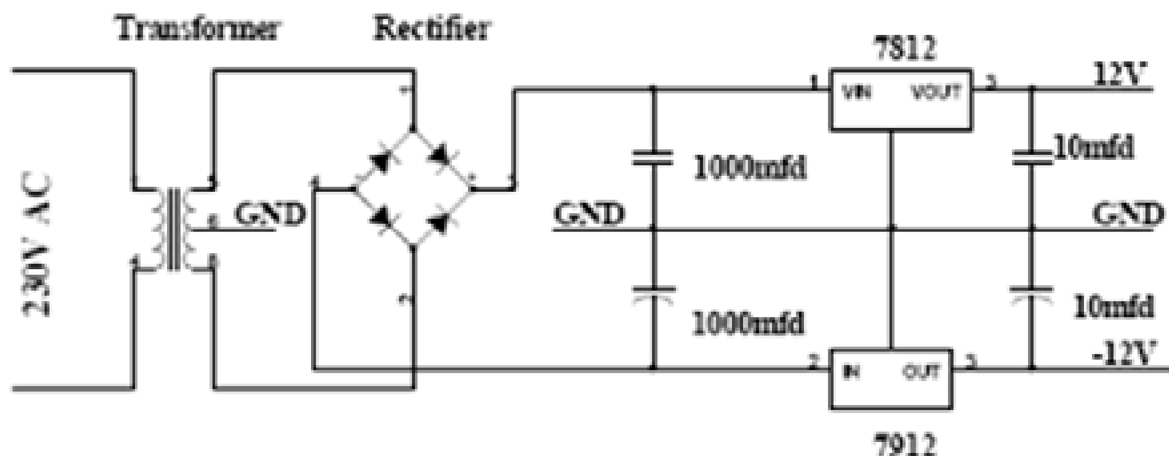

Fig.9 Schematic circuit diagram of fluorometer power supply

relevant concentration range <70 mgL^{-1} frequently found in contaminated potable water sources[10]. Spectroscopic methods of arsenic quantification are reliable and sensitive but do not fulfill the requirement of being affordable for routine monitoring of hundreds of thousands of drinking water wells in the most affected countries Bangladesh and Vietnam[4].

Biosensor measure bioavailable rather than total quantities of chemicals. They can be used

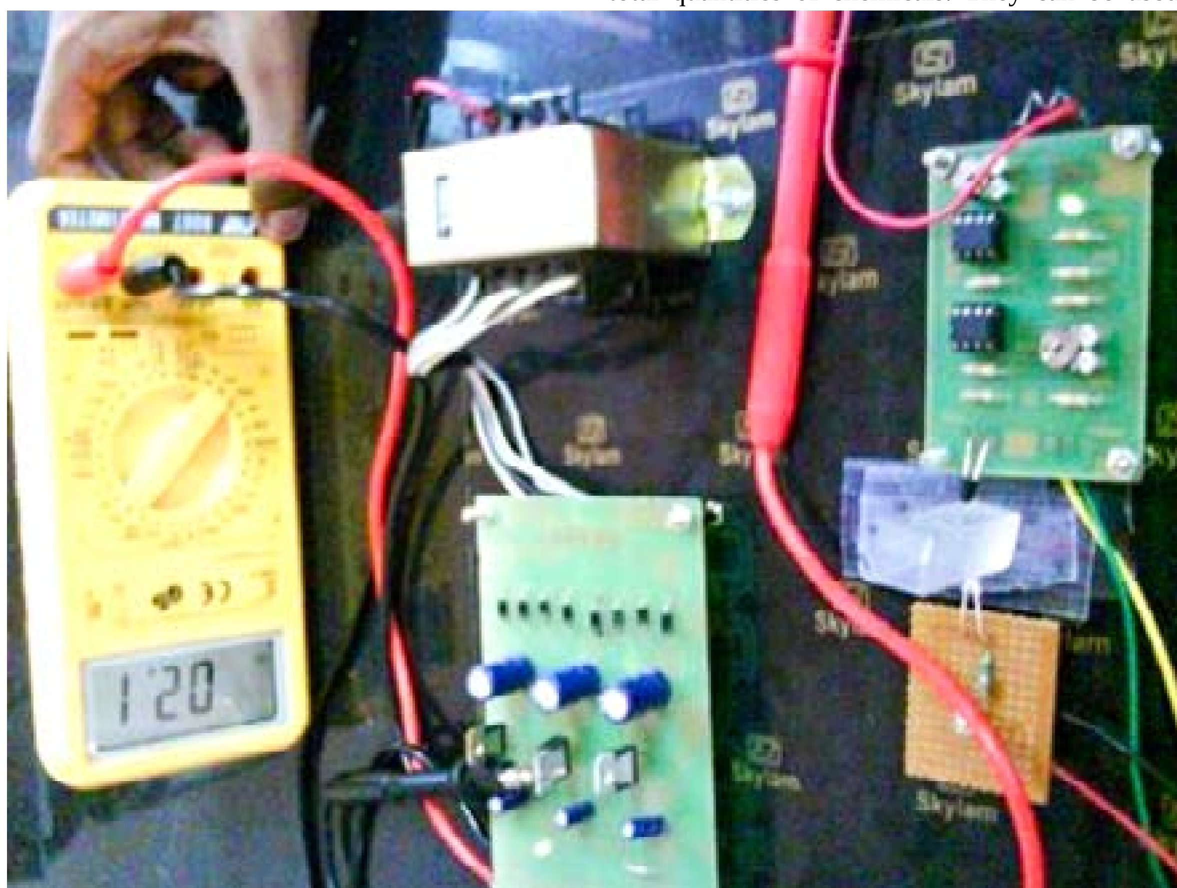

Fig. 10. Developed proto fluorescence sensing system showing deflection when arsenite solution placed in between transmitter and receiver circuit

for spatially restricted environments. Biosensor technology could be a rapid first-line screening for general toxicity or stress. A whole-cell biosensor using the bacteria as the host and a pigment gene/reporter gene as a novel reporter to detect environmental pollutants with high sensitivity[7]. Advantages of bacterial biosensor are Measures bioavailable fraction, inexpensive, produces real-time data, less labor intensive, more sensitive, suitable for field work. Limitations in bacterial biosensor are of short lifetime, lack of genetic stability, limited understanding of applicability to higher organisms, performance dependent on environment of procedure.

Bioreporters have made a brilliant career in research and are generally considered as valuable tools for toxicological and environmental research. Bioreporter organisms have thus little potential as mass products in research. Time also plays a role as well as the success and the public perception of demonstration applications. One promising field technique is a paper-strip test based on genetically engineered *E. coli* bacteria as biosensors. Also, in this case a color reaction indicates the concentration of arsenic in groundwater. The advantage of this technique is the reliable determination of concentrations between 2, 5-75 µg/L. After one-time application the paper-strip can be disposed. The used bacteria are not pathogenic, e.g. one drop of lemon juice makes them harmless. Moreover, this method is simple designed, and the cost of production are kept low (34 Cent a piece), because it is mainly intended for domestic purpose. Simple applications like biosensor field test kits or paper test strips are hampered by the difficulty to compare the resulting color signals with standardized color tables. Therefore, a robust and reliable system for internal calibration would be highly desirable[17].

The high sensitivity of the biosensor cells for arsenite has the additional advantage that unknown samples can be diluted, thereby also diluting possible inhibitory compounds. Qualitative measurements can be performed with the paper strip method, which could facilitate faster identification of samples containing arsenic concentrations substantially above drinking water standards and which subsequently could be analyzed in more detail. Furthermore, the paper strip method could also be used to test the effectiveness of local treatment methods, for instance those targeting the precipitation of arsenic with iron. Despite their potential[6], bacterial whole cell biosensor methods have not received great interest for practical applications until now and have remained mostly research laboratory-based methods. Mainly, this is because the physiological activity and maintenance of the microorganisms can be somewhat difficult to control.

However, almost no reported bacterial biosensors can detect analytes at concentrations below 0.1 µM[9]. Extensive efforts are necessary to increase the sensitivity of bacterial biosensors for widespread use. Arsenite measurements have been performed before with *lacZ* bioreporters under control of the *arsR* promoter using classical spectrophotometrically, chemiluminescence and electrochemical detection[11,12]; or by chromogenic visualization using X-gal[14]. The leaky expression from the *arsR* promoter must be reduced to obtain gradually intensifying blue color formation from X-gal as a function of arsenite concentration. This was accomplished by placing a second *arsR* DNA binding site downstream of *arsR*[14].

The *E. coli* arsenite biosensor cells could be used to produce a color in response to arsenite. For this purpose, β-galactosidase would be a useful reporter protein since many different substrates are available for this enzyme, which will be converted to insoluble colored products. Unfortunately, also the arsenite sensor using *arsR* transcriptionally fused to *lacZ* produced too high background levels of β-galactosidase in the absence of arsenite. Therefore, a second binding site for the *arsR* protein downstream of *arsR* and in front of *lacZ* was introduced. The use of the substrate X-Gal to assess β-galactosidase levels produced intracellularly by the sensor cells. Increasing shades of blue arise at increasing arsenite concentrations to which the cells have been exposed.

Fig. 11. Developed proto fluorescence sensing system showing deflection when arsenite solution placed in between transmitter and receiver circuit

Although the intensity of the blue color can be measured spectrophotometrically, producing similar calibration curves, the biosensor cells expressing β-galactosidase could also be used on a solid medium for a qualitative strip test. Here to small batches, were dried on a paper matrix. When the paper strips were placed for 30 min in a tube with 1mL of aqueous sample containing arsenite, then taken out and the substrate X-Gal added, blue spots appeared, the intensity of which was proportional to the arsenite concentration. At the amount of biosensor cells used here and after an incubation time of 30 min, the blue color was barely visible at an arsenite concentration around 10µg/L. This was designed in order to create the subjective interpretation of a "negative" test outcome at arsenite levels at or below the current drinking water limits of 10 µg of As/L.

The *arsR* gene controls expression of the reporter gene. In the situation with one *arsR* binding site (black vertical bar), some background expression occurs from the *arsR* promoter (P *ars*), symbolized as a wave line, which leads to the synthesis of *arsR* itself (symbolized as dimer protein) and of the reporter protein. *arsR* binds to its binding site but in the presence of arsenite (AsIII) will lose its affinity for its binding site on the DNA. In this case, repression will be relieved and transcription from the *arsR* promoter will be very high (thick wave line). In case of a secondary *arsR* binding site downstream of *arsR*, only the *arsRm* RNA will be produced as background. *arsR* will bind to both DNA binding sites and prevent RNA polymerase from reading the reporter gene. In the presence of arsenite, *arsR* will again lose its affinity, and expression from the reporter gene will be initiated (Fig.11).

The pUC18 is a high copy number plasmid and thus used to detect very low concentration of arsenite due to cloned *arsR* gene along with its promoter upon arsenite induction. The *gfp* needs no cofactors or substrates for its fluorescence, and exposure to oxygen leads to spontaneous maturation of the fluorophore in the cell. The *gfp* biosensor was developed in order to avoid the use of substrates that are needed for measuring the activities of the luciferase or *β*-galactosidase[14]. The sensitivity of the *gfp* biosensor to arsenite was less than that of the luciferase sensor, with a higher level of detection of 78 ppb. At arsenite concentrations above 7ppb increase of the fluorescence level of individual cells was observed. However, by varying the camera exposure time and induction time, this upper range of detection could be increased to 200 ppb[14]. Subsequent development of our biosensor aims to detect low level of arsenite contamination. The portability of instrument could be improved by acquiring knowledge from physics and electronics basics. The limitation in developing green fluorescence detector lies in the high sensitivity emission filter and optic fiber. These economic factors should be resolved for developing low cost portable sensor which could detect green fluorescence. Currently developed

biosensor could detect and display the values in terms of voltage for varying optical and fluorescence intensity.

The present study has shown that a nonpathogenic biosensor strain of *E. coli* can be controlled and maintained without great problems. These systems could be the basis for either larger scale production of such sensor devices as the paper strips/gadget are for local maintenance and production in quality control laboratories and hospitals, especially in developing countries, where the measurement of arsenic in domestic and drinking water samples is most urgent need of the hour.

CONCLUSION

Abatement of arsenite becomes unavoidable due to its abundance in nature reported since 1975 in Indian subcontinent. Arsenic has long held as serious public concern which affects biological systems upon ingestion. Arsenic contamination in deeper levels of groundwater due to the use of deep tube wells for water supply in the Ganges Delta. Detection of arsenic levels in potable water can be done with development of whole cell bacterial biosensor through recombinant DNA technology. Arsenite detection through presently available instrumental methods, chemical kits are unreliable. Isolated Pr-ABS-arsR gene was cloned in pBR322/pUC18 and confirmed by agarose gel electrophoresis. Similarly, reporter gene lacZ/gfp was also amplified and cloned in pBR322/pUC18-Pr-ABS-arsR construct. To reduce the background expression second copy of ABS inserted into pBR322/pUC18-Pr-ABS-arsR-lacZ/gfp and pBR322/pUC18-Pr-ABS-arsR-ABS-lacZ/gfp was obtained. The transformed cells with cassette pBR322-Pr-ABS-arsR-ABS-lacZ were selected by replica plating with Luria Bertani agar with ampicillin (100 µg/ml) and in tetracycline (50 µg/ml). The colonies which were not able to grow in tetracycline plate are selected. The selected single colony was used as whole cell bacterial biosensor for the strip preparation. The enzyme β-galactosidase that produces a color reaction in the presence of arsenic. These biosensor cells are also grown in liquid broth, harvested and mixed with ingredients such as sugars, amino acids and gelatin and were pipetted on paper strips and carefully dried at controlled temperature and in partial vacuum. For carryout an assay, thus prepared paper strip is placed in a vial with 1ml of aqueous sample, incubated for 30min at 30°C and taken out. A drop of substrate (X–gal) was added to paper strip the bacterial spot turns to blue color due expression of β-galactosidase. This paper strip may be used as a qualitative measurement of arsenite present in the water samples. In our studies the minimal arsnite detection was observed from 9µg/L. The transformed cells with cassette pUC18-Pr-ABS-arsR-ABS-gfp were selected by blue and white plagues on Luria agar with ampicillin (100µg/ml) and the blue plagues were selected and analyzed for fluorescence emission when exploited in the presence of arsenite. This shows the fluorescence from 7µM of standard arsenite stock solution.

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Fish Diversity of Selected Areas of Flood Affected Chalakudy River

Krishna A.M.[1] RevathyBabu[1]*, Ashitha Animon[1], HaripriyaRenjeev[1] and Arjun C.P. [2]
[1]Department of Zoology, Sree Sankara College, Kalady, Kerala,India.
[2]Ph.D Research Scholar, C.V Raman Laboratory of Ecological Informatics, Indian Institute of Information Technology and Management (IITM-K), Technopark campus, Karyavattom, Trivandrum.

Abstract

The freshwater biodiversity of the world is declining due to pollution, habitat loss, introduction of exotic species, over-exploitation etc., Several studies reported that the fish fauna of Chalakudy river, the fifth longest of the 44 rivers of Kerala, is at risk primarily due to pollution and introduction of exotic species. The recent flood in Kerala also had a devastating effect on Chalakudy river basin. In this regard, the present study was conducted to analyzethe fish diversity status of Chalakudyriver after flood. The study was conducted during the period August to December, 2018. Sampling was done twice a month from the selected three sites.A total number of 30 species, belonging to 10 orders, 22 families and 28 genera were recorded during the study period. Among the documented species, the order Perciformes was dominant (11 species) followed by Siluriformes (6species) and Cypriniformes (5 species). The order Anguilliformes was represented by 2 species and orders Beloniformes, Clupeiformes, Characiformes, Tetradontiformes, Mugiliformes and Elopiformes were represented by 1 species each. Of the 30 species recorded 3 are listed under various threatened categories on the IUCN Red List of Threatened Species. The present study also reported the occurrence of some coastal and estuarine species in the river. Rare species like *Pristolepis rubripinnis*, *Chelonodon patoca* were collected during the study period. 3 exotic fishes were also recorded from the study area.The study point to the fact that the Chalakudy river is rich in terms of fish faunal diversity however, the exotic fishes may pose threat to the native fish fauna and the associated biodiversity. We recommend performing periodic surveys in various regions of the river to get an accurate data of the fish diversity of Chalakudy river after flood.

Key words: Fish diversity, Chalakudy river, exotic species, critically endangered, *Pristolepis rubripinnis*.

Introduction

Fishes form a major vital part of the aquatic biodiversity. In the Western Ghats region, fresh water fishes form an important endemic vertebrate group. Rivers of Western Ghats region are exceptionally rich in biodiversity and a number of authors have described the icthyofaunal diversity in this region[1,2,3,4,5,13].The Chalakudy river which originates from the Western Ghats is one of the richest river systems in Kerala with regard to freshwater fish diversity and 71 fish species have been reported[1]. 83 fish species were recorded from Chalakudy river and altogether the biodiversity studies carried out in this river pointed that the icthyofaunal diversity encompasses 98 fish species[2]. Many taxonomists tried to explore the fish fauna of Chalakudy river to identify native, non-native and new species in order to prepare checklists of fish faunal resources. Several new species were recorded from this river during the 1990s, *Garra surendranathanii, Horabagrus nigricollaris, Osteochilichthys longidorsalis, Puntius chalakkudiensis* and *Travancoria elongata*. *Glyptothorax lonah* (Karappara tributary of Chalakudy river), *Barilius bendelisis* (Thekkady tributary) and *Macrospinosa cuja* were reported from Kerala fresh waters. Three new species namely

* *Corresponding author, E-mail: souparnika13revathy@gmail.com*

Osteochilichthys longidorsalis, *Travancoria elongate* and *Horabagrus nigricollaris* have been identified from the Chalakudy river in 1994[3]. *Hypselobarbus kurali, Euryglossa orientalis, Puntius dorsalis, Travancoria jonesi*, *Ompokma labaricus*, *Nemacheilus guentheri* and *Tetraodontravan coricus* were reported from Chalakudy River in 2000[4]. A new species *Pristolepis rubripinnis* was identified from Chalakudy River[5]. *Sahyadria chalakkudiensis* is a new fish species of cyprinidae family endemic to the Chalakudy[6]. The richest fish family in Chalakudy river is Cyprinidae, followed by Bagrid cat fishes and hill stream loaches[7]. Arunkumar *et al*, reported the presence of 6 endangered fish species in the Chalakudy river system which includes *Hypselobarbus curmuca*, *Hypselobarbus dubius, Tor malabaricus, Tor khudree,Travancoria jonesi*[8]. There are probably many more to be discovered here. Anyway, this is a good indication of the rich fish faunal diversity of Chalakudy river.

However, the fish fauna of Chalakudy river, is at risk due to habitat alteration, pollution, introduction of exotic species indiscriminate collection of threatened ornamental and endemic fish species and over exploitation of endangered food fishes by forest inhabitants and local fishers[9,10].The presence of exotic fishes like *Oreochromis mossambicus, Gambusia affinis, Osphronemus goramy, Xiphophorus maculates* and *Poecilia reticulata* has been reported in the Chalakudy River[1,11]. Illegally imported carnivorous fish species like African cat fish (*Clarias gariepinus*) and Red piranha has recently entered this river system and impacted the native species. It is generally agreed that exotic fishes are a menace to the indigenous freshwater fish fauna and it has been a major factor determining the overall health and functioning of the affected ecosystem. Apart from these, Chalakudy River basin was one of the badly hit river systems in Kerala flood,2018. Though, several researchers have assessed the fish faunal diversity of this river earlier no study was carried out after flood in 2018. Therefore, the current study was aimed to collect and identify the fish fauna from a few regions of Chalakudy river after the flood.

Materials and Methods

Study Area

Three flood affected sites (Site1: Pulikkakadavu;Site2:Thiruvarambikadavu ; Site3: Paneerkadavu) of Chalakudy River in a stretch of 5 km were sampled for the study. Site selection was based on convenience and sites were situated downstream to the Nitta Gelatin India Limited, Kathikudam.

Fish Sampling and Identification

Fish samples were collected from the selected three sites twice a month from August to December 2018. Fish fauna were collected with the help of local fishermen using cast nets, dip nets, drag nets of different mesh sizes. Fishing was noted along with the date and location. Fish fauna were photographed in their original color and morphological

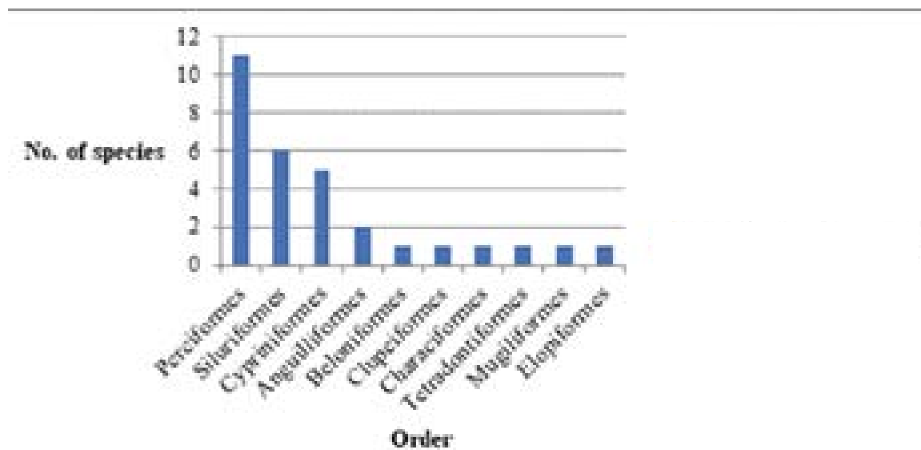

Fig.1. Distribution of the fish species recorded from the study areas

Fig. 2. Fish fauna of Chalakudy river
a. Stolephorus commersonni b. Megalops cyprinoides c. Pangasius pangasius d. Hypselobarbus thomassii e. Labeo cussumierri f. Glossogobius giuris g. Hyporhamphus limbatus h. Congresox sp.

Fig. 2. Fish fauna of Chalakudy river
a. Pygocentrus nattereri b. Mystus gulio c. Channa marulius d. Ambassis gymnocephalus e. Gerres filamentosus f. Lates calcarifer g. Anguilla benalensis h. Hetropneustes fossilis.

Fig. 4. Fish fauna of Chalakudy river
a. Etroplus maculatus b. Etroplus suratensis c. Rasbora daniconius d. Pristolepis rubripinnis e. Horabagrus brachysoma f. Lutjanus argentimaculatus g. Chelonodon patoca h. Channa striata.

Fig. 4. Fish fauna of Chalakudy river
a. Oreochromis mossambicus b. Dawkinsia filamentosa c. Systomus sarana d. Clarias gariepinus e. Ompok malabarius f. Mugil cephalus

characters were noted. Systematic identification of the fish species were carried out by using the standard keys[12] handbooks, and in consultation with experts. The conservation status of the fish species according to the IUCN Red List of Threatened Species was mentioned in our results.

Results

In the present study a total of 30 species, belonging to 10 orders, 22 families and 28 genera were recorded (Table.1). Among the documented species, the order Perciformes was dominant (11 species) followed by Siluriformes (6species) and Cypriniformes (5 species). The order Anguilliformes was represented by 2 species and orders Beloniformes, Clupeiformes, Characiformes, Tetradontiformes, Mugiliformes and Elopiformes were represented by 1 species each (Figure.1). Fishes listed under threatened categories on the IUCN Red List were *Hypselobarbus thomassi*,-belong to critically endangered category, *Horabagrus brachysoma*,belonging to vulnerable category and *Anguilla bengalensis* comes under near threatened category. Some estuarine and coastal

Table1.The list of fishes recorded during the study period in the selected areas and their conservation status

Sl. No.	Order	Family	Scientific name	Common name	IUCN red list category *DD,LC, VU, NT, NE, CR
1.	Anguilliformes	Muraenesocidae	*Congresoxsp* (Blecker,1853)	Indian Pike Conger	NE
2.	Anguilliformes	Anguillidae	*Anguilla bengalensis (J. E Gray, 1831)*	Indian mottled eel	NT
3.	Cypriniformes	Cyprinidae	*Hypselobarbus thomassiiF. Day, 1784)*	Red canarese barb	CR
4.	Cypriniformes	Cyprinidae	*Labeo dussumieri* (Valenciennes, 1842)		LC
5.	Cypriniformes	Cyprindae	*Rasbora daniconius* (Hamilton, 1822)	Black line rasbora	LC
6.	Cypriniformes	Cyprinidae	*Dawkinsia filamentosus (Valenciennes, 1844)*	Filament barn, Black spot barb.	LC
7.	Cypriniformes	Cyprinidae	*Systomussarana (F.Hamilton, 1822)*	Olive barb	LC
8.	Siluriformes	Siluridae	*Ompok malabaricus (Valenciennes, 1840)*	Goan catfish	LC
9.	Siluriformes	Clarridae.	*Clarias gariepinus (Burchell,182)*	African sharp tooth catfish	LC
10.	Siluriformes	Bagridae	*Horabagrus brchysoma* (Gunther ,1864)	Sun catfish	VU
11.	Siluriformes	Heteropneustidae	*Heteropneustes fossilis* (Bloch ,1794)	Asian Stinging Catfish	LC
12.	Siluriformes	Pungasiidae	*Pangasiuspangasius (Hamilton, 1822)*	Shark cat fish	LC
13.	Siluriformes	Bagridae	*Mystusgulio* (Hamilton,1822)	Long whispered catfish	LC
14.	Perciformes	Cichlidae	*Etroplus maculates* (Bloch,1795)	Orange chromidae	LC
15.	Perciformes	Cichlidae	*Etroplus surstensis* (Bloch, 1790)	Pearl spot	LC
16.	Perciformes	Cichlidae	*Oreochromis mossambicus* (Peters, 1852)	Mozambique tilapia	VU
17.	Perciformes	pristolepididae	*Pristolepis rubripinnis* (Britz, kumar&Baby, 2012)	Leaf fish	NE
18.	Perciformes	Lutjanidae	*Lutjanus arjentimaculatus* (Forsskal, 1775)	Mangrove Red snapper	NE
19.	Perciformes	Ambassidae.	*Ambassis gymnocephalus* (Lacepede, 1802)	Bald glassyperchlet	LC

Sl. No.	Order	Family	Scientific name	Common name	IUCN red list category *DD,LC, VU, NT, NE, CR
20.	Perciformes	Gerreidae	*Gerres filamentoses* (Cuvier, 1892)	Whipfin silver biddy	LC
21.	Perciformes	Latidae	*Lates calcarifer* (Bloch, 1790	Baramundi or Asian sea bass	NE
22.	Perciformes	Channidae	*Channa striata* (Bloch, 1793)	Banded snake head	LC
23.	Perciformes	Channidae	*Channa marulius* (F.Hamilton, 1822)	Bulls eye snake head	LC
24.	Perciformes	Gobiidae	*Glossogobius giuris* (Hamilton, 1822)	Tank goby	LC
25.	Beloniformes	Hemiramphidae	*Hyporhamphus limbatus* (Valenciennes, 1847)	Congaturi halfbeak	LC
26.	Clupeiformes	Engraulidae	*Stolephorus commersonni* (Lasepede ,1803)	Commerson's anchovy	LC
27.	Characiformes	Serrasalmidae	*Pygocentrus nattereri* (Kner,1858)	Red bellied piranha	NE
28.	Tetradontiformes	Tetradontidae	*Chelonodon patoca (Hamilton, 1822)*	Puffer fish	LC
29.	Mugiliformes	Mugilidae	*Mugil cephalus* (Linnaeus,1758)	Flathead grey mullet	LC
30.	Elopiformes	Megalopidae	*Megalops cyprinoides* (Broussonet, 1782)	Indo-Pacific tarpon	DD

*DD-data deficient LC- least concern NT- Near Threatened NE- not evaluated VU – Vulnerable CR- Critically endangered

fishes collected in the study were *Gerres filamentoses, Congresox sp. Stolephorus commersonnii, Lates calcarifer, Ambassis gymnocephalus* and *Lutjanus argentimaculatus*, Rare species documented were *Pristolepis rubripinnis* and *Chelonodon patoca*. Exotic species like *Oreochromis mossambicus, Clarias gariepinus, and Phygocentrus nattereri* were also recorded in the study.

Discussion

In the rivers of Western Ghats region biodiversity has been negatively impacted by industrial pollution, invasive species, agriculture and aquaculture practices[13]. Chalakudy river ecosystem, providing a habitat for stunning array of biodiversity and supporting the livelihood of many local people has been altered due to the construction of dams (Sholayar,Peringalkuthu) for hydroelectric projects and affected the local migration of fish species like Tor[8]. During the study period we could collect 30 species of fishes from the 3sampling sites. Here we reported the presence of some coastal and estuarine fishes which include *Gerres filamentoses*, *Congresox sp. Stolephorus commersonnii*, *Lates calcarifer* and *Ambassis gymnocephalus.* The presence of *L.argentimaculatus,G. filamentoses,S.commersonnii* have been reported in the Chalakudy river[1].However, we could collect fishes like *A. gymnocephalus*, *L.calcarifer* and *Congresox sp*. after the flood and this might have happened due to their displacement from the natural habitats as a consequence of flood. Flood impact assessment carried out by the Kerala state Biodiversity Board reported the presence of Red

bellied Pacu, Malaysian catfish, African Mushi, Sucker catfish, Giant gourami, Carps etc. in several water bodies of Kerala during flood[14]. 10% of the fishes collected were categorized as exotic species. It is reported that introduction of exotic species, intentionally or unintentionally, has resulted in the depletion of many native fish fauna in several parts of the world[15,16]. Moreover, several studies have reported the occurrence and the impact caused by exotic fish species in Kerala freshwaters, *Oreochromis mossambicus* in the Chalakudy river system[2,10,11], *Clarias gariepinus* in Vembanad Lake[17]. Predatory feeding habits and high growth rate helped *Clarias gariepinus* to establish in the reservoirs of South India[18]. According to the local fishermen these exotic species probably escaped from the local fish farms and entered the river system. Exotic fishes introduced in to the Poringalkuthu dam for enhancing fish production from the reservoir is also an important source of non-native fishes[9]. In order to conserve the biodiversity of freshwater ecosystems like Chalakudy river it is necessary to control the illegal introduction as well as culturing of exotic species by local farmers. Of the 30 species 3 species belong to the threatened categories of IUCN Red List of Threatened Species. *Hypselobarbus thomassi* is a critically endangered species endemic to the Western Ghats in Kerala and Karnataka.*Anguilla bengalensis*is a catadromous fish listed as a near threatened species. *Horabagrus brachysoma* has been assessed as a vulnerable species. Preferential cultivation of *Clarias gariepinus*in the Western Ghats region has been identified as the major reason for their declining population[15].

Conclusion

Chalakudy river is noted for the rich icthyofaunal diversity and presence of many endemic fish species. Our study revealed the current fish faunal diversity which encompasses many estuarine, exotic and threatened species. Therefore, Chalakudy river should be considered as a sensitive ecosystem and there is an urgency to conduct periodic surveys which are needed to unveil the actual biodiversity and to evaluate the impact of species invasion.

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Transmission of bacterial pathogens through Mobile phone cases–an emerging threat

P. Saravana kumari[1]* and M. Jagadeeswari[2]
[1&2]Departmentof Microbiology, Rathnavel Subramaniam College of Arts and Science, Coimbatore–641402, India.

Abstract

Recently, mobile phones have become a potent vector for the transmission of pathogens. The average person checks their mobile phone approximately once in every 12 minutes, or 80 times per day. This means our hands are on our devices nonstop, and they are likely accompanying us on nearly every errand and activity. Hands are in use constantly, touching everything from door handles to cash to sink taps. Along the way hands are capable of collecting enormous amounts of germs. So, touching the mobile devices handled by the person also has serious germ potential. Means that mobile devices act as potential source of germs that end on mobile phone while the person carries. A second reason that our phones are so dirty is the heat they produce. Warm environments are where bacteria thrive. This was ascertained in the current work by analyzing 22 mobile phone used by students community. Of the collected 22 samples, 6 samples showed overloaded number of population of organisms. The number of organisms exceeded to more than 1000 which is indicative of immediate requirement of safety measures. Few samples showed less than less than 1000 number of bacterial population, but the various types of bacteria were present. About 46 bacteria species were isolated and among them *Staphylococcus aureus* was identified as maximum (24%), followed by *Bacillus* sp., accounts for 20% followed by *E. coli* accounted for 15%, *Proteus* sp., unidentified organisms and *Staphylococcus citrus* for 9%, *Klebsiella* sp., for 6%, *Shigella* sp., for 4% and to the least level of saprophytic organism *Serratia* sp. The results indicated that mobile phone are much populated with bacterial pathogens and normal flora of the skin. Necessary measures need to be taken care to avoid such spread of pathogens and control the emergence of drug resistant organisms.

Keywords: Mobile phone, microbial load, identification of bacteria, transmission of bacteria.

Introduction

Mobile phone users in India are increasing gradually that accounts for about 550 million persons in the year 2020. Mobile phones are the hand held devices that are a major part of communication system. Apart from its function as means of communication, they have evolved due to human intelligence after the collaboration of various technologies within a single mobile device. They are used by all types of people irrespective of their social or occupational background[1]. This device keeps the population updated with the surroundings and had maintained the work pace of the cooperate world.

New applications and technologies helpful for the society and in another way also have trouble in the lives of human. They offer as a source of contamination. It is necessary to aware public regarding the prevention of infection and highlighting their duty to control infections. The vast majority of mobile phones are hand-held. Our phone remains in close contact with our body, majorly with our hands, mouth, ears and face. Apart from this, it is very common practice of carrying the phone to washroom. Also, people have habit of operating phone while consuming meals permitting the direct transfer of microorganisms from the mobiles to our gut[2]. Mobile phones are absolutely essential devices but also majorly operated in the areas of excessive microbial load.

Firm actions must be incorporated in our daily activities like proper washing of hands before and after meals, cleaning of phones with disinfectant. There is no such known practice to clean the mobile phones that result in its contamination by the microbes. Most microorganisms face a constant battle for resources and vast numbers of microbes are present in all environments close contact with human body[3]. We can view the dust by our naked

* *Corresponding author, E-mail: sarankumaribs@gmail.com*

eyes that get deposited on the mobiles surface but unable to see the microbes that would be flourishing on phone. Because of the achievements and benefits of the mobile phone, it is easy to overlook its hazard to health; this is against the background that many users may have no regard for personal hygiene, and the number of people who may use the same phone. This constant handling of the phone by different users exposes it to an array of microorganisms, and makes it a good carrier for microbes, especially those associated with the skin resulting in the spread of different microorganisms from user to user. Our research has shown that the mobile phone could be a health hazard with tens of thousands of microbes living on each square inch of the phone.[4]

These days, mobile phones used daily everywhere by households, workers, healthcare staffs, etc. as communication medium. No strict rules have been followed to avoid mobile phones in ICU where contamination risk is higher[5]. Mobile phones being in touch with our body and different objects are creating frequent health issues. Hand comes in contact with our phones very frequently therefore serving as an infectious source. The current research work investigate on bacterial transmission through mobile phone and identify the microbes regularly associated with mobile phones.

Materials and methods

Collection of mobile phones and swabs

Before taking a swab, both hands of laboratory technicians were cleaned using an alcohol-based hand sanitizer, and powder-free disposable gloves were worn per sample throughout the work to prevent cross-contamination. The samples of mobile phones, 22 devices were collected from the college students age ranging between 16 and 23 during three-week period of January 2020, in Coimbatore, India. Sterilized cotton swab moisten by sterile normal saline was rotated to swipe from overall (screen, keypad, sides, and back) area of the mobile phone. In case of mobile phones with covers, the swab taken from the outer surfaces of the cover, besides the screen.

Culture of bacteria and microbial count

Each collected swab placed immediately into sterile normal nutrient broth, transported to the Microbiology Laboratory and incubated at 37°C for an hour. The suspension was plated by spread plate technique on nutrient agar, Mac Conkey and Mannitol salt agar plates. The plates were incubated at 37°C for 48 hours and observed for growth and colonial description of the isolates. After incubation, each culture plate analysed for the number of colonies on the plate and different types

Table 1. Total count and number of organisms present on the mobile phone Sample

No	Total no. of organisms/ swab	Types of organisms	Sample identity
1	462	4	B-1,B-2, B-3, B-4
2	10064	1	B-5
3	347	4	B-6, B-7, B-8, B-9
4	5427	3	B-10, B-11, B-12
5	436	2	B-13, B-14
6	459	1	B - 15
7	764	4	B-16, B-17, B-18, B-19
8	75	2	B-20, B-21
9	548	1	B-22
10	4689	3	B-23, B-24, B-25
11	3906	2	B-26, B-27
12	278	1	B-28
13	659	2	B-29, B-30
14	678	1	B-31
15	3588	1	B-32
16	55	2	B-33, B-34
17	68	1	B-35
18	864	2	B-36, B-37
19	446	3	B-38, B-39, B - 40
20	754	2	B-41, B-42
21	642	1	B-43
22	3740	3	B-44, B - 45, B - 46

of microbial population using a colony counter. Each type of isolate subcultued and analysed by the standard microscopic and biochemical test for identification of common Gram negative and Gram positive bacterial pathogens[11].

Results and discussion

Total bacterial count

The samples of mobile phones used in the study were in regular use by the person and were not cleaned for past one months. Collected swab inoculated in nutrient broth immediately turned yellow to brown colour and also turned little turbid after one hour of incubation. Microbiological standards in hygiene are necessary for a healthy life. It is not uncommon, however to observe practices that deviate from normal standards of hygiene in both the developing and the developed world. This investigation confirms deviation, as variety of microbes were found on mobile phones.

Nutrient agar plates seeded with samples 2, 4, 10, 11, 15, and 22 showed overloaded number of population of organisms. Total number of bacterial exceeded more than 1000 which is indicative of immediate requirement of safety measures. Though the sample number 4 and 7 were having less than 1000 number of bacterial population, 4 different types of pathogens were recognized. The following table – 1 indicates the number and types of organisms isolated from the samples. Of the remaining 14 samples, three samples 8, 16 and 17 showed insignificant level of microbial population.

Biochemical test results

The isolated bacteria from the mobile devices subjected to standard biochemical tests[7] and identified list tabulated (Table 2). Table 2 showed that all isolates of *Klebsiella* sp., *Staphylococcus* sp., *Streptococcus* sp., and *Escherichia coli* were negative result for indole while positive by *E. coli*. Utilization of citrate is one of several important physiological test used to diagnose members of all Enterobacteriaceae except *E.coli* which is negative for citrate, while *P. aeruginosa* is positive for citrate. *Klebsiella* sp., produce CO_2, turns the pH indicator (bromthymol blue) from green to blue, reflecting it as positive for citrate test. Whereas in urease test, positive for *Klebsiella* sp., *Staphylococcus aureus* and negative for *E. coli*[8]. *Klebsiella* sp., can produce urease enzyme and gives urease positive test[6].

In motility test, *Klebsiella* sp., were non-motile. But the linear growth in SIM agar indicated negative result for *Klebsiella* sp.,[9] and positive for *E.coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp, and *Proteus* spp isolates[10, 11, 12]. Another way to confirm bacterial genus by catalase test, catalase positive and oxidase negative for *Klebsiella* sp., *E. coli*[13], whereas *Pseudomonas aeruoginosa* oxidase and catalase positive, while *Staphylococcus aureus* isolates were oxidase negative and catalase positive[14]. The coagulase test is specific to differentiate *Staphylococcus aureus* from other species[15].

Identification of bacterial isolates

Based on morphological, cultural, microscopic, physiological and biochemical tests list of bacteria identified in agreement with previous studies given in the table 3.

Among the 46 isolates, *Staph. aureus* was identified as maximum of 24%, followed by *Bacillus* sp., accounts for 20% followed by *E. coli* counted for 15%, *Proteus* sp., unidentified organisms and *Staphylococcus citrus* for 9% each, *Klebsiella* sp., for 6%, *Shigella* sp., for 4% and to the least level of saprophytic organism *Serratia* sp. The results indicated that mobile phone are much populated with normal flora of the skin such as *Staph. aureus, Staph. citrus* and *E. coli* (Fig. 1).

The heat generated by the phones creates a prime breeding ground for many microorganisms that are normally found on the skin. Staphylococci, particularly S. epidermidis are members of the normal flora of the human skin, respiratory and gastrointestinal tracts. Nasal carriage of S. aureus occurs in %50-20 of human beings. The hand serves as a major vehicle of transmission of various microbes including the enteric species.

Table 2. Biochemical test results of isolated organisms

	Identification	Staphylococcus aureus	Bacillus sp.	E. coli	Proteus sp.	Shigella sp.	Unidentified	Serratia sp.	Staph. citrus	Klebsiella sp.	Pseudomonas sp.
Biochemical test results	Starch hydrolysis	–	+	–	–	–	–	–	–	–	–
	Nitrate Reduction	+	+	–	+	+	+	+	+	+	+
	Urease test	+	–	–	–	–	+	–	+	–	–
	IMViC	_+ ++	_ _ ++	++ _ _	_ + _ +	_ + _ _	_ + _ _	_ _ + _	_ + _ +	_ _ + +	_ _ _+
	Catalase test	+	+	+	+	+	+	+	+	+	+
Microscopic tests	Spore staining	–	+	–	–	–	–	–	–	–	–
	Capsule staining	–	–	–	–	–	–	–	+	+	–
	Motility	–	–	+	+	–	–	+	–	+	+
	Gram staining	+	+	–	–	–	–	–	+	–	–
Colony morphology	Mannitol salt agar	Yellow colonies	No growth	No growth	No growth	No growth	No growth	No growth	Yellow colonies	No growth	No growth
	Mac Conkey agar	No growth	No growth	Pink colour colonies	Yellow colonies	Yellow colonies	No growth	Dark red colonies	No growth	Pink colonies	Yellow to green colonies
	Nutrient agar	Large, entire colonie	Large, irregular colony	Large, dome shaped colony	Wavery growth	Small, entire	Small, pinhead colony	Large, orange colony	Small, lemon	Large, mucoid	Green colony
Isolate No		B-1,5, 9, 10,11,23 26, 31, 35,37,42	B-2, 6, 27, 28, 29, 39, 41,43,45	B-3, 8, 16, 21, 33, 36, 44,	B-4, 12, 19, 24,	B-7, 18,	B-13,17, 30, 34	B-14	B – 15, 25,38, 46	B – 20, 22, 32,	B – 40,

Table 3. List of organisms identified from the isolated organisms

Sample No.	Types of organisms	Genus name of the isolate
1	4	B1- Staphylococcus aureus B-2 - Bacillus sp. B-3 – Escherichia coli B-4 – Proteus sp.
2	1	B-5- Staph. aureus
3	4	B-6 - Bacillus sp. B-7 – Shigella sp. B-8 – E. coli B-9 - Staph. aureus
4	3	B-10 - Staph.aureus B-11– E. coli, B-12 - Proteus sp.
5	2	B-13 - Unidentified, B-14 – Serratia sp.
6	1	B - 15 – Staph. citrus
7	4	B-16 – E. coli B-17 – Unidentified B-18 – Shigella sp., B-19 – Proteus sp.,
8	2	B-20 – Klebsiella sp., B-21 - E. coli
9	1	B-22 - Klebsiella sp.,
10	3	B-23 –Staph. aureus B-24 - Proteus sp. B-25 - Staph.citrus
11	2	B-26 – Staph.aureus B-27 – Bacillus sp.
12	1	B-28 – Bacillus sp.
13	2	B-29 – Bacillus sp. B-30 – Unidentified organism
14	1	B-31 - Staph.aureus
15	1	B-32 – Klebsiella sp.
16	2	B-33 – E. coli B-34 – unidentified
17	1	B-35- Staph. aureus
18	2	B-36 - E. coli B-37- Staph. aureus
19	3	B-38 –Staph. citrus B-39 – Bacillus sp. B-40 – Pseudomonas sp.
20	2	B-41 – Bacillus sp. B-42 – Staph.aureus
21	1	B-43 - Bacillus sp.
22	3	B-44 - E. coli B-45- Bacillus sp. B-46 - Staph. citrus

Proteus mirabilis is one of the most common Gram-negative pathogens encountered in clinical specimens. It can cause a variety of community or hospital-acquired infections, including those of the urinary tract, respiratory tract, wounds and burns, bacteraemia, neonatal meningoencephalitis, empyema and osteomyelitis[16]. Next to *Staph. aureus*, E.coli and P. mirabilis belonging to Enterobacteriaceae most often isolated in European clinical microbiology laboratories[17], accounting for ~3% of nosocomial infections in the United States[18]. *Pseudomonas aeruginosa* is a metabolically versatile γ-Proteobacterium, which inhabits terrestrial, aquatic, animal, human, and plant -host-associated environments[19].

Previous similar study also indicated that *Staph. aureus, Staph. epidermidis, P. aeruginosa, Neisseria sicca, Micrococcus luteus, Proteus mirabilis, Bacillus subtilis* and *Enterobacter aerogenes* are the major bacterial isolates frequently associated with mobile phones. These organisms may probably have found their way into the phone through the skin and from hand to hand. This is because the isolated bacteria are a subset of the normal microbiota of the skin as advanced by earlier researchers[20]. Frequent handling by many users with different hygiene profiles producing regular skin contact with the phones may have resulted in the frequency and the degree of population of the isolates. This has many health implications.

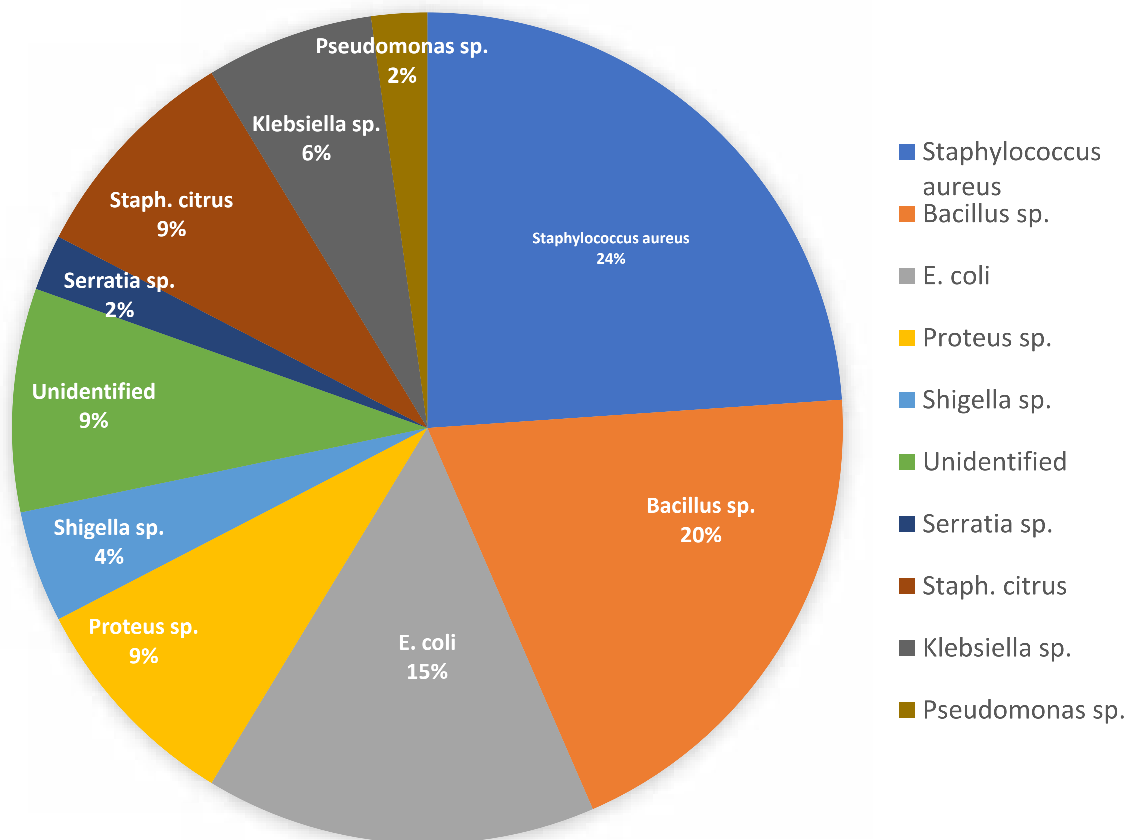

Fig. 1. Chart showing distribution of different bacteria on mobile phone

Staphylococcus aureus is known to cause illnesses ranging from pimples and boils to pneumonia and meningitis, a scenario supported by the high population of colony isolates.

The overall implication of these results is that mobile phones which make communication easy and accessible also form good carriers of pathogenic agents of disease transmission. If care is not taken, they could be vehicles for the transmission of biological weapons. Karabay *et al.*, in 2007 reported that mobile phones may get contaminated with such bacteria as *E. coli, P. aeruginosa* and *K. pneumoniae*, which cause hospital borne infections, and may serve as a vehicle for the spread of nosocomial pathogens[21]. Users of mobile phones are found everywhere: in the market, the home, hospitals, and schools. They could therefore, be the cause of the spread of the infection in the community.

Today, mobile phones are important equipment for physicians and other health workers. Since restrictions on the use of mobile phones is not a practical solution, many researchers suggest that adherence to such infection control precautions as hand hygiene should be strict. In addition, people should be informed that these devices may be a source for transmission of hospital-acquired infections to and from the community. Further studies for the possible means of decontamination of mobile phones, such as the use of alcohol and/or disinfection tissues, should be found and employed in hospitals that have large bed capacities and Intensive Care Units.

Karabay *et al.*, in 2007 found that most of the organisms isolated were skin flora. However, %16.7 of the samples were positive for pathogens known to be associated with nosocomial transmission[21], such as *Enterococci* spp, *S. aureus* and *K. pneumonia*. Vancomycin-Resistant Enterococci (VRE) and Methicillin-Resistant *S. aureus* (MRSA) were not isolated. Other investigators reported that telephones, intercoms, dictaphones and bedpan

flusher handles may be contaminated with potentially pathogenic bacteria[22, 23, 24, 25]. Jeske *et al.*, in 2007 also reported that bacterial contamination of anesthetists hands by personal mobile phones occurred, (38/40 physicians, 4/40 with human pathogen bacteria) in the operating theatre[26]. The use of mobile phones in the Intensive Care Unit, burn wards and operative rooms may have more serious hygiene consequences, because unlike fixed phones, mobile phones are often used close to patients. Intensive Care Unit patients and burn patients are very vulnerable to infectious diseases, so the risk of transmission of organisms associated with nosocomial infections is increased[27].

Since the restriction of the use of mobile phones by health care workers is not effective for the prevention of the spread of nosocomial infections, it is also necessary to develop effective preventive strategies that will include environmental decontamination, hand hygiene, surveillance, and contact isolation for the prevention of these nosocomial infections[28]. Simple cleaning of computers and telephones with 70% isopropyl alcohol may decrease the bacterial load[29].

Control measures are quite simple and can include engineering modifications, such as the use of hands-free mobile phones, surfaces that are easy to clean and disinfect, hand washing, and the wearing of gloves by the appropriate personnel. In general, resident infection control staff of the medical facility can advise on the routine control practices for medical devices. Observance of these simple control procedures can decrease morbidity and mortality and thereby reduce medical care costs for hospitals and other care providers.

Conclusion

The mobile cases were analysed for microbial population, among the 46 isolates, *Staph. aureus* was identified as maximum of 24%, followed by *Bacillus* sp., accounts for 20% followed by *E. coli* counted for 15%, *Proteus* sp., unidentified organisms and *Staphylococcus citrus* for 9%, *Klebsiella* sp., for 6%, *Shigella* sp., for 4% and to the least level of saprophytic organism *Serratia* sp. The results indicated that mobile phone are much populated with normal flora of the skin such as *Staph. aureus*, *Staph. citrus* and *E. coli.* The overall implication of these results is that mobile phones which make communication easy and accessible also form good carriers of pathogenic agents of disease transmission. If care is not taken, they could be vehicles for the transmission of biological weapons.

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Bismuth triiodide: Ab-initio simulations to spray cast thin films for optoelectronic applications

Sebin Devasia[1], Anjali A. Ramachandran[1], S. Shaji[1,2], D. A. Avellaneda[1], J.A. Aguilar Martinez[1,3], Bindu Krishnan[1,2,*]

[1]Facultad de Ingeniería Mecánica y Eléctrica, Universidad Autónoma de Nuevo León. San Nicolás de los Garza, Nuevo León, México, 66455.

[2]Centro de Innovación, Investigación y Desarrollo en Ingeniería y Tecnología (CIIDIT)- Universidad Autónoma de Nuevo León, Parque de Investigación e Innovación Tecnológica (PIIT), Apodaca, Nuevo León, 66600, México.

[3]Centro de Investigación e Innovación en Ingeniería Aeronáutica (CIIIA); Facultad de Ingeniería Mecánica y Eléctrica, Carretera a Salinas Victoria, Apodaca, Nuevo León, 66600, México.

Abstract

In this report, we discuss the computational as well as experimental research done on the metal halide perovskite bismuth iodide.We employed DFT calculations to explain the spin-orbit coupling effects in BiI_3 in lowering the CBM and thus, obtained an optical band gap of 1.44 eV having indirect nature. Further, we synthesized BiI_3 powder from bismuth nitrate and potassium iodide. Then, we fabricated BiI_3 thin films via a scalable ultrasonic spray deposition method using the powder as precursor. Further, we studied the structure, morphology, composition, absorption and photoresponse of the thin film. The film possessed dendritic-like morphology with an indirect bandgap of 1.42 eV. Moreover, it displays a very good response to the illumination from white light and LEDs with a sensitivity of 4890 %.

Keywords: Bismuth iodide, DFT, Thin films, Ultrasonic spray coating, photoresponse.

Introduction

The layered bismuth tri-iodide (BiI_3) with its intermediate bandgap from strong spin-orbit coupling is an interesting semiconductor for structural and electronic exploration[1–8]. Their potential semiconducting character is complemented by a rich structural diversity in the inorganic framework arising from the different degrees of distortion of bismuth halide octahedra (BiX_6). Such bismuth halide octahedra can connect by corner-, edge-, face- sharing to form distinct networks of bismuth halogen anions like BiX_4^-, BiX_5^{2-}, BiX_6^{3-}, $Bi_2X_9^{3-}$, $Bi_2X_{11}^{5-}$, $Bi_{13}X_{12}^{3-}$, $Bi_4X_{18}^{6-}$, $Bi_5X_{18}^{3-}$, $Bi_6X_{22}^{4-}$ and $Bi_8X_{30}^{6-1}$. Taking advantage of the intermediate bandgap, high density and high effective atomic number, it has been used for room temperature gamma-ray detection[2], scintillation[3] and x-ray digital imaging[4]. In addition, the material has also found potential applications in photodetectors, LEDs and photovoltaics[5], owing to the recent interests to replace toxic Pb in the hybrid lead halide perovskites.

At room temperature, BiI_3 has a rhombohedral structure which belongs to the trigonal crystal system with six formula units per unit cell. Within each unit cell, BiI_3 adopts a layered structure with highly ionic Bi-I bonds in the layer and weak van der Waals bonding between the layers[8]. As a matter of fact, the non-toxic and stable bismuth (Bi^{3+}) cation has an electronic configuration of $[Xe]4f^{14}5d^{10}6s^2 6p^0$ which is isoelectronic to Pb^{2+}[6]. In BiI_3, the vacant Bi 6p orbitals account for the conduction band minimum (CBM) and the occupied Bi 6s and I 5p orbitals for the valence band maximum (VBM). The strong antibonding character of Bi s and I p in the VBM of the material can lead to shallow defect states and longer carrier lifetimes. Besides, strong absorption can be expected

* *Corresponding author, E-mail: k_binduk@yahoo.com*

owing to the high density of states of p-orbitals in the conduction band minimum (CBM)[7,9].

Noticeably, the band gap values determined from experimental and computational studies of BiI_3 spread across a wide range of values from 1.43 eV to 2.2 eV. Schluter et al. used the empirical pseudopotential method to calculate the electronic structure of BiI_3 and obtained a direct bandgap of 2.2 eV[10]. However, Yorikawa and Muramatsu observed a much lower bandgap of 1.6652 eV but indirect in nature[11]. The first-principles of linearized augmented plane-wave (LAPW) calculations using three different potentials yielded band gaps of 1.43, 1.67 and 1.82 eV[3]. The band gaps determined by Podreza et al. from the optical and computational methods were indirect with a value of 1.55 and 1.67+/- 0.09 eV, respectively, whereas the spectroscopic ellipsometry results showed a direct transition of 1.94-1.96 eV[8]. Recently, the studies conducted by Ma et al. suggests strong spin-orbit coupling (SOC) in the BiI_3 monolayer leading to an indirect bandgap of 1.57 eV[12].

Predominantly, the earlier studies were focused on the single crystals of BiI_3 synthesized by vapor transport[1314], Bridgman method[8] and single diffusion techniques [15]. Meanwhile, thin films of BiI_3 have been realized through methods like hot wall technique [16–18], chemical methods [19], thermal evaporation [20,21], physical vapor transport [5,225] and mostly by spin coating [20,23,24]. However, such deposition methods cannot promise low-cost devices due to the constraints faced in large scale production. A truly scalable ultrasonic spray coating is indeed a versatile technique that can offer large area deposition at a fast rate and low cost. Ultrasonic spray coater uses a piezoelectric transducer to break the precursor solution into a fine mist of uniform micron sized droplets in contrast to the aperture based conventional spray coaters that utilize compressed air. Consequently, thin films are deposited with better uniformity[25–27]. Lately, the organic lead halide perovskites have been deposited by the method and effectively integrated into photovoltaic structures[28–35] to attain PCE as high as 19.4%[25].

The experimental and computational studies conducted over the time reveal that there is a strong disagreement in the values and nature of the bandgap for the BiI_3. Here, we follow a synergetic approach towards the computational and experimental study of BiI_3 which yielded results that are quite in agreement with each other. We used VASP codes for computation and ultrasonic spray deposition (USD) for fabricating pure BiI_3 thin films. The solution-based synthesis route of thin films relies on nontoxic ethanol solvent different from the widely used dimethylformamide (DMF). Unlike BiI_3 thin films deposited by other methods, the stability and purity of our BiI_3 thin films in ambient conditions as well as their high absorption coefficients are noteworthy.

Materials and Methods

Computational Methods

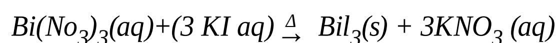
Initially, a generalized gradient approximation (GGA) to the exchange-correlation energy is used with the Perdew-Burke-Ernzerhof (PBE)[36] functional for geometry optimizations. We used the most reliable VASP electronic structure method[37,38] in the platform of MedeA software[39]. Here, we allowed the relaxation of atomic positions, cell shape and volume to create a stress-free unit cell of BiI_3. The structure was optimized using a planewave cutoff energy of 500 eV to relax all the structural parameters until the force and energy were converged to 0.02 eV/Å and 1×10^{-5} eV, respectively. The blocked Davidson algorithm was used for the convergence of electronic iterations. The k-points were sampled at 3×3×3 mesh both for SCF and non-local exchange with the Monkhorst-Pack method. The linear tetrahedron method with Blöchl corrections was used for Brillouin zone integration and the smearing width was 0.2.

Subsequently, we used the minimized structure for the calculation of electronic band structure and density of states (DOS) to determine the optical band gap of BiI_3. The generalized gradient approximation (GGA) in the Perdew-Burke-Ernzerhof (PBEsol) form within the projector augmented wave method[40] was employed as

exchange-correlation functional in the DFT calculation. Further, we included spin-orbit coupling to analyze the effects of heavy Bi and I atoms on the observed optoelectronic properties.

Synthesis of bismuth iodide powder

The BiI_3 powder was prepared by mixing 1M bismuth nitrate ($Bi(NO_3)_3$, 394.99 g/mol) (24.25 g) and 3M potassium iodide (KI, 166.0028 g/mol) (24.9 g) in 50 ml water for the following reaction,



$$Bi(No_3)_3(aq)+(3\ KI\ aq) \xrightarrow{\Delta} BiI_3(s) + 3KNO_3\ (aq)$$

Fig. 1. Bismuth iodide powder obtained from the reaction of $Bi\ (NO_3)_3$ with KI.

The BiI_3 precipitate was filtered and washed with distilled water and then dried in a hot air oven at 120°C for 1 h. The brownish-black BiI_3 powder (fig. 1) was carefully grinded and stored.

Deposition of bismuth iodide film by ultrasonic spray method

A 0.01 M precursor solution was prepared by dissolving 0.295 g of BiI_3 powder in 50 ml ethanol after stirring well for 2 h. The deposition was done using the ultrasonic spray pyrolysis coater (CY-USP130-A, Zhengzhou CY Scientific Instrument Co., Ltd.) which employs a 40 kHz, 130 W ultrasonic nozzle for atomization[41]. The cleaned glass substrates were pre-heated to 150°C. The spray rate, air pressure and the current supply for ultrasonic nozzle were set to 6 ml/min., 0.1 MPa and 0.05 A, respectively. The solution was sprayed onto the glass substrates for 30 mins during which the speed of the spray head was 50 mm/s in the x-axis and 20 mm/s in the y-axis. Immediately after deposition, the glass substrates coated with films were transferred from the heater and stored (Fig. 2).

Fig. 2. The as-deposited BiI_3 thin film on glass substrates by ultrasonic spray deposition.

Characterization

The X-ray diffraction study was done with the PANalytical EMPYREAN diffractometer employing Cu Kα radiation (λ=1.5406 Å). The Thermo Scientific K-alpha equipment (Al K_α =) was used for X-ray photoelectron spectroscopy. For the XPS data, a charge correction to the binding energy was done referencing to the C-C/C-H peak of adventitious carbon at 284.6 eV in addition to the charge compensation by a flood gun. Further, the peak modelling was done using the Shirly-type background and the Gaussian-Lorentzian sum function. The film morphology was examined from the scanning electron micrographs obtained using SEM-Hitachi SU8020 equipment. A Jasco V770 spectrophotometer was used for absorption measurements. Photoresponse of the thin film was measured by an arrangement involving a Keithley 6487 Picoammeter, contact electrodes and 50 W tungsten halogen lamp or LEDs.

Results and Discussion

Computational results

The geometry optimized structure of BiI_3 unit cell containing 6 formula units is shown in fig. 3**.** The bulk BiI_3 structure consists of layers of highly ionic Bi-I bonds stacked in ABC order with weak van der Waals interaction between the layers. The BiI_3 has an R-3H symmetry with 6 Bi atoms at the 6c Wyckoff positions and 18 I atoms occupy the 18f Wyckoff positions.

The structure optimizations relaxed an energy of -0.019026 eV for $(BiI_3)_2$. The final energy and

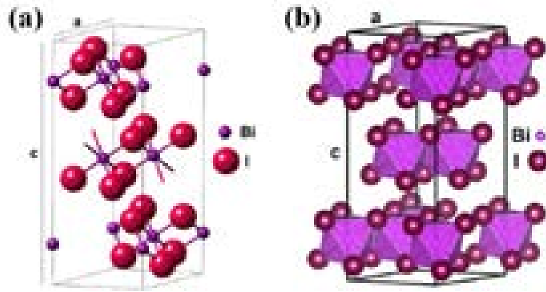


Fig. 3. (a) The R-centered hexagonal unit cell of BiI_3 after structural optimization. (b) hexagonal unit cell displaying the high symmetry (BiI_6) octahedra

the density of the unit cell $(BiI_3)_6$ are -68.011136 eV and 5.819 gcm^{-3}, respectively. Further, more parameters related to the optimized cell can be referenced in Table 1.

Table 1. The unit cell parameters obtained from the structure optimization of the BiI_3 unit cell.

Parameter	Original (Å)	Final (Å)	Change (%)
a	7.516	7.523913	0.1
b	7.516	7.523913	0.1
c	20.718	20.595052	-0.6
α	90	90	0
β	90	90	0
γ	120	120	0
Volume	1013.565929	1009.673714	-0.4

Figure 4(i) & (ii) illustrates the electronic band structure for high symmetry directions in the Brillouin zone for the BiI_3 exhibiting the spin-orbit coupling (SOC) effects. It is observed from the calculations that the spin-orbit coupling significantly lowers the conduction band minimum to lower energy, a characteristic of heavy atoms like Bi and I. The calculations without SOC have resulted in a large indirect bandgap of 2.387 eV with valence band maximum near (0.20, 0.20, 0.20) at -0.053 eV and conduction band minimum near (0.40, 0.40, 0.40) at 2.334 eV with respect to the Fermi level. On the other hand, the inclusion of SOC for optical band gap calculation revealed a shorter indirect transition of 1.439 eV between the VBM (0.33,0.33,0.33) and CBM (0,0,0) near the Γ point, in the ZΓ direction.

In addition to the observed indirect transition, low band dispersion in VB suggests the possibility of direct transitions of marginally higher energy. The pronounced band dispersion in CB and the flat VBM indicates a low effective mass of electrons with respect to the holes in BiI_3[42–44]. The calculations suggest that the electrons could be contributing more to the transport properties of the material[45].

Correspondingly, the partial and total density of states from the single point calculations are displayed in fig. 4(a), (b) and (c). A careful analysis

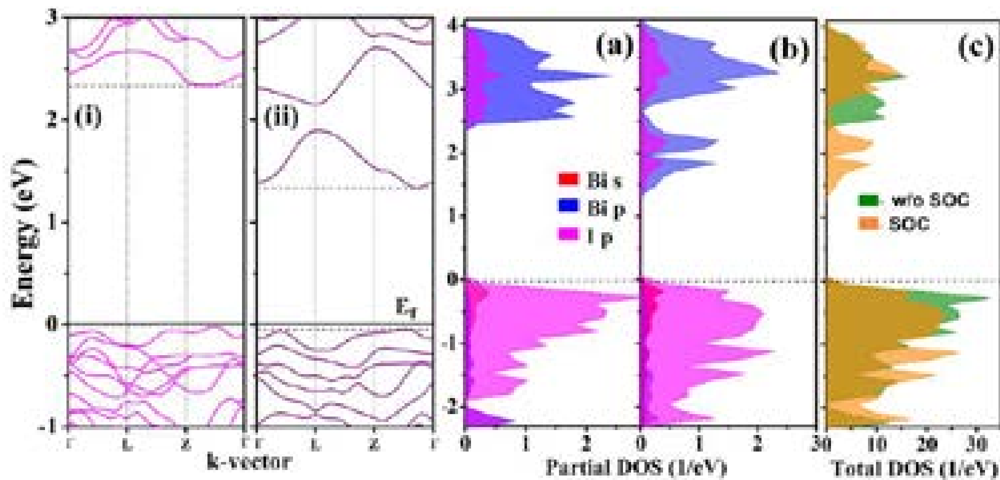


Fig. 4. The band structure for high symmetry directions in the Brillouin zone of BiI_3 (i) without spin-orbit coupling and (ii) with spin-orbit coupling. Corresponding partial density of states for calculations (a) without SOC and (b) with SOC as well as their (c) total density of states.

indicates that the valence band maximum (VBM) is mainly composed of I p states (hybridized with Bi s state) and conduction band minimum (CBM) is composed of Bi p states (hybridized with I p states). Therefore, light-induced excitation from the valence band to the conduction band occurs mainly from the occupied I p with a small Bi s contribution into the empty Bi p +I p states[46]. The high density of states of p orbitals in the conduction band minimum can contribute to strong absorption[7]. Significantly, two additional broad peaks have been emerged in the CB due to the spin-orbit splitting, first one at ~2.18 eV above E_F with a width of ~0.70 eV and the latter at ~1.83 eV with ~0.60 eV width, predominantly composed of Bi p states in hybridization with I p. It is evident from our DFT calculations that the low bandgap observed in BiI_3 is a consequence of strong spin-orbit coupling from the heavy elements, Bi and I.

Bismuth iodide powder

Structural analysis (XRD)

The XRD pattern of the laboratory prepared BiI_3 powder is shown in fig. 5. While most of the diffraction peaks are well in match with the JCPDS file no: 48-1795 of BiI_3, two low intense peaks at 29.64° and 31.64° corresponds to the (102) and (110) planes of tetragonal BiOI phase

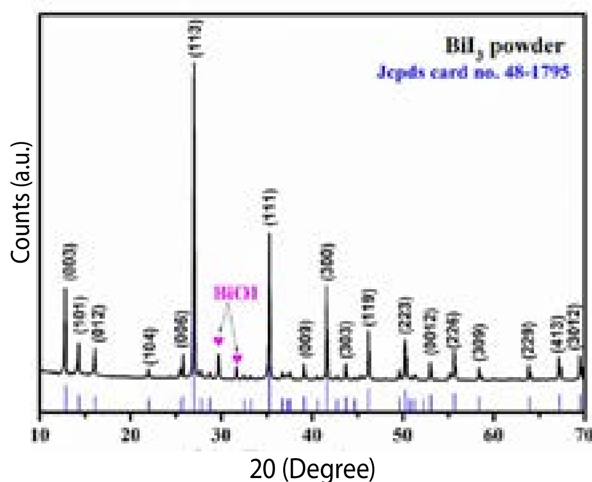


Fig. 5. The XRD pattern of laboratory synthesized BiI_3 powder. The heart shape denotes the peaks from the BiOI phase present in the powder.

(10-0445). Here, the polycrystalline BiI_3 has the rhombohedral structure with the space group R-3, which is usually represented by an R-centered hexagonal unit cell (Fig. 3).The most intense peak was observed at 26.97° corresponding to the (113) plane signifying the preferential orientation of crystallite growth in synthesized powder. Other major peaks observed are from (003), (111) and (300) planes at 12.79°, 35.25° and 41.57°, respectively.

Chemical state analysis (XPS)

The powder samples were dissolved in acetone and then drop-cast on a glass slide for X-ray photoelectron spectroscopy (XPS) measurements. The surface survey spectrum in fig. 6 identifies the presence of Bi, I, O and C and confirms the absence of K, nitrates or any other elemental impurities from the reactants.

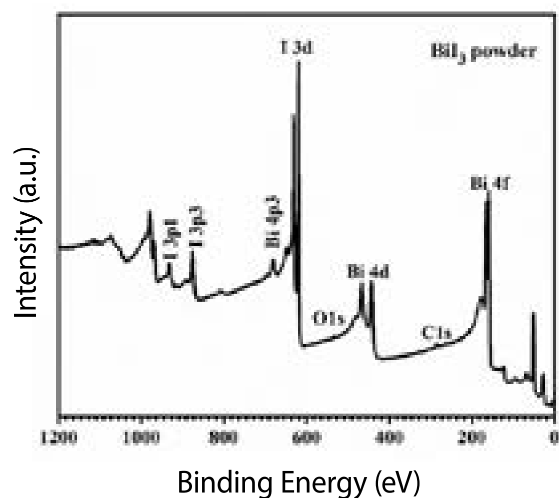


Fig. 6. Survey spectrum collected from the surface of BiI_3 powder drop-cast on a glass substrate.

The high-resolution spectra of Bi and I are illustrated in fig. 7. Bismuth has two peaks at 159.06 and 164.37 eV corresponding to $4f_{7/2}$ and $4f_{5/2}$ levels with a spin-orbit splitting of 5.31 eV which are in agreement with the doublets for Bi^{3+} state in BiI_3[47]. The I $3d_{5/2}$ and I $3d_{3/2}$ are located at 619.36 eV and 630.85 eV with energy splitting of 11.49 eV due to spin-orbit interactions[47]. The 1s state of oxygen has peaks located at 530.28, 531.86 and 533.03 eV. The peak at 533.03 eV

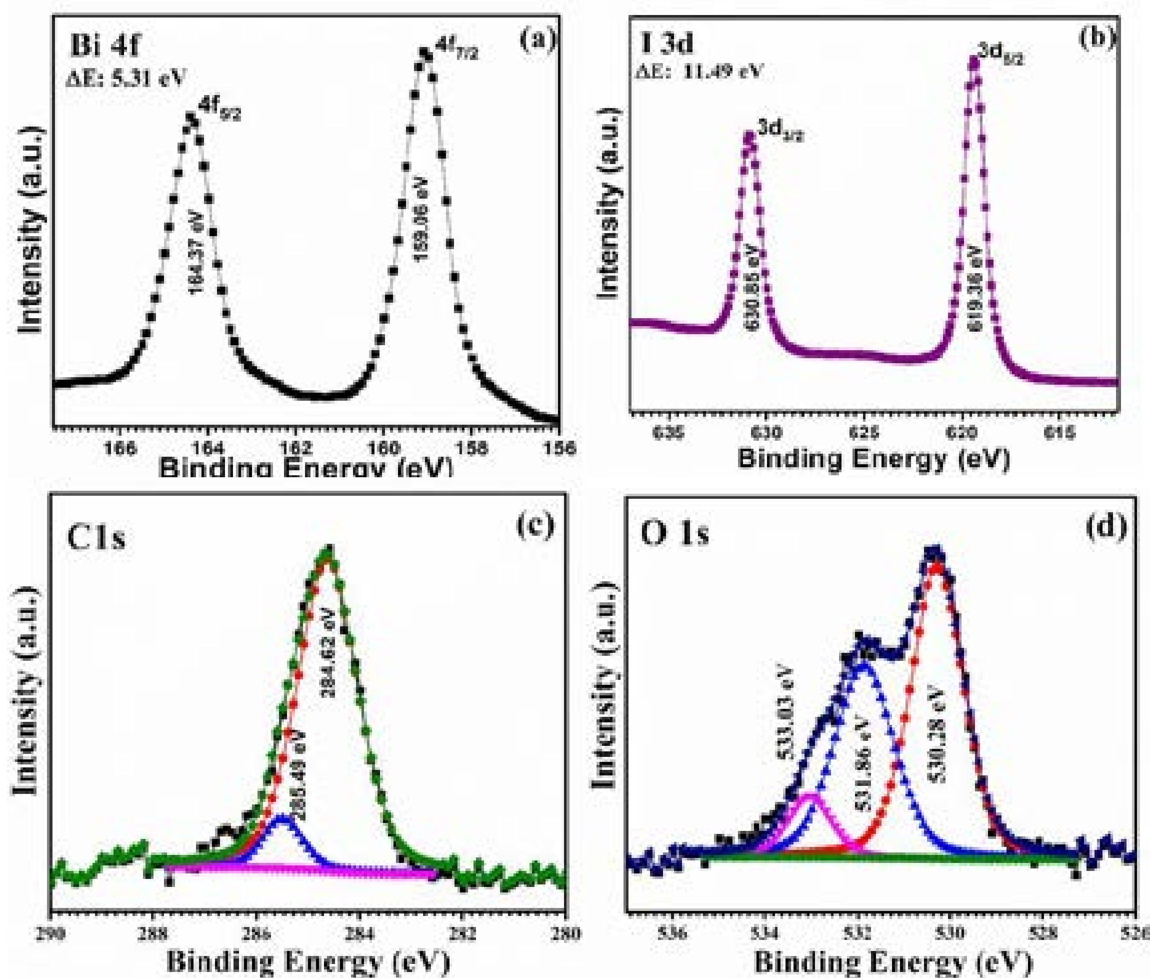


Fig. 7. The high-resolution spectra of (a) Bi 4f., (b) I 3d(c) C 1s and (d) O 1s from synthesized BiI_3 powder.

could be from glass substrate whereas the peak at 531.86 eV could be due to the C-O, C-O-O or O-H on the surface. The peak at 530.28 eV could be from the Bi-O in bismuth oxyiodide[47,48]. Thus, the XRD and XPS results together conclude to BiI_3 phase in the laboratory synthesized powder with a very little oxide impurities.

Ultrasonic spray deposited BiI_3 thin film

Structure (XRD)

X-ray diffraction of the thin film is presented in fig. 8 with appropriate indexing. The reflections in the pattern are well in match with the JCPDS card 48-1795 indicating R-3 rhombohedral BiI_3 in the trigonal crystal system. Unlike the bulk BiI_3, preferential orientation is in the (003) direction for the polycrystalline thin film. The other major intense peaks observed correspond to (006), (113),

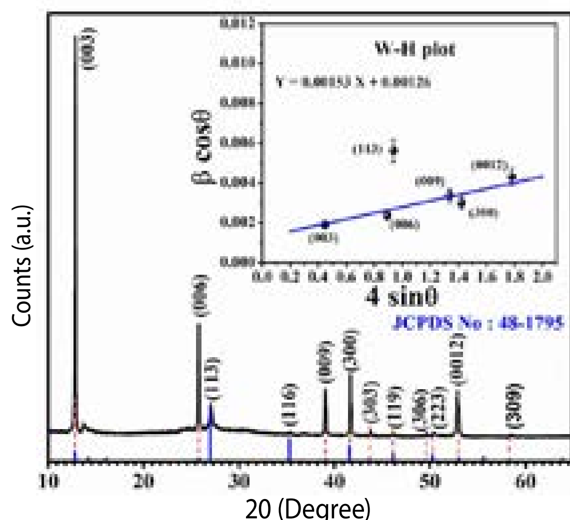


Fig. 8. XRD pattern of the spray-cast BiI_3 thin film compared with the JCPDS file of rhombohedral structure. (inset) Linear plot for Williamson-Hall equation.

Table 2. The structural properties derived from the X-ray diffraction analysis.

Diffracted planes	2θ (°)	FWHM (°)	Texture coefficient (TC_{hkl})	Crystallite size (nm)	Average crystallite size (nm)	Crystallite size (W-H plot) (nm)	Lattice strain
(003)	12.87	0.1094	0.34	73.06	46	110	1.53×10^{-3}
(006)	25.78	0.1393	0.33	58.51			
(113)	27.07	0.3315	0.01	24.64			
(009)	39.05	0.2037	0.18	41.38			
(300)	41.70	0.1831	0.03	46.42			
(0012)	52.88	0.2761	0.12	32.14			

(009), (300) and (0012) planes [19,21] along with mild peaks from (116), (303), (119), (306), (223) and (309) planes. Although we have observed BiOI impurity peaks in the as-synthesized BiI_3 precursor powder (Fig. 5), the thin film XRD reveals phase-pure BiI_3. The deposition at relatively high temperature (150°C) and the rapid nucleation followed by recrystallization of BiI_3 has effectively suppressed the BiOI phase present in the BiI_3 precursor powder.

The average crystallite size (D) was calculated using the Scherrer equation (1)[49],

$$D = \frac{K\lambda}{\beta Cos\theta} \quad (1)$$

Where K is the crystallite-shape factor (~0.9 for spherical), λ is the wavelength of Cu K_α radiation (1.5406 Å), β is the full width at half maximum of diffraction peaks in radians and θ is the Bragg angle. Further, the Williamson-Hall (W-H) plot following the equation (2) was used for accurate crystallite size and stress calculation[50],

$$\beta Cos\theta = 4\epsilon \sin\theta + \frac{K\lambda}{L} \quad (2)$$

Here, the broad (113) peak leads to the small R-square (0.522) of the linear fit in the W-H plot (Fig. 8 inset) from which the crystallite size was calculated to be 110 nm as compared to the 46 nm obtained from Scherrer equation. This appreciable variation in average crystallite size values suggest the presence of intrinsic strain in the structure. Such large crystallite sizes calculated from W-H plot may be comparable to the large particles observed in the SEM images. The texture coefficient represents the preferential orientation of crystallite growth in the sample which can be deduced from the equation (3),

$$TC_{hkl} = \frac{I(hkl)/I_o(hkl)}{\Sigma^N_{n=o} I_i(hkl)/I_o(hkl)} \quad (3)$$

where, I(hkl) is the intensity of the peak, $I_0(hkl)$ is the intensity of the corresponding peak in the JCPDS file and N is the number of peaks. Table 2 displays the structural properties of the spray-coated BiI_3 thin film.

Morphology (SEM)

Fig. 9 displays the scanning electron micrographs of the spray deposited BiI_3 thin film at various magnifications. The images reveal a dendrite-like branched network spread throughout the surface. Besides, the distribution of large particles can be seen between these branches. To the best of our knowledge, such kind of interesting morphologies has never been observed in BiI_3 thin films. We believe that the in-situ crystallization at 150°C during the deposition might have resulted in the agglomeration of crystallites and thus the resulted morphology.

Chemical state (XPS)

The survey scan spectra of BiI_3 thin film with Al K_α radiation at room temperature in the full energy scale can be seen in fig. 10, displaying the

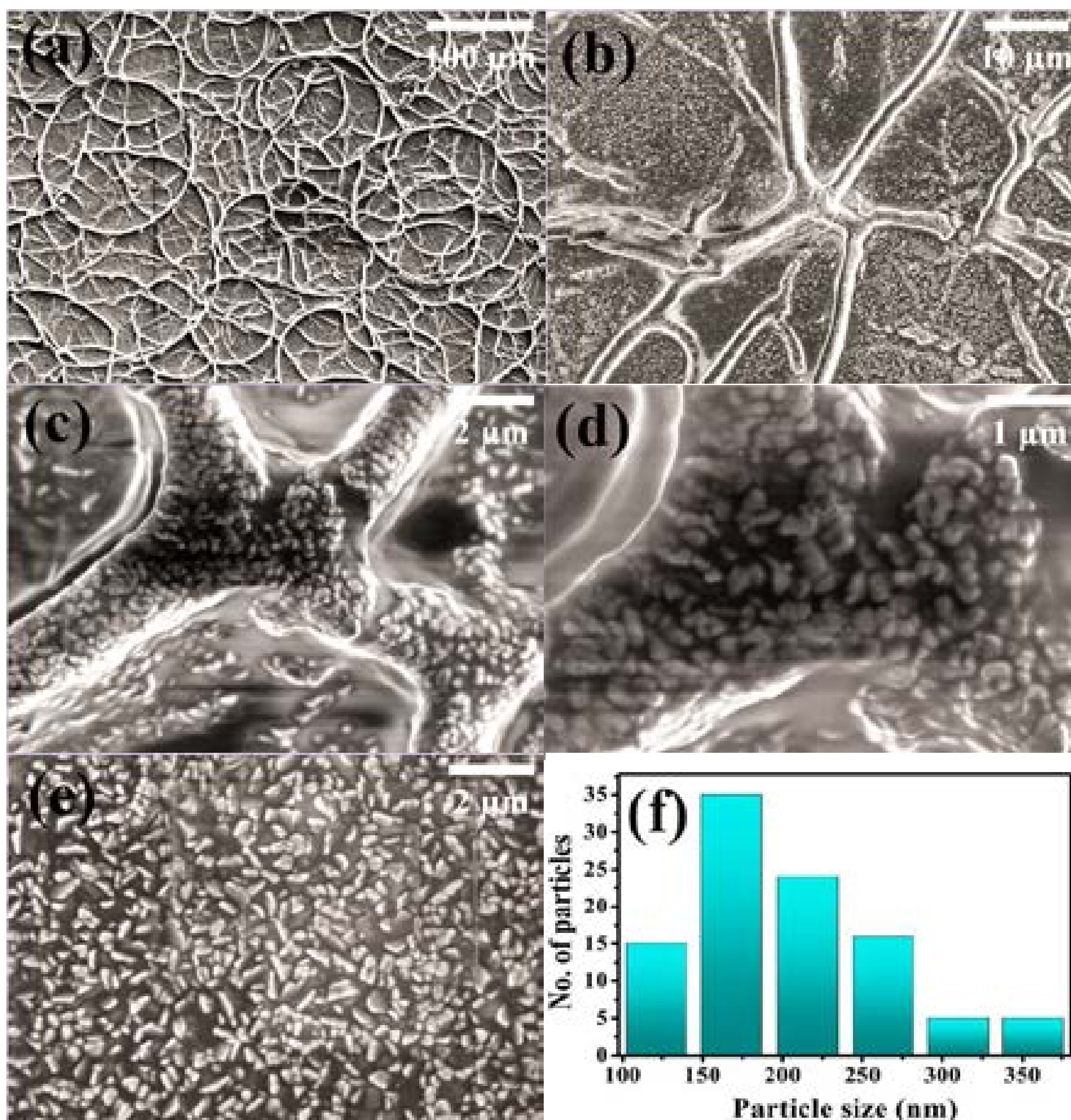


Fig. 9. (a-e) SEM images of spray-cast bismuth iodide thin film at different magnifications. (f) Particle size distribution for 100 particles measured.

important photoemission lines corresponding to Bi, I, C and O in the samples.

In order to check the relevent lines of Bi 4f, I 3d, C 1s and O 1s, high resolution scans were performed in the specified energy ranges and the obtained spectra are deconvoluted while taking care of the intensity ratios, FWHM and spin-orbit splitting.

The high-resolution Bi 4f scans show three doublets corresponding to $4f_{7/2}$ and $4f_{5/2}$ with a spin-orbit splitting of 5.3 eV (Fig. 11(a)). The peaks at 164 eV and 158.66 eV indicate the 3+ state of Bi species in BiI_3, whereas the peaks at 162.3 eV and 157 eV are due to the elemental Bi induced during the Ar-ion etching in the XPS measurement[4751]. Besides, it is reported that long exposure to X-ray radiation can lead to more Bi^0 species in the sample[52]. Further, the peaks at 157.97 eV and 163.29 eV may have resulted from bismuth oxides formed from the long ambient exposure. However, the presence of oxides couldn't be observed in the

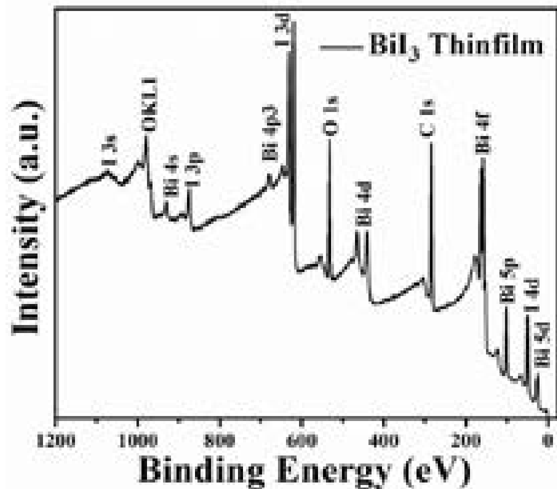


Fig. 10. The XPS survey spectrum of BiI_3 thin film by ultrasonic spray deposition.

XRD pattern. The peaks corresponding to I $3d_{5/2}$ and $3d_{3/2}$ were observed with a spin-orbit separation of 11.49 eV (Fig. 11 (b)). The peaks at 618.92 eV and 630.39 eV can be attributed to I^- in BiI_3 thin film[51]. The peaks at 631.79 eV and 620.39 eV can be attributed to I in I_2[47].

Optical property (UV-Vis-NIR Spectroscopy)

The UV-Vis-NIR absorbance spectrum of BiI_3 thin film with an absorption edge of ~822 nm is shown in fig. 12. The absorption spectrum reveals the strong absorbing nature of the ultrasonically spray deposited BiI_3 thin film.

We then calculated the optical bandgap from the tauc relation (4) given by,

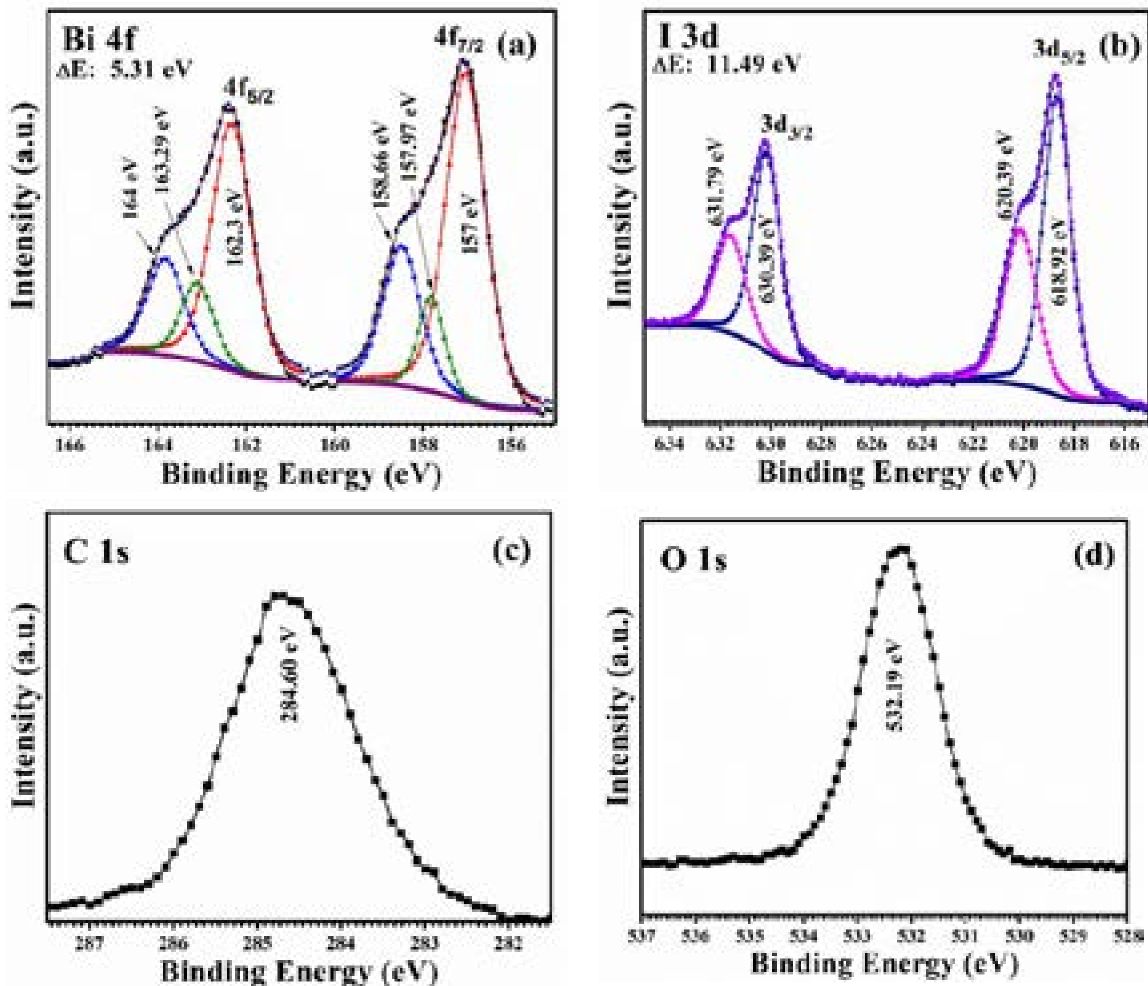


Fig. 11. High-resolution spectra of Bi 4f, I 3d, C1s and O1s states in BiI3 thin film after etching.

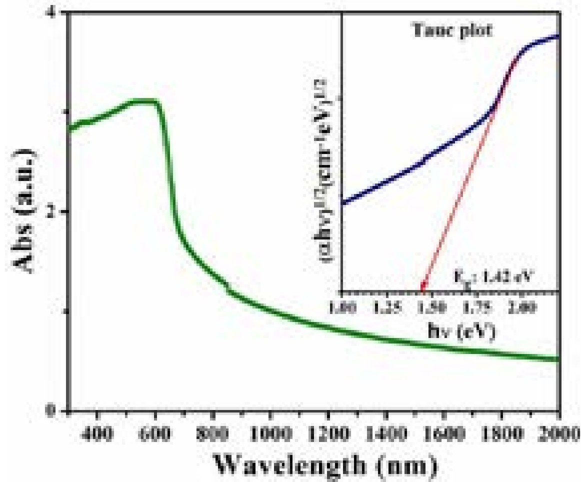


Fig. 12. The absorption spectrum of BiI_3 thin film. (Inset) Tauc plot showing the calculated indirect bandgap.

Where E_g is the optical band gap, h is the

$$B(h\vartheta - E_g)^n = \alpha h\vartheta \quad (4)$$

planks constant, ν is the photon frequency, B is called the band tailing parameter and n can have values corresponding to direct allowed (n=1/2), indirect allowed (n=2), direct forbidden (n=1/3) and indirect forbidden (n=3) transitions. α is the absorption coefficient which was estimated from the formula (*5*),

$$\alpha = \frac{1}{t} \times 2.303A \quad (5)$$

Where, A is the absorbance and t is the thickness of the thin film which was measured to be around ~1 µm using a stylus profilometer.

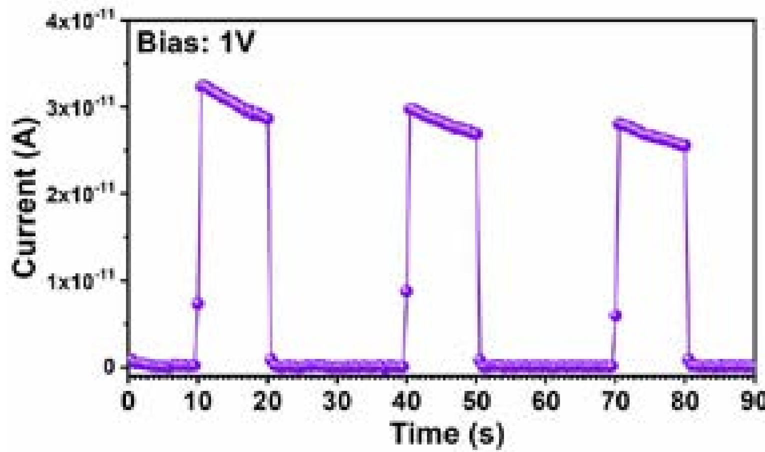


Fig. 13. Photoresponse measurement of BiI_3 thin film under 50 W halogen lamp.

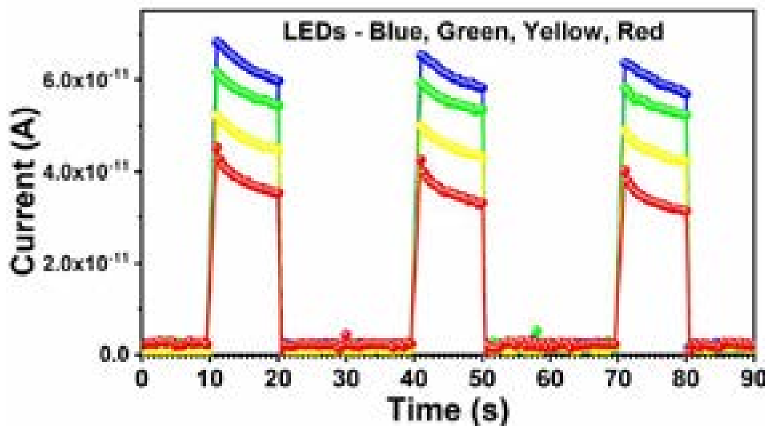


Fig. 14. Photoresponse measurements of BiI_3 film under LEDs at 1V bias. The color of the curves represent the LED wavelength.

The tauc plot between $(\alpha h\nu)^{1/2}$ and hν reveals an indirect allowed transition of 1.42 eV which is interestingly in correlation with the 1.44 eV predicted by the DFT calculations. As discussed earlier, the lower indirect bandgap is a direct consequence of spin-orbit magnetic effects from heavy Bi and I elements in BiI_3. As a matter of fact, this bandgap is very close to the optimum bandgap (1.40 eV) for maximum power conversion efficiency as per the Shockley-Queisser limit[53].

Photoresponse

The fabricated thin films are photoconductive with a very fast response to light. The measurements were carried out under a 50 W halogen bulb and different LEDs with 1 V bias. The measurements show a dark current of 6.1×10^{-13} A which was rapidly increased to 3.1×10^{-11} A under illuminated halogen lamp (Fig. 13).

Further, we have measured the response of the film to blue, green, yellow and red LEDs (50 W) and

Table 3. The sensitivity of BiI_3 thin film to different illuminations under a bias of 1 V.

Sample Illumination	I_d (pA)	I_{ph}(pA)	Sensitivity (%)
Halogen lamp	0.61	29.9	4890
Blue LED	2.24	61.1	2730
Green LED	2.24	55.0	2460
Yellow LED	2.24	45.3	2020
Red LED	2.24	36.0	1610

the results are depicted in fig. 14. The sensitivity (S) of the film under different illuminations are calculated from the equation (6)[54].

$$S\,(\%)=\frac{I_{ph}}{I_{dark}}\times 100 \qquad (6)$$

Where the photocurrent, $I_{ph}=I_{light}-I_{dark}$ The obtained data is presented in table 3.

Conclusions

DFT calculations on the BiI_3 resulted in the better understanding of the electronic structure of the material. Motivated from the computational results, BiI_3 powder was prepared and was used as a precursor for depositing thin films by the ultrasonic spray technique. The obtained phase pure films had a dendritic like morphology with an indirect band gap of 1.42 eV which was well in agreement with the DFT results. Further, the film demonstrated excellent response to illumination from halogen lamp and LEDs at low bias voltages. The interesting properties showcased by the spray deposited film are truly inspiring for further optimization and incorporation into optoelectronic devices.

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Diversity of Odonata in Palakkal Kole wetland, Thrissur, Kerala before and after deluge

Siji P. K.* and Bijoy C.
Department of Zoology, Christ College, Irinjalakuda, Kerala- 680125

Abstract

A study was conducted to analyze the impact of the deluge on the Odonata diversity at Palakkal kole wetland, Thrissur, Kerala. The paper deals with the study of Odonata diversity of Palakkal Kole wetland, Thrissur before and after Kerala deluge, 2018. The comparison of Odonata diversity was made between 4 months (September to December) of 2017 and 2018. The diversity of Odonata of pre-deluge was 21 species and post-deluge was 22 species and the abundance were1659 and 1042 respectively.

Keywords: Odonata, deluge, kole wetland, diversity

Introduction

Insects are the most diverse group of animals on the planet, including more than a million described species and representing more than half of all known living organisms[1]. Order Odonata is a primitive group of carnivorous insects encompassing the dragonflies (Anisoptera) and the damselflies (Zygoptera) which have existed since the Triassic period. Odonates are the most dominant invertebrate predator in any ecosystem[2]. There are approximately 6000 species under 600 genera in 29 families described all over the world[3]. 474 species belonging to 142 genera in 18 families exist in India[4]. 193 species under 83 genera in 14 families are known from the Western Ghats[5]. Of this, 168 species are reported from Kerala[6]. This rich diversity is fast disappearing due to the destruction of their breeding and resting habitats.[7]

Dragonflies are considered as bio-indicators. Some species are habitat-specific and so can be used for mapping of the habitats which they represent[7]. Economically they are of great importance in destroying noxious flies and mosquitoes, as well as the smaller moths which are regarded as pests[8]. Dragonflies, therefore, have potential health and economic value, which is not yet fully exploited[7]. Palakkal kole wetland of Thrissur, our study area was completely flooded during devastating deluge of August 2018. We tried to analyze if there is any effect of the deluge on the diversity of Odonata, by compairing post deluge diversity data (2018) with diversity data of the year previous to deluge (2017), which was already taken.

Materials and Methods

Study area (Fig. 1)

Palakkal kole wetland was monitored for Odonata diversity. Palakkal is situated at a distance of 6 km from Thrissur, at geographical coordinates of 10°28’ 15’’N and 76°12’ 40’’E. The study site (Fig. 2) includes deep and shallow waters, open mudflats, grassland and paddy fields. It also includes

Fig. 1. Study area with Odonata transect: Palakkal Kole wetlands, Thrissur

* Corresponding author, E-mail: siji1437@gmail.com

bunds, dykes and trees which provide different types of microhabitat for the odonates.

Fig. 2. Study site

Data collection and analysis

The fieldwork was done for four months from September to December in 2017 and September to December in 2018 which include pre-deluge data and post-deluge data respectively. Odonata specimens were sampled twice in a month. The observation started at 9.30 am and it was continued upto 12.00 pm. The area under study was surveyed using the line-transect method (Fig. 1). Specimens were photographed using Nikon B700. If found necessary, sweeping was carried out using an insect net to collect odonates. The day to day observations was noted in the field book and a data-sheet is prepared in Microsoft excel. The field photographs were used for identification of species with the help of the photographic field guide of dragonflies and damselflies of Kerala[9].

Diversity and dominance were estimated using statistical indices such as Dominance Index and Shannon-Wiener Index.

$$\text{Dominance index} = 1 - \left(\frac{\Sigma_i n_i(n_i - 1)}{N(N - 1)}\right)$$

$$\text{Shannon index} = -\sum_i \left(\frac{n_i}{N}\log_2\left(\frac{n_i}{N}\right)\right)$$

n_i = the total number of individuals of each species

N= the total number of organisms of all species

Results

A total of 24 species of odonates under 19 genera belonging to 4 families namely, Libellulidae, Coenagrionidae, Gomphidae and Platycnemididae are recorded during the study period in two consecutive years. Dragonflies dominate with 17 species, 16 of which belong to family Libellulidae. The second dominant family was Coenagrionidae which belongs to damselflies with 6 species.

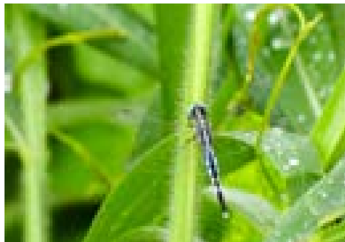

Fig. 3. ***Acisoma panorpoides*** **Rambur**

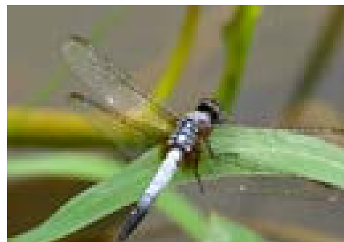

Fig. 4. ***Brachydiplax chalybea*** **Brauer**

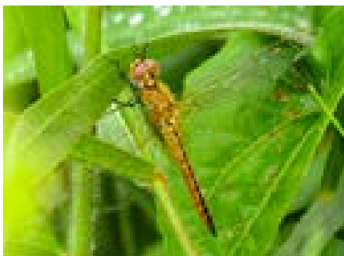

Fig. 5. ***Pantala flavescens*** **Fabricius**

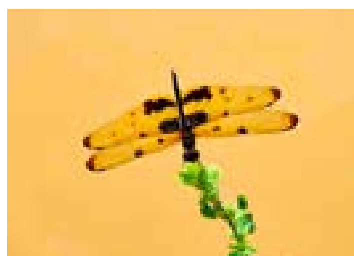

Fig. 6. ***Rhyothemis variegata*** **Linnaeus**

Pre-deluge period (September to December 2017): 21 species under 18 genera belonging to 4 families were recorded from the study area. Out of 21 species, dragonflies dominate with 15 species. Damselflies like *Copera marginipes* and Ceriagrion olivaceum were recorded only in this period. *Rhyothemis variegata (342) (Fig. 6) and Acisoma panorpoides (293) (Fig. 3)* were the most abundant species recored during this period. In pre-deluge, dominance index was 0.8785 and Shannon index was 2.347.

Post-deluge period (September to December 2018): 22 species under 19 genera belonging to 3 families were

recorded from the study area. Out of 22 species, dragonflies dominate with 17 species. Two dragonfly species; *Diplacodes trivialis*, *Tetrathemis platyptera* and a damselfly species, *Agriocnemis keralensis* were additionally recorded (endemic to Western Ghats[10]. *Brachydiplax chalybea* (113) (Fig. 4) and *Brachythemis contaminata* (112) were the most abundant species of this period. In Post-deluge, dominance index was 0.9287 and Shannon index was 2.735.

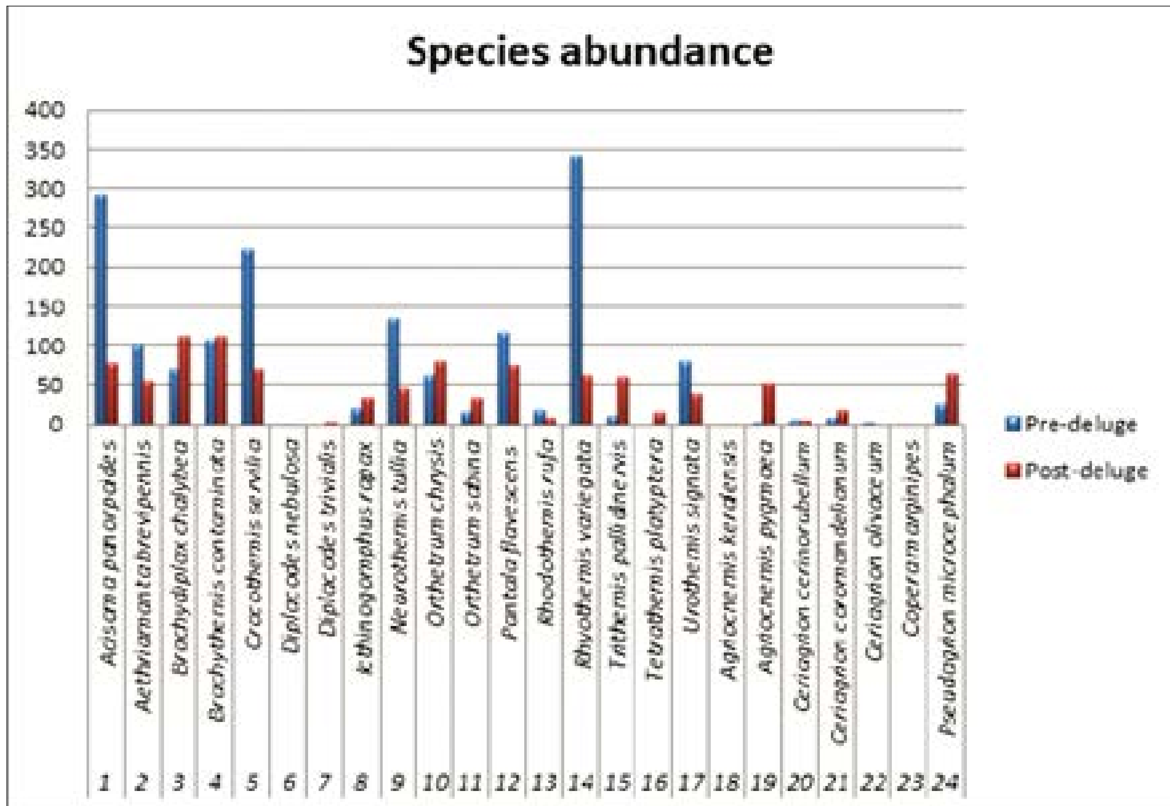

Fig .7. Diversity and abundance of odonata species recorded from study area

Discussion and conclusion

A study conducted by Gigi *et al.*[11] at Muriyad kole wetlands of Irinjalakuda in 2016 recorded 12 species of dragonflies whereas in this study, 17 species of dragonflies were recorded. Even though a significant difference cannot observe on the Odonata diversity from the data collected in two different years at Palakkal kole wetland, more abundance of Odonata was observed in pre-deluge data (1659) compared to that of post-deluge data (1042).

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Table 1. Diversity and abundance of odonata species recorded from study area

Sl. No	Species	Family	Pre-deluge	Post-deluge	Total
1	*Acisoma panorpoides* Rambur, 1842	Libellulidae	293	78	371
2	*Aethriamanta brevipennis* (Rambur, 1842)	Libellulidae	101	57	158
3	*Brachydiplax chalybea* Brauer, 1868	Libellulidae	71	113	184
4	*Brachythemis contaminata* (Fabricius, 1793)	Libellulidae	108	112	220
5	*Crocothemis servilia* (Drury, 1770)	Libellulidae	225	71	296
6	*Diplacodes nebulosa* (Fabricius, 1793)	Libellulidae	1	2	3
7	*Diplacodes trivialis* (Rambur, 1842)	Libellulidae	0	5	5
8	*Icthinogomphus rapax* (Rambur, 1842)	Gomphidae	21	35	56
9	*Neurothemis tullia* (Drury, 1773)	Libellulidae	134	45	179
10	*Orthetrum chrysis* (Selys, 1891)	Libellulidae	63	80	143
11	*Orthetrum sabina* (Drury,1770)	Libellulidae	17	35	52
12	*Pantala flavescens* (Fabricius, 1798)	Libellulidae	118	75	193
13	*Rhodothemis rufa* (Rambur, 1842)	Libellulidae	20	10	30
14	*Rhyothemis variegata* (Linnaeus, 1763)	Libellulidae	342	64	406
15	*Trithemis pallidinervis* (Kirby, 1889)	Libellulidae	12	61	73
16	*Tetrathemis platyptera* Selys, 1878	Libellulidae	0	16	16
17	*Urothemis signata* (Rambur, 1842)	Libellulidae	81	39	120
18	*Agriocnemis keralensis* Peters, 1981	Coenagrionidae	0	2	2
19	*Agriocnemis pygmaea* (Rambur, 1842)	Coenagrionidae	4	53	57
20	*Ceriagrion cerinorubellum* (Brauer, 1865)	Coenagrionidae	7	6	13
21	*Ceriagrion coromandelianum* (Fabricius, 1798)	Coenagrionidae	9	18	27
22	*Ceriagrion olivaceum* Laidlaw, 1914	Coenagrionidae	4	0	4
23	*Copera marginipes* (Rambur, 1842)	Platycnemididae	2	0	2
24	*Pseudagrion microcephalum* (Rambur, 1842)	Coenagrionidae	26	65	91

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Land use pattern and biodiversity value of Siruvani Watershed Area, Western Ghats

Suganthasakthivel, R., Sreekumar, V. B.* and Sreejith, K. A.
Forest Ecology and Biodiversity Conservation Division
Kerala Forest Research Institute, Peechi, Thrissur, Kerala – 680 653, India

Abstract

The landscape in the Siruvani watershed experienced large scale human disturbance in the past through various activities including dam construction and selection felling. A large-scale degradation of natural forests resulted in disruption in the rainfall patterns and subsequent changes in landscape. This paper deals with the present land use pattern and significance of biodiversity of Siruvani watershed area. The decline in forest cover and disruption of rainfall had affected the hydrological regimes in different landscape units. The land use cover changes had resulted in the deforestation in the Attapady valley where the Siruvani watershed is located. Through remote sensing satellite image analyses, this paper finds out the present land cover information and by collating the floral and faunal resources existing in the remnant forest patches, highlights the significance of biodiversity of this study area and its conservation.

Keywords: Siruvani watershed, Bhavani River Basin, Muthikkulam, biodiversity

Introduction

The Siruvani watershed is situated on the Bhavani river basin falling in the Attapady Block of Palakkad district in Kerala State. The watershed is located between 10° 56’and 11° 9’ N latitudes and 76° 34’ and 76° 44’ E longitudes. The River Siruvani, originating from the high, rain drenched and heavily forested Muthikkulam High Value Biodiversity Area, descends rapidly and flows across Attappady and joins the River Bhavani. The total geographical area of Siruvani watershed is around 215 km^2. Muthikulam is a part of Attappady Reserve and was under private ownership and subjected to intensive cultivation in the past[1]. These areas were reserved by the British in the early 1900s. The following description, as given in the notification of 1901 and cited by Ayyar[2] gives an idea of the destruction of these forests.

“Unscientific forestry, the ravages of timber thief, the destructive *Ponam* (shifting) cultivation fatal to tree growth, the average *Jenmis*’ (landlord) anxiety to turn his trees into money with the least possible delay, the *Moppila* (Muslim trader) in the guise of honest merchant removing on payment of *Kuttikanam* (stump fee) three times as many trees as he has paid for – all these contributed to the slow but steady denudation of the forests in the accessible areas, and these gradually became almost destitute of good timber”. Dietrich Brandis in Madras Government Records[3] stressed the need to protect the evergreen forests which formed the water resources of the Bhavani and its tributaries.

Forests affect the hydrology of watersheds in various and complex ways. Increasing evapo-transpiration, increasing soil infiltration, intercepting cloud moisture, reducing the nutrient load of run-off are some of the services available through the forests to the watershed. Destruction to the natural forest may result in drastic environmental changes including change in hydrological regime. The relationship between the forest degradation and disruption of the rainfall regimes is a proven hypothesis[4].

* *Corresponding author, E-mail: sreekumar@kfri.res.in*

It leads to increased runoff, desertification, loss of specialized microclimates etc. The loss of biodiversity with the removal of keystone species, the change of land-use pattern such as conversion of forest land for agriculture practices, grazing, and invasion by alien plant species may all have profound effects on the functioning of watersheds. A sustainable co-existence of land, water and vegetation is a prerequisite for environmental security and livelihood dependence for local community. As a part of preparation of the management plan for the Muthikkulam High Value Biodiversity Area, we anlaysed the pattern of current land-use system and the existing flora and fauna of Siruvani watershed. The existing land-use information of the available forest area and its potential in supporting a wide range of plant and animal resources of this watershed are largely unknown. Despite the services of this watershed in supporting the irrigation and drinking water needs of the population that spreads across two states of Kerala and Tamil Nadu, a comprehensive understanding on its present landuse and biodiversity status is also not available. In this context, this paper attempts to provide insight to the current land use pattern and biodiversity value of Siruvani water shed.

Materials and Methods

The Siruvani watershed boundary was delineated using the Survey of India toposheets at the scale of 1:25,000. The topo-sheets used are 58 A 12 and 58 B9, four sub-sheets each. The toposheets were geo-rectified with the software ArcGIS and vector mask of the watershed was prepared. The general land-use/land-cover information on the maps was used as the reference with the satellite images at the preliminary stage. Then the area was visited and different land uses were recorded with the handheld Global Positioning System (Garmin Map 60 CSX). The field verification was carried out by repeated field visits and the landuse of the area was classified into five thematic classes on the basis of a comparison with the older landuse maps. The categories in the thematic classes are forested patches, scrubs, plantation/ agriculture areas, barren land and water bodies.

The Landsat 7 Enhanced Thematic Mapper along with satellite images of the March 2001 of the Nilgiris region was used and made a subset of the Siruvani Watershed in Erdas Imagine software. The bands 4, 3 and 2 was used to discriminate the forest related landuse categories in the study region and performed a supervised classification with the maximum likelihood algorithm to develop thematic classes of the landscape and estimated the classification accuracy with the transformed divergence index for seperability implemented in the software Erdas Imagine software.

Results and Discussion

The understanding the present landuse pattern and biodiversity significance of Siruvani watershed is

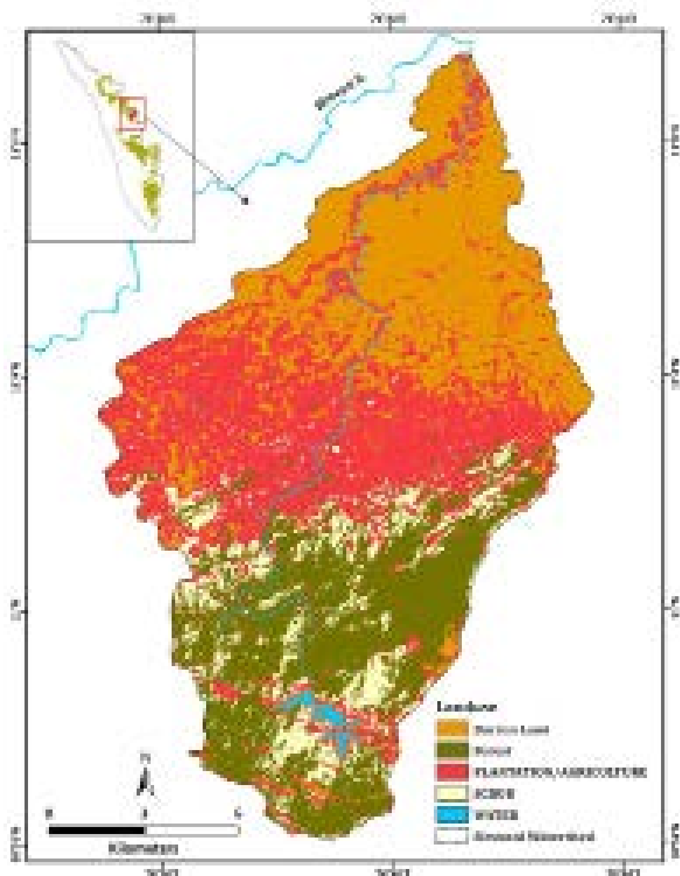

Fig. 1. Landuse Map of Siruvani Watershed, Kerala

important considering of its water scarcity, drastic climate variation, high runoff potential, slide susceptibility and land degradation. According to Sankar and Muraleedharan[5], this area has experienced high rate of deforestation, since the second quarter of the century, with large areas of mature forests being converted to agricultural lands, waste lands and pastures. Unscientific management practices, large-scale human interventions produced barren lands leading to large scale soil erosion in this area[6]. In the present study, the maximum likelihood classification accuracy is significant than expected, which is demonstrated in the Erdas Imagine as the separability index of the average and minimum transformed divergent indices are 1929 and 1358 respectively. The land uses of the Siruvani watershed are predominantly of plantation/agriculture followed by barren lands (Table 1) in the northern half of the watershed (Fig. 1). The intact forests are found in the southern half which forms the 'Muthikulam High Value Biodiversity Area' with an area of 52.5651 km^2. This forests acts as a critical component of Siruvani watershed and also forms the connectivity between forests of north of the Palakkad gap and Silent Valley forests which is the lifeline of Siruvani shed.

However, the major change on the pattern of land use, its fragmentation due to selection felling, encroachments, fire and forest degradation and various human disturbances seriously affected the continuous connectivity between Attappady Block VI and Silent Valley forests. Phyto-geographically the vegetation structure of this area shows more affinities to that of tropical Asia and Sri Lanka, thereby suggesting the existence of land connections in the past. The comparison of floristic diversity of Silent Valley, Muthikulam and Nelliampathy forests indicates[7] that the flora of these areas is of a higher Simpson's index of above 0.87. The main species associations in the study area is *Myristica-Mesua-Aglaia* and these three species constitutes 50% of the tree species community. Moreover, this forests hosts 77 species of micro and macro lichens,[8,9] 83 species of pteriodophytes and 488 species of flowering plants. Recently, new species of *Ophiorrhiza* (Rubiaceae)[10] and *Chlorophytum* (Asparagaceae)[11] and rediscovery of rare plants like *Gnidia glauca* var. *sisparensis* (Thymelaeaceae)[12] suggests the floral significance of this area. Similarly, this area is home to diverse wild aromatic and medicinal plants and the Muduga tribe of this area has a rich ethnic knowledge[13] of these plants. NTFP collection, including the medicinal plants is their main source of income. Gajathippali (*Balanophora fungosa* J. R. & G. Forst. ssp. *indica* (Arn.) Hansen), Thelli (*Canarium strictum* Roxb.), Manjakoova (*Curcuma zanthorrhiza* Roxb.) Cheenikka (*Acacia sinuata* (Lour.) Merr.), Maravettikkuru (*Hydnocarpus alpina* Wight) Oken,), Urunjikai (*Sapindus emarginatus* Vahl), Kallurvanchi (*Rotula aquatica* Lour.), Analivenga (*Pittosporum neelgherrense* Wight & Arn.) and Kattupavakka (*Momordica dioica* Roxb. ex Willd.) are collected from the forests. The other important medicinal plant resources are *Coscinium fenestratum* (Gaertn.) Colebr., *Dicliptera cuneata* Nees, *Gymnostachyum febrifugum* Benth. var. *bracteatum* V.S. Ramach., *Justicia procumbens* L., *Rungia parviflora* (Retz.) Nees, *Dioscorea pentaphylla* L., *Dioscorea oppositifolia* L., *Elettaria cardamomum* (L.) Maton, *Amomum pterocarpum* Thw., *Curcuma neilgherrensis* Wight, *Artocarpus hirsutus* Lam., *Phyllanthus amarus* Schum. & Thonn., *Cinnamomum sulphuratum* Nees, *Piper hymenophyllum* Miq., *Piper trioicum* Roxb., *Piper nigrum* L. var *nigrum* Hook. f., *Piper galeatum* Cas., *Myristica malabarica* Lam., *Bacopa monnieri* (L.) Pennell, *Sida cordifolia* L., *Sida acuta* Burm. f. and *Naravelia zeylanica* (L.) DC.

The study area is also rich with high faunal diversity which includes rare species such as Nilgiri Tahr and Lion-tailed macaque. The presence of extensive patches of almost undisturbed medium elevation evergreen forests (*Cullenia-Mesua-Palaquium* type)[14, 15] provides an ideal habitat for the Lion-tailed macaque. Similarly the mountain goat endemic to the Nilgiri Hills and the southern portion of the Western Ghats, the Nilgiri Tahr (*Nilgiritragus hylocrius*) is also found from higher reaches of Elivalmala with around 60 individuals. Populations of these animals are

small and isolated, making them vulnerable to local extinction. The studies on bird diversity of Siruvani and Muthikulam hills and reported a total 158 species of which 14 species are endemic to the Western Ghats[16]. The discovery of Nilgiri Laughingthrush (*Garrulax cachinnans*) an endangered species, with 200–250 individuals in these hills is significant in establishing the importance bird communities of these habitats. The Western Ghats endemic and Vulnerable Nilgiri marten (*Martes gwatkinsii*)[17] is sighted from South Reserve forests of Muthikkulam along with Attapady Reserve forests and Silent Valley National park[18]. The studies on butterflies diversity in Muthikulam, Mukkali and Chindakki areas reported around 84 species of butterflies with the maximum diversity was found in Muthikulam Reserve Forest (3.48) followed by Mukkali (3.41) and Chindakki areas (3.13)[19]. The surveys conducted in 1998[20] and 2007[21] recorded 52 reptile species with 17 Western Ghats endemic species from Muthikkulam hills. This includes one species of fresh water turtle, 21 species of lizards, and 30 species of snakes. Lion-tailed macaque (*Macaca silenus*) is a flagship species of Western Ghats classified as Schedule-I (highly protected) species in the W (P) A, 1972 and declared as an endangered species by IUCN[22]. The distribution of this species is restricted to the tropical evergreen forests of Kerala, Karnataka and Tamil Nadu. The population in the Muthikulam HVBA is reported to be severely fragmented[23] and isolated due to conversion of medium elevation evergreen forests into reservoirs and forestry and commercial plantations. The presence of extensive patches of almost undisturbed medium elevation evergreen forests (*Cullenia-Mesua-Palaquium* type) provides an ideal habitat for the species, since they mostly feed on fruits and young leaves of *Cullenia exarillata*[24]. Other common higher animals include, Bonnet macaque, Nilgiri langur, Elephant, Gaur, Sambar deer, Spotted deer, Barking deer, Mouse deer, Wild pig, Malabar giant squirrel, Ruddy mongoose, Indian civet, Sloth bear, Jungle cat and Wild dog etc.

Various anthropogenic disturbances in the past caused fragmentation of landscape and further reduction and isolation of primary forests and population of many species. The populations of endangered and endemic species like *Vateria macrocarpa*, *Dipterocarpus bourdilonii* and *Dysoxylum malabaricum* become isolated to small patches confined to limited localities. In addition to the alteration of habitats by dam construction, selection felling and other human interventions caused serious damage to the ecosystem by removing species key to the function of ecosystem. Still, the natural patches in Karimala, Vellingirimala and Elival mala provide more or less continuous forest cover with different types of forests including dense primary evergreen forests with *Cullenia exarillata – Mesua ferrea – Palaqium ellipticum* type vegetation which is relatively undisturbed and require high conservation value as a unique biodiversity repository in Western Ghats. There are six eucalypts plantations raised between 1962 and 1987 within Muthikulam HVBA, covering an area of 92.5 ha in grasslands. These plantations are under stocked and belong to the failed category which can be either restored by indigenous species or the natural grassland conditions can be maintained as such. Similarly the invasive weeds like Mikania *micrantha*, Lantana *camara*, *Mimosa diplotricha* var. diplotricha and Chromolaena *odorata* , which are a major threat to the native flora which should be removed by uprooting during monsoon in phased manner but continuously for three years, then alternate year, wherever needed.

Table 1. Land-use of Siruvani Watershed

Sl. No	Land use type	Area (km^2)	Per centage
1	Forest	52.5651	24.42
2	Scrub jungle	19.5684	9.09
3	Plantation/Agriculture	71.6265	33.28
4	Barren land	61.3683	28.51
5	Water	10.0891	4.69
	Total	215.2174	100

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