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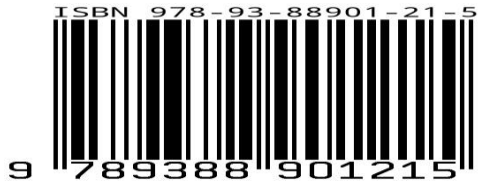

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PREFACE

*We are delighted to publish our book entitled "**Ecology Research (Volume III)**". This book is the compilation of esteemed articles of acknowledged experts in the fields of ecology providing a sufficient depth of the subject to satisfy the need of a level which will be comprehensive and interesting. It is an assemblage of variety of information about advances and developments in ecology. With its application oriented and interdisciplinary approach, we hope that the students, teachers, researchers, scientists and policy makers will find this book much more useful.*

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for compilation of such nice data in the form of this book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editorial Team

Ecology Research (Volume III)

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CONTENTS

Ecology Research (Volume – III)
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ENVIRONMENT DNA (e-DNA) AS MARKER FOR SUSTAINABLE ECOSYSTEM

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Abstract:

Sustainable ecosystem is a self-sustaining system where normal cycle of disturbance maintains biodiversity of that region along with all biogeochemical cycling. For maintaining biodiversity, knowledge of phylogeny is very important for the organisms. Environment DNA gives us the area to know more about the organisms which are surviving in the particular ecosystem. Environment DNA (eDNA) can be extracted from the samples like soil, water, faeces or any remnants of plants or animals. By doing the phylogenetic studies the organisms present in environment can be easily identified up to species level. In population genetic studies also eDNA can be used for finding the species-specific alleles. Organisms are seasonally distributed and pattern of distribution also can be noted by comparing the level of eDNA by collecting sites from different sites. Migratory patterns of organisms in aquatic system especially fishes can be enrooted by analysing the eDNA pattern. Present paper through light on eDNA tool as an easily available marker without disturbing the organism in environment. By knowing systematic status, a conservation strategy can be easily planned looking into status of organism in ecosystem

Keywords: Sustainable ecosystem, Marker, eDNA, Conservation, phyllo -genetic studies

Introduction:

Sustainable development of a region can be treated as economic development by providing security by giving good education among society members, better health for human population and other biotic and abiotic factors in the natural environment to balance the ecosystem. For conservation of the ecosystem, knowledge of phylogeny of flora, fauna and other organism is very important as it gives us idea of the role of living organism in ecosystem. Every ecosystem features key functions such as primary production and

nutrient cycling, which give rise to ecosystem services that improve human wellbeing such as the provisioning of clean water, fertile soils, timber and capture fisheries (Ehrlich and Mooney, 1983; Seddon *et al.,* 2016). Environmental DNA (eDNA) analysis can increase the ability to detect and quantify biodiversity, as it overcome challenges by collecting the data by survey method. eDNA technique and its application in ecology and conservation is increasing in recent years. Present paper is discussing application and advantages of using eDNA technology and conclude by suggesting eDNAasa marker for sustainable ecosystem.

Discussion:

Environment DNA (eDNA) obtained directly from environmental samples can be used to evaluate species distributions. These methods have recently been developed and are considered to be useful techniques (Rees *et al.,* 2014; Goldberg *et al.,* 2015; Thomsen and Willerslev, 2015; Deiner *et al.,* 2019). From 2012 many studies were conducted using eDNA technology and fishes were most studied group of macro-organisms in aquatic ecosystems with respect to e DNA (Tsuji *et al.,* 2019) For example, Fish species were detected using eDNA by (Minamoto *et al.,* 2012, Mahon *et al.,* 2013). High-throughput parallel DNA sequencing (HTS) has been applied in eDNA studies to examine community composition from eDNA samples (Deiner *et al.,* 2019). This eDNA technique with HTS sequencing and DNA-based species identification is called eDNA metabarcoding and is considered to be a useful method for assessing aquatic communitiesin evaluations of the effectiveness of this tool. Season wise occurrence of organism was also able to note by studying the eDNA in the environment. As Spear *et al.* (2014) found that abundance of eDNA for the Eastern Hellbender (*Cryptobranchus alleganiensis*) was highest in the autumn breeding season for this large aquatic salamander relative to the summer. Laramie *et al.* (2015) similarly reported that eDNA concentrations for Chinook Salmon *(Oncorhychnus tshawytscha*) and invasive Bigheaded Carp species (*Hypophthalmichthys* spp.) peaked during the spawning season of themin the Mississippi River. Fujii *et al.* (2019) reported that eDNA metabarcoding of fish communities can be performed similarly through multiple capture methods in backwater lakes, as traditional fish-capture methods are more time-consuming and more effort in field is required for collection of samples. In their work they showed that eDNA in 1 L water samples had a similar detectability to that of traditional methods of fish capture, suggesting the usefulness of eDNA in detecting fish community in the habitats. Belly *et al.* (2019) reported eDNA as appropriate standards for fish survey studies. Taberlet *et al.* (2012) reported HTS as advantageous as cloning and sequencing by Sanger sequencing through eDNA technology. Lu shu et al, 2020 described standards for methods utilizinge DNA for detection of Fish Species. ThiseDNA technology is successfully utilised in migration studies of fishes by Thalinger *et al.* (2019) in protandrous fishes. Silje *et al.*

(2020) revealed the reason for decreasing European Eel (*Anguilla* sps) is blocking of migration path way by hydroelectric power construction. According to Jayasankar *et al.* (2017) no eDNA studies were conducted in India in relation to fishes and they will impact profound advancement for management of fisheries. They also reported a lab experiment which proved the success of the eDNA technology in small scale.

Apart from fishes, Clark *et al.* (2020) used environmental DNA (eDNA) metabarcoding to examine the response of eukaryotic (18S rRNA), diatom (rbcL) and bacterial (16S rRNA) communities and provided a base for molecular-based estuary monitoring tools, which suggest more holistic and standardized approach for health assessment of ecosystem with faster turn- around times and lower costs.

eDNA technology is also used in population genetic studies as Rusello *et al.* (2004) and Smith and Wang (2014) used for genetic diversity studies in inbreeding population and Sharma *et al.* (2011) used as evolutionary significant units.As per Stoeckle *et al.* (2016) Detection of species by eDNA technology has many advantagesbut this method cannot fully substitute classical monitoring, for population genetics studies for conservation management. Fujii *et al.* (2019) also reported some disadvantages of the eDNA metabarcoding such as PCR inhibition for eDNA analysis and false positives of some marine species originating from wastewater contamination.

Zhang (2019) suggested use of eDNA technology as effective and efficient tools to evaluate the effects of chemical pollutants on wildlife population, there importance in ecological communities and function of them in the ecosystem. They conceptualised adverse outcome pathways using molecular biology tool in ecosystem level.

Conclusion:

Despite its technical challenges, eDNA remains a promising and powerful tool for fish monitoring and conservation. In the last decade, eDNA methods have been increasingly utilized in fish detection for monitoring and conserving fish diversity. Fujii *et al.* (2019) highlighted difficulties faced by researchers choosing technique with respect to its reliability. They also reported a standard technology from the collection of water sample, eDNA capture and extraction, genetic marker selection, and eDNA detection in fish surveys. also highlighting key standards for reducing or avoiding false and negative detection in eDNA studies. They suggested method for eDNA technique as reported by many previous reports for fish identification.They summarised with the following method, collection of 1 or 2 L surface water and followed by filtration using 0.7 μm GF filters, followed by extraction with the DNeasy Blood and Tissue Kit or PowerWater DNA Isolation Kit can be performed to obtain high-quality eDNA for reliable results. Subsequently, using specific primers based on Cytb for species-specific qPCR assay or using universal primers

based on both 12S rRNA and 16S rRNA for eDNA metabarcoding via HTS is effective for target species identification or species richness assessment. Also established quality controls to minimize detection errors by mitigating contamination including spatial separation, surface cleaning, and negative control in each step of eDNA analysis, performing PCR replication such as triplicate PCRs for each sample, and using both 12S and 16S markers in multiple primer sets. Decontamination and negative control are aimed at decreasing false-positives, whereas PCR replication and using multiple genetic markers are aimed at decreasing false-negatives. Nevertheless, these standards must be adapted occasionally considering technical progress. The emergence of the portable field-based eDNA platform and portable sequencing technology suggest that with additional development and improvements, eDNA techniques can be used successfully to rapidly evaluate environmental samples.

Also, eDNA samples from soil, water, or air are useful when individual traces cannot easily be identified and sampled. For example, eDNA can be used to target sites of suspected occupancy before intensive, invasive sampling effort is carried out in difficult-to-sample habitat (Goricki *et al.,* 2017). With eDNA metabarcoding of environmental samples,comparing the use of traditional methodology in tandem repeat study will confirm the technology and species identification as suggested by Stat *et al.* (2018)

It is seen in references that eDNAsample can be collected without visualising the specimen and it can be used as biodiversity and biosecurity monitoring tool with a strong taxonomic focus;hence it can be utilised as a marker for species biodiversity and ecological biodiversity in an ecosystem which indicates sustainability of the organism in that particular ecosystem.

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ICTHYOFAUNA OF GENUS: PUNTIUS HAMILTON-BUCHANAN 1822, RECORDED IN RIVER SIANG OF ARUNACHAL PRADESH, INDIA

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Abstract:

Puntius is a genus of cyprinid fishes known as the spotted barbs for the predominant pattern, though many have vertical black bands instead. The maximum size for an adult of this genus is less than 25 cm (9.8 in), typically 7–15 cm (2.8–5.9 in), and many species only achieve around 5 cm (2.0 in) adult length. In appearance they may resemble miniature carp and are often brightly coloured or patterned. These fishes are omnivorous; their diet includes small invertebrates and plant matter. Breeding is by egg scattering and takes place close to the bottom, near or within areas of dense plant growth. They do not show parental care, and adults may eat the young. There are currently 57 recognized species in this genus, but there are four species of *Puntius* are recorded in River Siang of Arunachal Pradesh, these are *Puntius chola, Puntius sophore, Pethia ticto* and *Systomus sarana.*

Keywords: *Puntius,* Cyprinidae, River Siang, Arunachal Pradesh.

Introduction:

River Siang, a hill-stream of 1st order river; had colluvial valley segment and pool-riffle type of reach. Pools, riffles and runs were generally found to dominate the micro-habitat type with frequent occurrence of trench pools. River Siang was said to be more entrenched based on V-shaped valley segment. The substrate type had been found to be dominated by gravels and cobbles with frequently occurring quite large number of boulders and some bed rocks. River Siang was the unique in the ichthyofaunal diversity. Fish sampling was carried out with the help of different kinds of nets such as cast net, gill net and traps, lines and hooks, *etc*. More than 50% of fish species of River Siang belongs to the Order Cypriniformes whereas other fishes were represented by the Orders *viz.,* Siluriformes, Perciformes, Clupeiformes, Synbranchiformes, Osteoglossiformes,

Tetradontiformes and Beloniformes. In the present study on fish diversity, it was revealed that the number of fishes was recorded higher in pre-monsoon and monsoon seasons in all the study years. In this regard, the present objective is the numbers of different species of *Puntius* are available in River Siang of Arunachal Pradesh.

Materials and Methods:

Study Site:

The River Siang, is largest river of Brahmaputra river system, originates from Chema Yungdung Glacier near Kubi at 5150 m in Tibet. In Tibet it is popularly known as Tsang-Po, flows in West–East direction. After traversing a distance of about 1(**C** ı river in Tibet and then it takes a turn in south direction, enters the territory of India near Tuting in the Upper Siang district of Arunachal Pradesh and flows through North–South direction in East Siang district towards Assam and finally it merges with Lohit and Dibang in Assam and it becomes the mighty River Brahmaputra (Das *et. al.* 2014 a, b; Das and Kar 2015) (Figure 1).

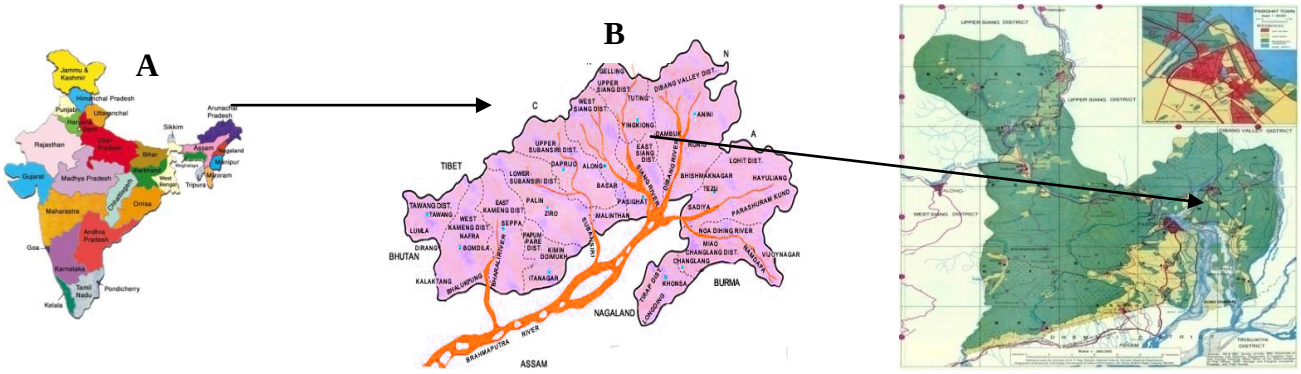

Figure 1: Map of (A) India indicating Arunachal Pradesh, (B) Arunachal Pradesh indicating to East Siang District, (C) In East Siang district highlighting River Siang (Study Area) of Arunachal Pradesh

Freshwater Survey:

Fish samples were collected from River Siang during January 2012 to December 2014 through experimental fishing; using cast nets (dia.3.7 m and 1.0 m), gill nets (vertical height 1.0 m- 1.5 m; length 100 m -150 m), drag nets (vertical height 2.0 m), triangular scoop nets (vertical height 1.0 m) and a variety of traps and with hook and lines in certain places (where netting is not possible). River was surveyed and classified into different habitat units based on morphology (Bisson *et al.,* 1982) and finally divided in to six (6) different study sites covering upstream, mid-stream and downstream stretches of the river.

General survey of the fish biodiversity was done using standard procedures (Armontrout, 1990).

Fish Measurement:

The morphometric study included measurement of Total length (TL), Standard length (SL). Body depth (BD) Snout length, Post orbital length, Head length (HL), Pre dorsal length, Prepelvic distance, Eye diameter (ED), length of Caudal Peduncle, and Length of caudal fin. SL was the distance from the tip of the snout to the mid base of the caudal fin and TL was the distance from the tip snout to the furthest tip of the caudal fin. BD was the greatest vertical distance across the body. The measurements were done using Vernier Calliper Scale and Digital Sartorious Electronic Balance.

Fish Preservation and Identification:

Fish species had been preserved, at first, in concentrated formaldehyde in the field. After that, the fishes were transferred to laboratory and preserved in 10 % formalin. The small size fishes were preserved in 5% aqueous formalin solution and big size fishes in 10% aqueous formalin solution and kept in the air-tight plastic bottles.

In the laboratory, the fishes were identified by following standard literature, notably, Day (1878), Rainboth (1996), Sen (2000), Talwar and Jhingran (1991), Jayaram (1999, 2010), Nath and Dey (1997, 2000), Vishwanath (2000, 2002), and Kar (2007, 2013) and www.fishbase.org. All the fishes were kept in the Assam University Fish Museum (AUFM) for preservation and record. After labeling the fishes were drawn and photographed with the help of digital camera (Nikon Coolpix L-810).

Results and Discussion:

In River Siang we had recorded *Puntius chola, Puntius sophore, Pethia ticto* and *Systomus sarana.* They are described as follows:

Genus: *Puntius* Hamilton-Buchanan 1822:

Puntius Hamilton-Buchanan, 1822, *Fish Ganges*, pp. 310 (type species, *Cyprinus sophore* Hamilton- Buchanan, by subsequent designation).- Jayaram, 1991, *Occ. Papers ZSI, No. 135.* pp. 1-78 (revision).

Diagnosis:

Body short to moderately elongate, deep, compressed. Abdomen rounded. Head short. Snout conical, occasionally with pores or tubercles. Eyes moderate to large, dorsolateral and not visible from below ventral surface. Barbels none. Dorsal fin short, inserted nearly opposite pelvic fins with 9 rays, (7 branched). Anal fin short with 7 rays (5

branched). Caudal fin forked. Scales small to moderate, with few and strongly divergent striae. Lateral line incomplete with 24 scales.

***Puntius chola* (Hamilton-Buchanan, 1822):**

Key to Species:

One pair of barbels present. Lateral line with 24 scales. Last unbranches dorsal fin ray strongly osseous. Body with two conspicuous dark blotches generally between 21st and 23rd scales and another near gill opening. A dark mark at base of anterior dorsal fin ray also present (Figure 2 and Plate 1).

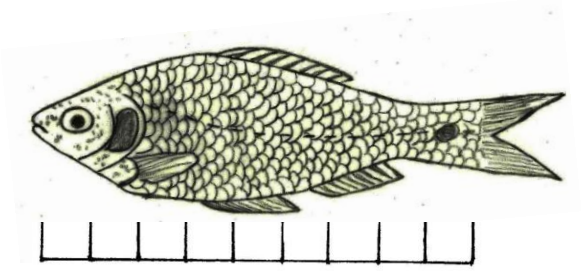

Figure 2: *Puntius chola*

Plate 1: *Puntius chola*

Snout length = 0.8 cm, Post orbital length = 1.4 cm, Head length= 2.3 cm, Pre-dorsal length = 4.2 cm, Pre-pelvic distance = 4 cm, Standard length = 7.5 cm, Total length = 9.9 cm, Eye diameter = 0.6 cm, Length of caudal peduncle = 1.6 cm, Length of caudal fin = 2.7 cm, Body depth = 3 cm and Weight = 12.50 g.

Distribution:

River Siang, Barak, Brahmaputra, Lohit India. Bangladesh. Myanmar. Nepal. Pakistan. Sri Lanka. Thailand.

***Puntius sophore* (Hamilton-Buchanan, 1822):**

Key to Species:

Barbels absent. Complete lateral line with 24 lateral line scales and Posterior dark blotch on 22nd to 24th scales. Presence of 9 circumpenduncular scales (Figure 3 and Plate 2).

Snout length = 0.4 cm, Post orbital length = 0.7 cm, Head length = 1.3 cm, Pre-dorsal length = 2.3 cm, Pre-pelvic distance = 2.0 cm, Standard length = 3.9 cm, Total length = 5.2 cm, Eye diameter = 0.5 cm, Length of caudal peduncle = 0.9 cm, Length of caudal fin = 2.7 cm, Body depth = 1.9 cm and Weight = 11.89 g.

Distribution: River Siang, Barak, Brahmaputra, Lohit India. Bangladesh. Myanmar. Nepal. Pakistan. Sri Lanka. Thailand.

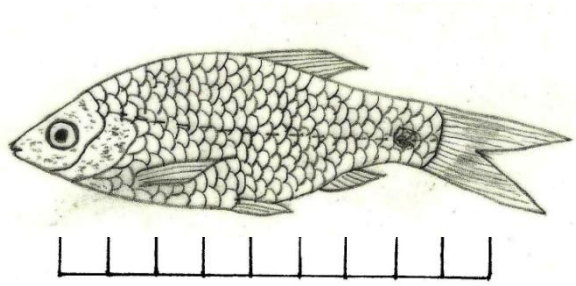

Figure 3: *Puntius sophore*

Plate 2: *Puntius sophore*

***Pethia ticto* (Hamilton-Buchanan, 1822):**

Key to Species:

Barbels absent. Dorsal spine strong, osseous, serrated. Lateral line incomplete having 23 scales. Anterior colour spot present. Pre-anal scales 13. Dorsal spine short, equal to head length and body depth.Body with two vertical bands.Presence of two black blotches on body (Figure 4 and Plate 3).

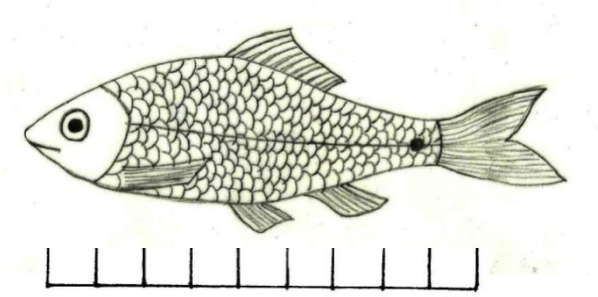

Figure 4: *Pethia ticto*

Plate 3: *Pethia ticto*

Snout length = 0.4 cm, Post orbital length = 1 cm, Head length = 1.5 cm, Pre- dorsal length = 2.7 cm, Pre-pelvic distance = 3 cm, Standard length = 4.4 cm, Total length = 4.7 cm, Eye diameter = 0.6 cm, Length of caudal peduncle = 0.8 cm, Length of caudal fin = 1.83 cm, Body depth = 1.5 cm and Weight = 3.61 g.

Distribution:

River Siang, Barak, Brahmaputra, Lohit India. Bangladesh. Myanmar. Nepal. Pakistan. Sri Lanka. Thailand.

***Systomus sarana* (Hamilton-Buchanan, 1822):**

Key to Species:

Barbels present, two pairs of barbels, lateral line scales 32. Last un-branched dorsal ray osseous, strong (Figure 5 and Plate 4).

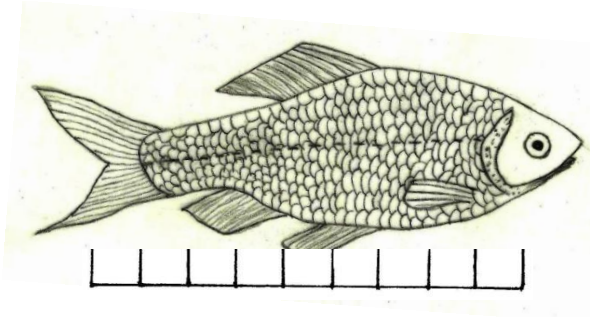

Figure 5: ***Systomus sarana***

Plate 4: ***Systomus sarana***

Snout length = 0.4 cm, Post orbital length = 1 cm, Head length = 1.5 cm, Pre-dorsal length = 2.7 cm, Pre-pelvic distance = 3 cm, Standard length = 5.4 cm, Total length = 6.7 cm, Eye diameter = 0.6 cm, Length of caudal peduncle = 0.8 cm, Length of caudal fin = 1.83 cm, Body depth = 1.5 cm and Weight = 13.61 g.

Distribution:

Arunachal Pradesh, Assam, North-East, West Bengal of India; Bangladesh, Myanmar, Nepal, Pakistan, Sri Lanka and Thailand.

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THE SUNDARI ON THE BRINK OF EXTINCTION IN THE SUNDARBANS

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Abstract:

The Sundarbans- the largest mangrove ecosystem on the planetEarth, meaning the "beautiful forest" derives its name from the Sundari (*Heritiera fomes*) trees found here but off lately enlisted as endangered species. The Sundarbans is intersected by a complex network of tidal waterways, mudflats and little islands of salt-tolerant mangrove forest formed by the super confluence of the rivers- Ganges, Brahmaputra and Meghna in the Bay of Bengal. This unique diverse ecosystem is understood for its wide selection of flora and fauna -the most famous being the man-eating Royal Bengal Tigers besides numerous species of birds, spotted deer, crocodiles, snakes etc. Due to its extremely rich diversity and uniqueness, Sundarbans has been declared as a UNESCO World Heritage Site.Over the centuries, these mangroves have been extensively exploited for timber, fish, prawns or converted for paddy and aquaculture, as a result, itis now facing the intense challenge for its existence. The constantly growing human population, global climate changes particularly the ocean level rise and increasing salinity are some of the main threats to this vast magnificent biodiversity. Further loss of this precious mangrove forest will ultimately result in the reduced protective biological shield against disastrous cyclones and tsunamis which would not only greatly endanger its diverse flora and fauna but also put the encompassing coastal communities at high risk of survival.Therefore, strict measures should be taken to conserve this valuable biodiversity, decrease the man-animal conflict and save the Sundari from its extinction.

Keywords: Sundari, Sundarbans, mangrove, ecosystem, biodiversity, endangered

Introduction:

The Sundarbans- the world's most important and largest mangrove ecosystem, meaning the "beautiful forest" derives its name from the Sundari (*Heritiera fomes*) trees found here.[1]The Sundari is the dominant mangrove tree species of the Sundarbans of India and Bangladeshand according to IUCN red list Conservation Category, H. fomes is assigned with endangered status.[2]If adequate measures are not taken then very soon this species might become extinct as their number is declining fast due to several factors and are visible in only 6% of the sampling sites in India.[3]

The Sundarbans Mangrove ecosystem is a unique and highly productive ecosystem within the sea-land interphase with the conglomerations of plants, animals and microorganisms acclimatized within the fluctuating environment of the tropical intertidal zone and protect the coastal areas against natural hazards like cyclones and tsunamis.[4-7] It is intersected by a complex network of tidal waterways mudflats and little islands of salt-tolerant mangrove forests This ecosystem is highly valued in terms of economy, environment and ecology.[8-14] In comparison to the Bangladesh part, the Indian component of the Sundarbans has poor forest formation due to higher salinity and biotic interactions leading to different growth pattern and ecological succession. Being on the land-sea interface, mangroves are always associated with and subjected to saline seawater. However, the saline condition is not a prerequisite for their development; rather mangroves choose saline conditions to avoid the competition with the more vigorous terrestrial plants. and the rising sea level. A large portion of the silt is deposited on the eastern side causing land accretion, particularly in the south-eastern region; and compensatory erosion in the southwestern part, thereby pushing the coastline towards the sea. Mangroves are rich in polyphenols and tannins. Phenols and flavonoids present in mangrove leaves serve as UV-screen compounds. The primary threats to all mangrove species including H. fomes are habitat destruction and removal of mangrove areas for conversion to aquaculture, agriculture, urban and coastal development, and overexploitation.[3]

Area:

The Sundarbans is found within 21°32′ to 22°40′N and 88°05′ to 89°51′E and covers an areaof roughly 10,000 km of which 62% lies within Bangladesh and only 38% in India.[15]

Formation of Sunderban delta:

This ecosystem has been formed by the super confluence of the rivers-Ganges Brahmaputra and Meghna. [1]The mighty Indian river, the Ganges and its associated estuaries like Muriganga, Saptamukhi, Bidyadhari, Haribhanga, Thakuran etc, open into the Bay of Bengal having a north-south direction of water flow. The silt and loam carried by these estuaries were deposited on the salt marsh eventually resulting in the formation of a mosaic of 102 deltaic islands of which 54 are reclaimed for human habitation. Rivers within the Sundarbans are meeting places of saltwater and freshwater[15-16] actually it's a region of transition between the freshwater of the rivers originating from the Ganges and the saline water of the Bay of Bengal.[16]

Uniqueness:

This unique diverse ecosystem is understood for its wide range of flora and fauna -the most famous being the man-eating Royal Bengal Tigers besides numerous species of birds, spotted deer, crocodiles, snakes etc. The extremely rich biodiversity and uniqueness of Sundarbans has earned it the prestigious tag of UNESCO World Heritage Site and natural wonder of the planet. [1,15]

Importance of this ecosystem:

The environment of the Sundarbans mangroves—both in India and Bangladesh—is densely populated. This population being impoverished depends heavily on the forests for their livelihood and sustenance. [17-19] This ecosystem maintains the agricultural economy by providing timber and fuelwood, faunal resources like fishes, honey etc, protects the coast from erosion, mitigates flood and maintains estuarine flow. Mangrove trees are used for timber and construction material (e.g. for houses, boats, traps) also for fuel and charcoal production. Apiculture is widespread within the Sundarbans mangrove forests and provides honey and wax. A large number of people are engaged in beekeeping within the Indian Sundarbans, producing approximately 90% of the entire natural honey production in India.[13] Apart from these useful resources, mangrove trees also provide tannins for leather production and are home to a wide array of medicinal plants. Innumerable varieties of sea creatures like crabs, molluscs, shrimps and fish are caught within the brackish waters [1] which also acts as nursery grounds for several commercially important fish species. One of the most important beneficial effects of this mangrove is that it acts as buffers against

cyclones, storms etc. The 2004 tsunami could have caused more destruction of lives and property had the buffering capacity of the forest been not present.[20] Furthermore, in May 2009, much of the momentum of cyclone Aila was absorbed by the mangroves, saving the city of Kolkata from clutches of greater loss.

Biodiversity:

This ecosystem harbours thousands of flora and fauna in its diversified habitats along with the highest number of mangrove tree species which accounts for one-third of the global total, high biodiversity within the Sundarbans is additionally represented by other groups with more than 200 additional plant species, more than 400 species of fish, over 300 species of birds, 35 species of reptiles,42 species of mammals, also countless invertebrates, bacteria, fungi, etc.[21-22] But now the very Sundari after which the name of Sundarbans has been coined is at the brink of extinction along with other species due to various factors.[3]

Endangered Sundari (*Heritierafomes*) trees of the Sundarbans:

Characteristics:

Sundari is a mangrove buttressed tree of 10 to 25 m tall with dense, robust pneumatophores about 50 cm in height. It is the only *Heritiera* species that produces pneumatophores. The roots do not penetrate deep into the soil, but spread on the surface with numerous stout offshoots and often with narrow ridges forming plant-like projections above the soil and also form flat narrow buttress to the basal trunk. It prefers freshwater and is fast-growing in low-saline environments. The species is commonly found along the tidal creeks and channels of the coastal swamps and regenerate naturally through seeds.[23] The species is found in the upstream estuarine zone in the high intertidal region and adapted best along the seashore.

(Source: https://www.google.com/search?q=images+of+sundari+tree)

Utility:

The chemicals produced from the species can be used for gastrointestinal disorders (including dysentery, diarrhoea, indigestion, colic, acidity, constipation, bloating, lack of appetite, stomachache). Besides this, it can be used to treat hepatic disorders (including jaundice and hepatitis), insect repellent and skin diseases (including eczema, abscess, acne, boils, scabies, itch, infections, dermatitis, rash, sores, scar, warts, etc.).[3]

Reasons for extinction:

The species is now on the extinction threat in West Bengal due to overcutting and increased salinity. Unlike other mangrove species, H. fomes prefer extremely low saline condition (5 – 15 psu) and hence can act as a biological indicator of climate change-related to sea-level rise. In the highly populated Bengal (India and Bangladesh) the dry season demand for freshwater has increased dramatically; major rivers have been dammed and the downstream effects are becoming apparent with increasing soil salinities and unexplained 'top dying' disease is threatening the H. fomes population. The first factor is anthropogenic; the second, although aggravated by upstream diversions of Ganga water, is largely due to long-term geomorphic processes.[3]

Salinity Increase:

H. fomes flourishes well under low salinity conditions and various studies have revealed that they are salt-tolerant but not salt lovers as a result this species of tree isat the brink of extinction owing to the increasingsalt level of the ocean. This saline condition retards the optimal growth and development of the trees. An increase in salinity leads tostunting, reduces the rate of photosynthesis andlater decline in the number of species.[3]According to Dr SwapanSarker, an associate professor at the Forest Environment Science of Shahjalal University of Science and Technology (SUST),a research work mentioned that the diversity in the Sundarbans took a heat during 1986-2014. The number of Sundari, Passur, Shingra, Amur, Dhundal and Kakra trees are on the decline. Noted water expert Ainun Nishat said Sundari trees have been dying due to the adverse effect of Farakka and lack of sweet water. "There are now 85.67crore Sundari trees in the forest which have been on extinction." [24]

Industrial Pollution:

Oil or gas exploration, petroleum production and accidents by large oil tankers cause significant damage to mangrove ecosystems, causing defoliation of trees, the mortality of all

sessile and benthic organisms and contamination of many waterfowls with a minimum recovery period of 10years. In Indian Sundarbans, several industrial effluents are released into the adjacent coastal water bodies,these compounds hamper the circulatory system of the mangroves species such as H. fomes.[3]

Disease and Infestation:

Top dying of Sundari in the Sundarbans is considered to be the most important of all the diseases and disorder of tree. It has been estimated that about 45 million trees have been affected by the top dying in the Sundarbans.[25] The top-dying disease is believed to be caused by an array of factors viz., increased soil salinity due to reduced water flow, reduction in periodic inundation, excessive flooding, sedimentation, nutrient imbalances, pathogenic gall cankers, and cyclone-induced stress. When the salinity increases, the species becomes stunted, rare and ultimately disappears. Sundari affected by top dying, where the death of twigs and small branches gradually reduce the canopy and destroys the growth potential of such trees which may also suffer from the death of the top of the main stem and be truncated while the remaining portion of the main stem remains healthy. Moreover, Sundari trees are attacked by borers and wood decay fungi. A large number of dead Sundari trees are affected by root rot disease. In general, top dying Sundari trees have a dead and truncated top with accompanied death of twigs and branches to a varying degree leaving a variable extent of the healthy canopy.[3]

Causes of Top-Dying disease:

(i) increase in soil salinity, (ii) burial of pneumatophores, reduction in number (iii) deficiency of micronutrients and presence of high level of calcium, (iv) greater opening in the canopy, Loranthus infestations (v) Once top-dying starts several fungi degrade wood of the tree, and (vi) insect infestation of sapwood and wood decay fungi. Biological invasions are now considered one of the main threats to the world's biodiversity.[26] A July 2018 report revealed that "In the last 30 years, 1.44 million cubic meters of Sundari trees, worth 2,000 crores Bangladeshi Taka, have been lost to "top-dying disease" from the Bangladesh Sundarbans.[24]

Climate and weather effects:

The Sundarbans is already affected by climate change and extreme weather events such as tropical cyclones and storms. Mangrove forests protect all types of coastal communities from the fury of extreme weather events through their mere presence by

providing the best shelterbelt. Tropical cyclones and storms are more common in the Bay of Bengal, severely affecting the eastern coast as compared to that of the western coast. These cyclones with tremendous speed hit the coastline and inundate the shores with a strong tidal wave, severely destroying and disturbing coastal life.Thus, the protective role of mangroves depends on: (i) vegetation characteristics such as density, height, species composition, density of forest, diameter of mangrove roots and trunks, and elevation of habitats, as well as the status of ecological degradation of the forests; and, (ii) tsunami wave characteristics .[3]The mangrove has been struck off lately – by Cyclone Bulbul in November 2019 and Amphan in May 2020. These tropical cyclones like Amphan have led to huge catastrophic damages including loss of human lives, property and millions displaced from their homes as well as an unimaginable loss of natural resources of the Sundarbans.[27]

Illegal trading:

In the Bangladesh Sundarbans, illegal logging of Sundari trees continues unabatedly. "Before 1985, Sundari logs were sold openly, but in that year, in response to declining numbers, the species was protected, with the felling, sale and transportation of Sundari logs banned. It's now 31 years later and their sale is yet to cease" – the report stated.[27]

(Source:https://www.google.com/search?q=images)

Threats to this biodiversity and ecosystem:

During the past three decades, large parts of the remaining Sundarbans are protected for wildlife conservation, particularly tigers, through the creation of several sanctuaries and biosphere reserves.However, the biodiversity and basic fabric of

ecosystem functioning are being threatened due to several reasons like[1]: reclamation of the deltaic island for human use, deforestation, erosion and unwanted accretion, embankment (polder) erosion/waterlogging salinity invasion,[28] freshwater reduction from the north (eg. Farraka barrage),[1,25,29]overexploitation of natural resources, illegal shrimp fry collection, hunting and tree felling. Practices H. fomes is the single most important species of the Sundarbans, but the dominance of Heritiera forest is decreasingTop dying in Sundri (*Heritierafomes*) trees[25,29], construction of embankments,[30] ecotourism[31] pollution and sea-level rise, global climate change and heating[32]have aggravated the matter.

Biodiversity conservation:

As biodiversity conservation measure new habitats are being provided to the wildlife of the Sundarbansthrough mangrove plantations. At the same time parts of the mangrove forest and plantations are declared protected areas like wildlife sanctuaries, national park and ecologically critical areas.[24]to protect the diverse species of flora and fauna present here. To increase protective efficiencyof the mangrove differentmodelling studies are being carried out in the Indian Sundarbans, to find the optimum plantation width and the number of rows.[3]Both the Bangladesh as well asthe Indian government have been taking significant conservation stepsto preserve the magnificent biodiversity of the Sundarbans, yet the mangrove is slowly being destructed due to increasing global warming, drastic climate change, human encroachment of forest, man and animal conflict, illegal felling of trees including Sundari ,poaching of animals, rampant deforestation for personal gains etc. The World's most valuable mangrove which not onlyprovides food, water, other natural resources and occupation to millions for their livelihood for their sustenance but also shelters several species of plants, birds, reptiles, insects, animals, including the protected Royal Bengal tigers. Mangroveacts as a biological shield for its inhabitants andprotects the adjoining areas from the worst effects of cyclones and tsunamis.Yet, its sustainability is in question due to rampant pilferages of the valuable species found there.[33]

Conclusion:

Conservation programs are necessary to increase the supply of freshwater to the Sundarbans because various studies and satellite images have indicated towards fast extinction of *H.fomes*.If we don't stop now and act immediately, a time may come soon when

the majestic Sundari will no more be visible in the Sundarbansof both the countries and the Sundarbans will lose the essence of its name.

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BIOINFORMATICS ANALYSIS OF CANCER HEALTH EFFECTS OF ENDOSULFAN-AN ORGANOCHLORINE PESTICIDE

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Abstract:

There is a growing concern regarding the environmental exposure of pesticides with increasing cases of cancer. Several available *in vitro* studies support the link of pesticides with cancer disease. Many pesticides are declared carcinogenic and banned. Among pesticides, one lesser-known but widely used pesticide is organochlorine endosulfan. Humans are exposed to this contaminant directly through the farm or indirectly via food crops. It causes several health effects to the human-like endocrine disruption, infertility, immune system disturbances, and premature abortion however its carcinogenic nature is not widely reported. In this context, we selected the putative genes involved in several cancer diseases from a comparative toxicogenomics database. Further, pathway analysis was done for genes involved in carcinoma hepatocellular using PANTHER software. An initial database search shows the role of endosulfan in 325 different cancer diseases. Among them, the top 5 diseases were prostatic neoplasm, carcinoma hepatocellular, breast neoplasm, lung neoplasm, and stomach neoplasm. Pathway analysis for carcinoma hepatocellular shows the involvement of 62 pathways in which the most upregulated pathways were, CCKR signaling pathway, gonadotropin-releasing hormone receptor pathway, inflammation, apoptosis, and angiogenesis pathway. In addition, we also analyzed the genes present in most upregulated pathways and found the involvement of the Fos gene. In conclusion, this study shed the light on various molecular pathways of hepatoma carcinoma influenced by endosulfan and imparts information about possible genes or pathways which might be targeted to control. It also highlights the importance of in silico approach to interpret the toxicological impacts of harmful chemicals at the molecular level.

Keywords: Endosulfan, Cancer, comparative toxicogenomics database, PANTHER, Fos

Introduction:

A group of chlorinated chemicals used as pesticides comes under the class of organochlorine pesticides. These chemicals are very persistent due to their very long half-life and classified as persistent organic pollutants (POPs). Mostly organochlorine pesticides are insecticides used efficaciously to control the insect in food crops or no food crops and also to control malaria and typhus in African countries (Aktar *et al.,* 2009). However, now most of the insecticides are banned due to their off-site impacts and persistency. But still, reported data on the worldwide use of pesticides shows that among total used pesticides, 40% pesticide is organochlorine (Gupta, 2004; FAO, 2005). Further, these organochlorine pesticides such as DDT, hexachlorocyclohexane (HCH), aldrin, dieldrin, and endosulfan are very popular in developing countries of Asia as these are very cheap and have broad specificity against various pests and insects (Gupta, 2004; FAO, 2005; Lallas, 2001). In context to India, the first pesticide imported and used was DDT for the control of malaria in 1948. Later, the production of these substances in India started in 1952 (Goeland Aggarwal 2007).

Currently, 234 pesticides are registered in India. Out of these, 4 are WHO Class Ia pesticides, 15 are WHO Class Ib pesticides, and 76 are WHO Class II pesticides, together constituting 40% of the registered pesticides in India. In terms of consumption too, the greatest volumes consumed are of these poisons. As per official data of the Directorate of Plant Protection, Quarantine and Storage, Govt of India, endosulfan were most dominantly consumed organochlorine insecticide with 15537 tonnes during 2005-06 to 2009-10). This insecticide has a long half-life and persists in the soil for years after its application. Endosulfan passes to the human directly during its application and through farm visits and indirectly through food crop (Briz, 2011). It is recognized as an endocrine disruptor, neurotoxin, and immune suppressor (USEPA, 2010). However, its carcinogenic nature is inconclusive. Though, increased cancer risk among pesticide applicators and nearby people is well known (Alavanja *et al.,* 2013). Still, pinpointing cancer-causing environmental contaminants or pesticide is a very laborious, time-consuming, and challenging process. Though, in the modern era, several animal-free computational system analysis approaches are available which can predict the toxicity of the chemicals. Approaches like New methodology philosophies (NAMs) that incorporate computational models, *in vitro* high throughput screening (HTS), omics considers (Hartung *et al.,* 2017), and the recently proposed 3S methodology (orderly, systemic, and frameworks science and toxicology) (Smirnova *et al.,* 2018). NAMs guarantee to predict the impact of synthetic compounds in people and may assist with supporting substance wellbeing evaluation (Andersen *et al.,*

2019). In this perspective, the present work is intended to explore the pathways involved in the cancer health effects of endosulfan with the help of a comparative toxicogenomics database (CTD) and PANTHER software to boost more research in this area and aware the public for pesticides exposure and their health effects.

Material and Methods:

A schematic representation of the work plan followed to perform this study is given in the form of a flowchart in Figure-1. Initially, a comparative toxicogenomics database (CTD) was assessed and already available genes involved in endosulfan's cancer health effects were retrieved. CTD is a publically available scientific database that contains all information related to environmental chemical's exposure routes, and their health effects in different species all affecting genes and predicted diseases based on experimental studies. Based on the database, we selected all genes affected by endosulfan toxicity in the top 10 cancer diseases. Further, we studied hepatoma carcinoma genes for pathway analysis in PANTHER software under functional classification viewed in genes list selecting Homo sapiens species. Moreover, statistical overrepresentation test analysis was also done. From this, the top 5 pathways were selected for gene analysis. Molecular function, biological process, and the protein encoded by these genes were also evaluated.

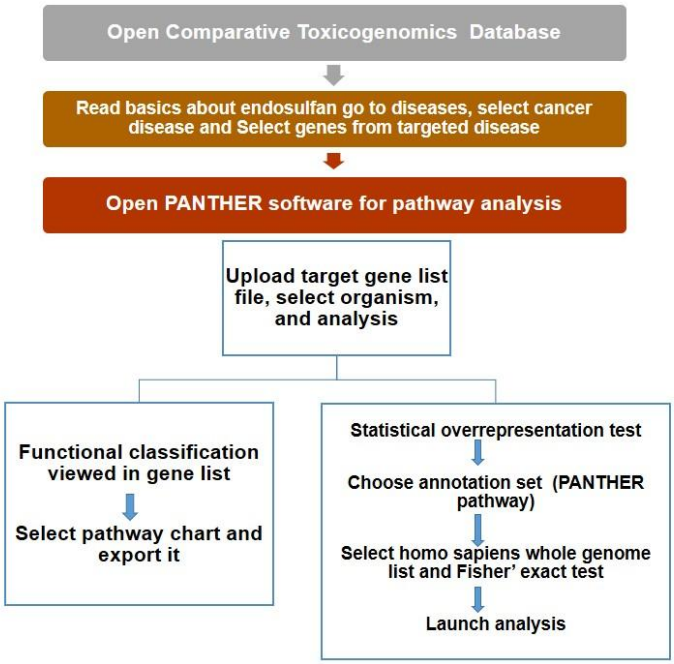

Figure 1: Flowchart of the Insilico approach to analyze pathways involved in the cancer health effects of endosulfan

Results:

Table 1: Genes involved in a cancer health effects of endosulfan retrieved from the comparative toxicogenomics database

Sr. No.	Diseases	Genes
1	**Prostatic neoplasm**	ABCC4 | ACHE | ACSM3 | ADAM28 | ADAM9 | ADRB2 | AHR | AKAP13 | AKT1 | ALOX12B | ALOX5 |ALOXE3 | ANXA1 | ANXA3 | APPL2 | AR | ARHGEF5 | ATF3 | ATM | ATP7B | BAD | BAX | BCL2 | BIRC5 | BNIP3 | BRAF | BRCA1 | BRCA2 | BRPF1 | CALR | CASP9 | CBR1 | CCND1 | CD276 | CDH1 | CDH12 | CDK2AP2 | CDKN1A | CENPF | CHD6 | CHEK2 | CHST14 | CLDN3 | COL5A1 | COL5A3 | COMT | CPNE3 | CREG1 | CRYAB | CRYL1 | CST6 | CTNNB1 |CTSB | CX3CL1 | CXCL12 | CXCL8 | CYP11B2 | CYP17A1 | CYP19A1 | CYP1A1 | CYP2A6 | CYP2E1 |CYP3A4 | CYP3A5 | CYP7B1 | DAG1 | DCXR | DEFB1 | DNAJC3 | DNMT1 | DNMT3B |EGR1 | EHHADH | EI24 | EMP1 | EMP3 | EPHX1 | ERBB2 | ERBB3 | ERG | ESR1 | ESR2 | ETV4 | EZH2 | FBLN1| FGFR4 | FOLH1 | FOXO1 | GADD45A | GALNT3 | GDF15 | GGT1 | GHR | GJA1 | GNG5 | GNMT | GRB7 | GREB1 | GSK3B | GSTK1 | GSTM1 | GSTM3 | GSTP1 | GSTT3 | HAO1 | HDAC6 | HERPUD1 | HMGN5 | HMOX1 | HNRNPH1 | HPN | HSD17B1 | HSD3B1 | HSP90B1 | HSPA1A | ICAM1 | IDH1 | IGF1 | IGFBP6 | IGFBP7 | IGSF5 | IL17RC | IL17RD | IL1RN
2	**Hepatoma cellular carcinoma**	A2M | AADAT | ABCB1 | ACACA | ACLY | ACTB | ADAMTS1 | ADD1 | ADH4 | ADRA1A | ANGPTL6 | AR |ASF1B | ASPG | ASPM | ATM | BCL2L1 | BCO2 | BID | BIRC5 | BMPER | BRAF | BTG2 | CASP8 | CBR1 | CCL3 | CCN1 | CCND1 | CCNF | CCR1 | CD274 | CD276 | CD34 | CDC25C | CDC6 | CDCA2 | CDCA3 | CDCA5 | CDK1 | CDK14 | CDKN3 | CDT1 | CEBPA |CEBPB|CENPA|CENPF|CEP55|CFP|CHAF1B |COMT | CP | CRP | CSMD1 | CSPG4 | CSRNP1 | CTNNB1 | CXCL12 | CXCL8 | CYP17A1 | CYP1A1 |CYP1A2 | CYP2B6 | CYP2E1 | CYP39A1 | DNASE1L3 | DTL | E2F1 | ECT2 | EGR1 | EGR2 | EHD3 |EPHX1 | ESM1 | ESR1 | EXO1 | EZH2 | FABP5 | FAM111B | FAM180A | FANCD2 | FASN | FBP1 | FDFT1 | FGF4 | FOS | FOSB | FOXM1 | FST | GDF15 | GHR | GJB1 | GLUL | GMNN | GNMT | GPM6A | GSTM1 |GSTP1 | GYS2 | HAMP | HAO2 | HJURP | HMGCR | HOXA10 | HSPA5 | HSPA9 | HSPB1 | IDH1 | IGF1 | IGFALS | IL1RN | IL6 | INPP4B | IQGAP2 | IQGAP3 | IRS2 | ISG15 | JUN | KBTBD11 | KCNN2 | KDM8 |KIF23 | KIFC1 | KMT2A | LRRC1 | LRRC59 | MAPT | MAT1A | MBTPS1 | MBTPS2 | MCM2 | ME1 | MFSD2A | MKI67 | MMP9 | MT2A | MVK | MYC | NDC80 | NEIL3 | NFE2L2 | NFKBIA | NR1H4 | NUF2 | NUSAP1 | OLFML2A | OLFML2B | ORC1 | PAMR1 | PCK1 | PDGFB | PDGFRL | PDIA3 | PHLDA1 | PIK3CA |PKM | PKMYT1 | PKP1 | PLXDC1 | PPARG | PPP1R1A | PRDX1 | PRDX6 | PTEN | PTGS2 | PTH1R | PTK2 | PYCARD | PYGL | RARA | RB1 | RCAN1 | RNF157 | RTP3 | SCD | SFN | SKP2 | SLC22A1 | SLC22A10 | SLC25A47 | SLC2A1 | SLC2A2 | SOD2 | SREBF1 | SRPX | STAT1 | TACC3 | TAGLN2 | TALDO1 | TEDC2 |TERT | TFPI2 | TGFB1 | THY1 | TK1 | TLR7 | TMEM70 | TNF | TNFSF10 | TONSL | TP53 | TTC36 | TYMS | UBD | UBE2T | UCHL1 | UROC1 | USP2 | VASN | VCAM1 | WDR76 | ZWINT

3	**Breast neoplasm**	ABCB1 | ABCG2 | ACHE | ACTA2 | ADAMTS1 | ADAR | AHR | AKAP12 | AKT1 | ALDOA | ANGPTL4 | APOBEC3B | AR | ARHGDIA | ARRDC3 | ATG10 | ATM | ATP6AP1L | ATP7B | B4GAT1 | BAX | BCAR3 | BCHE |BCL2 | BCL2A1 | BIRC5 | BMP2 | BMP4 | BRCA1 | BRCA2 | CADM1 | CASP7 | CASP8 | CAT | CCL20 | CCND1| CD40 | CDH1 | CDH2 | CDH5 | CENPF | CHEK2 | CLDN1 | CLDN4 | COMT | COTL1 | CSF1 | CSF2 | CSF3 |CST6 | CTNNB1 | CXCL12 | CXCL2 | CXCL8 | CXCR4 | CYP17A1 | CYP19A1 | CYP1A1 | CYP2B1 | CYP3A4 | DDIT3 | DEPP1 | DES | DHFR | DNMT1 | DNMT3A | DNMT3B | DSC3 | DYNC2H1 | E2F1 | EDNRB | EEF2 |EFEMP1 | EFNA1 | ELK3 | EPHB4 | EPOR | ERBB2 | ERBB3 | ESR1 | ESR2 | ESRRA | ETV4 | EVL | EXO1 | EZH2 | F3 | FASN | FGF4 | FGFR2 | FHL2 | N1 | FOS | FOXM1 | FST | GJA1 | GPER1 | GPNMB | GPX1 | GPX2 | GRB7 | GSTP1 | GUCY1A2 | H12 | H6PD | HADHB | HEY2 | HMOX1 | HNRNPL | HRG | IGF1 | IGFBP5 | IGFBP7 | | IL6 | JAG1 | JUN | KCNH1 | KIT | KRT14 | LDHB | LEF1 | LLGL2 | LOXL2 | LPAR1 | MAL | MALAT1 | MAP2K7 | MAP3K1 | MDM2 | MECOM | MIR132 |MKI67 | MME | MMP1 | MMP14 | MMP3 | MMP9 | MTR | NCOA1 | NDRG1 | NECTIN2 | NFE2L2 | NFKBIA | NOS2 | NOS3 | NQO1 | NQO2 | NR2F1 | NR2F6 | NRCAM | NRG1 | NUDT17 | OCLN | PDE2A | PER3 | PGR | PHGDH | PIK3CA | PIM1 | PLEKHD1 | PPARGC1B | PRSS46 | PTEN | PTGS1 | PTGS2 | PTPRD | RAD51 | RAF1 | RARA | RARB | RB1 | RELA | RGS2 | RIBC2 | RPLP2 | RXRB | SERPINB2 | SETBP1 | SHMT1 | SLC10A6 | SLC16A3 | SLC2A1 | SLC2A10 | SLC2A2 | SLCO1B1 | SNAI1 | SNAI2 | SNX32 | SOD2 | SPP1 | SRC | STAT3 | STC2 | STXBP4 | SULT1A1 | SYNJ2 | TANK | TBX3 | TERT | TFPI2 | TGM2 | TNF | TNFSF10 | TNIP1 | TOX3 |TP53 | TRIM47 | TUBB3 | TYMS | UBD | VDR | VEGFB | VIM | WT1 | XRCC2 | XRCC3 | ZEB2
4	**Colorectal Neoplasms**	ABCA1 | ABCA4 | ABCA6 | ABCA8 | ABCB1 | ABCC2 | ABCC3 | ABCC4 | ABCC6 | ABCC8 | ABCD2 | ABCD3 | ABCG1 | ABCG2 | ACHE | ACKR3 | ADH1B | AKT1 | ATP7B | BAX | BCL2 | BIRC5 | BMP2 | BMP4 | BRAF | CABLES1 | CALR | CASP8 | CCND1 | CDH1 | CDH5 | CHEK2 | CNPPD1 | CSF2 | CTNNB1 | CXCL8 | CYP1A2 | CYP2A6 | DCLK1 | DHFR | DLC1 | EFEMP1 | EPHA1 | ESR2 | EVL | EXO1 | EYA4 | FADS1 | FADS2 | FBN2 | FEN1 | FGFR3 | FHL3 | FLCN | FLNC | FOXL2 | GNB5 | GPNMB | GSTM1 | GSTM3 | GUCY1A2 |GUCY2C | KLF2 | KLF5 | LPAR1 | MCC | MKI67 | MLH1 | MMP1 | MYC | NAMPT | NME2 | NQO1 | RCAM | NUSAP1 | PAX8 | PDGFRL | PIK3CA | PMM2 | POLD3 | PON1 | PPARG | PROM1 | PTGS2 | PTPRD | PTPRJ | PYCARD | RASL11A | RET | RHPN2 | SATB2 | SELENBP1 | SESN2 | SFRP4 | SLCO2A1 | SMAD7 | SOD2 | SOX17 | SPARC | SRC | SULT2B1 | TFPI2 | TGFB1 | TK1 | TLR2 | TNF | TNFSF10 | TNFSF13 | TP53 | TXNRD1 | TYMS | WIF1 | XAF1 | ZFP36L2
5	**Stomach Neoplasms**	ACTC1 | ADRB1 | ADRB2 | AHR | ALB | ALDOB | ALOX5 | ATM | ATP6V0D2 | BCL2L1 | BDNF | BID | BIRC5 | BLVRB | BMP2 | BNIP3 | BOP1 | CASP8 | CCND1 | CD44 | CDH1 | CDH2 | CDKN1A | CHEK2 | CHFR | CKB | CLDN3 | CLN3 | CTNNA2 | CTSC | CTSL | CXCL8 | CYP2A6 | DES | DLC1 | DNMT1 | DNMT3B | ERBB2 | ERCC1 | FADS1 | FAT4 | FBP1 | FCGBP | FGFR2 | FGG | FST | GADD45A | GSTP1 | HMOX1 | HNRNPL | HSPA8 | HSPB1 | ICAM2 | ID4 | IGFBP7 | IL1B | IL1RN | IL6 | IRS2 |ITGA5 | ITGA8 | JUN | KMT2A | LGALS3 | MAPK1 | MAPK3 | MDM2 | MMP7 | MRPS18B | MT2A | MTSS1 | MUC1 | MX1 | MYC | NOS3 | NT5E | PIK3CA | PLCE1 | PPARG | PRDX5 | PTGS2 | PTOV1 | PTPRF | PTPRG | PYCARD | RAD23A | RARB | RARRES1 | REG4 | RGS2 | RHOA | RORA | RPL18 | RRP9 | RXRB | SCRN1 | SELENBP1 | SERPINB2 | SERPINE1 | SLC16A3 | SNAI1 | SOD2 | STAT3 | SYMPK | TBX3 | TFAP2C | THBD | TIMP3 | TNF | TNFRSF9 | TNFSF9 | TP53 | TWIST1 | TYMS | WIF1 | XAF1 | XRCC3

Extraction of specific data from comparative toxicogenomics database:

General toxicity analysis of endosulfan from CTD shows that it interacts with 5274 different kinds of genes in the various species. The top 10 interacting genes were ESR-1, CYP3A4, ABCB1, MAPK-1, CYP2B6, NR1I2, CAT, PGR, and CASP-3. Further, the effect of endosulfan on disease status displays that it involves in 2913 different types of diseases. Among all diseases, we targeted cancer disease considering the inconclusive carcinogen nature of endosulfan and found involvement of endosulfan in 325 different types of cancer diseases. The top 5 cancer diseases based on inference score were prostatic neoplasm, carcinoma hepatocellular, breast neoplasm, lung neoplasm, and stomach neoplasm. Genes for Top 5 cancer diseases are given in Table 1. Among all cancer diseases, we specifically targeted carcinoma hepatocellular for pathway analysis.

Analysis from Protein Analysis Through Evolutionary Relationships (PANTHER) Classification system:

Pathway analysis by gene list analysis:

Based on genes extracted from a comparative database, pathways regulated by these genes were analyzed by the use of the PANTHER tool. In carcinoma hepatocellular total of 218 genes were affected by endosulfan which hits 63 total pathways. The top 5 upregulated pathways were the CCKR signaling pathway, gonadotropin-releasing hormone pathway, Inflammation mediated by chemokine and cytokine signaling pathway, apoptosis signaling pathway, and angiogenesis pathway. Results are shown in Figure 2. At the cellular level, most of the genes were involved in forming cellular anatomical entity while at the molecular level most of the genes were involved in binding and catalytic activity (Figure 3A and B). Moreover, biological process analysis shows that endosulfan affects 14 different processes in the pathophysiology of carcinoma hepatocellular among them highly affected processes were cellular process and biological regulation (Figure 3C). Protein classes encoded by target genes were also analyzed. The finding shows that a total of 218 genes were encoding the 18 classes of protein in which Scaffold/ adaptor protein was highest (Figure 3D). Further, we evaluated the genes specifically include in the top 5 upregulated pathways. In the CCKR signaling pathway, 27 genes were found and all expressed equally (Figure 4). In the gonadotropin releasing hormone receptor pathway, 27 genes were found among them Fos gene was the most upregulated gene while in inflammatory chemokine and cytokine signaling pathway highly affected genes were IL-8 and CCL-3 (Figure 5 and Figure 6). Similarly, in the apoptosis signaling pathway, 11 genes were involved in which

the highly upregulated gene was Fos while in the angiogenesis pathway a11 genes were included in which C-Fos was the most upregulated gene (Figure 7 and Figure 8).

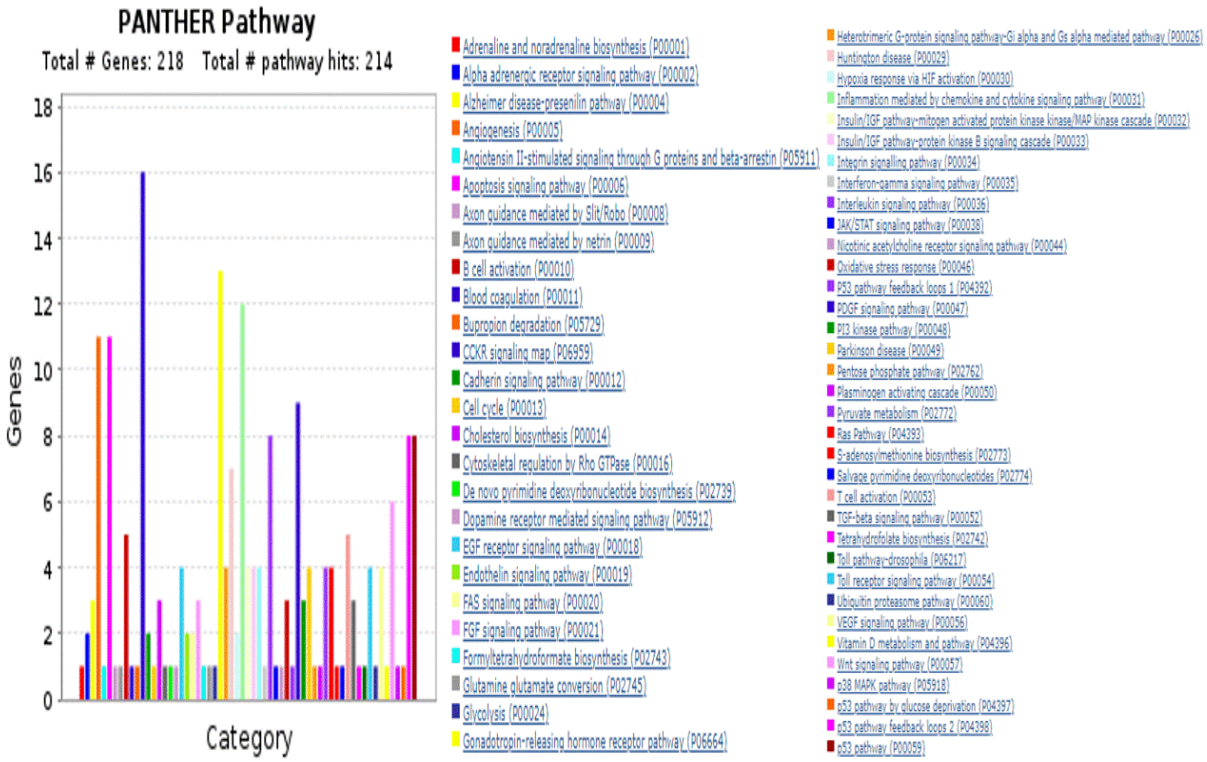

Figure 2: Gene list test analysis of genes involved in Carcinoma hepatocellular by PANTHER pathway for total pathways hits by selected genes

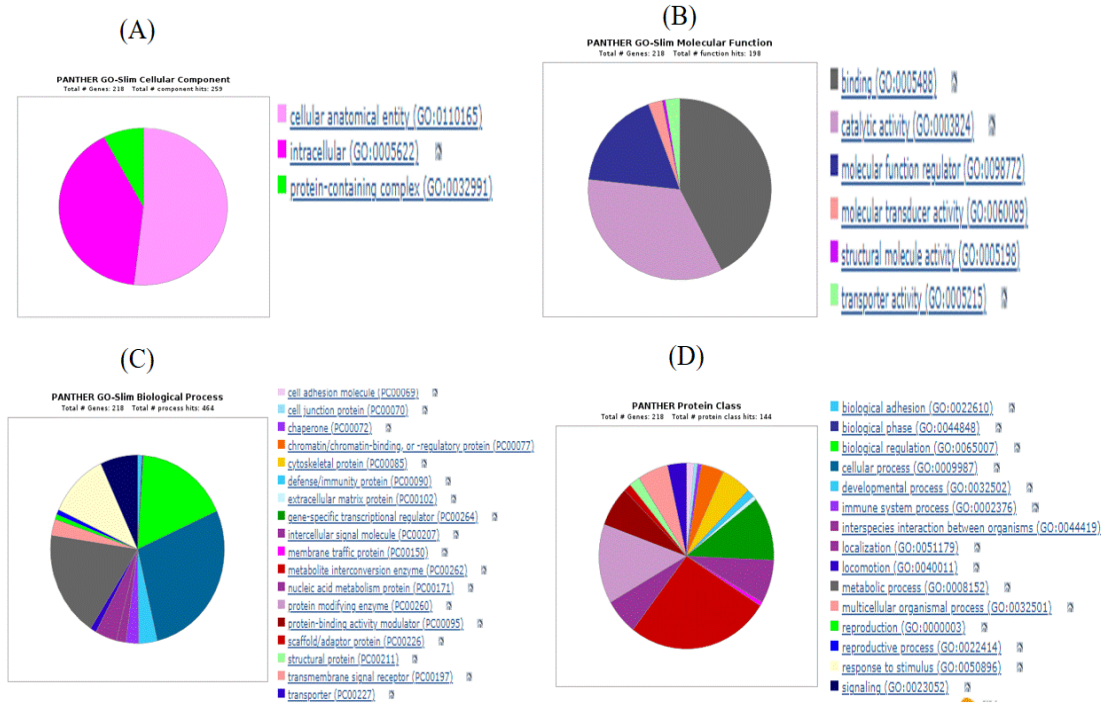

Figure 3: Go slim analysis of genes for different functions. (A) Cellular component (B) Molecular function (c) Biological processes (D) Panther Protein

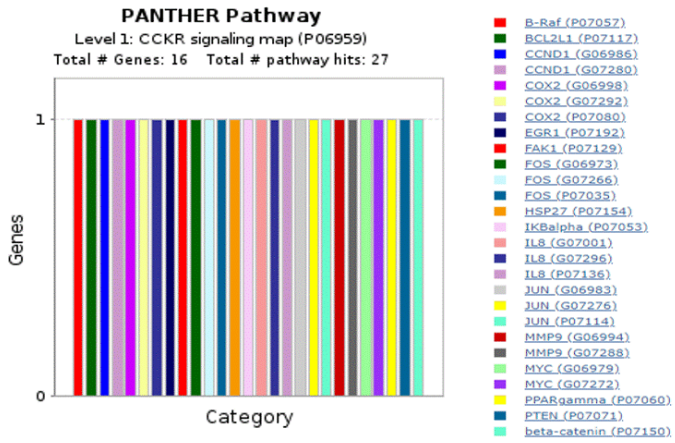

Figure 4: Statistical overrepresentation test analysis of genes involved in CCKR signaling pathway

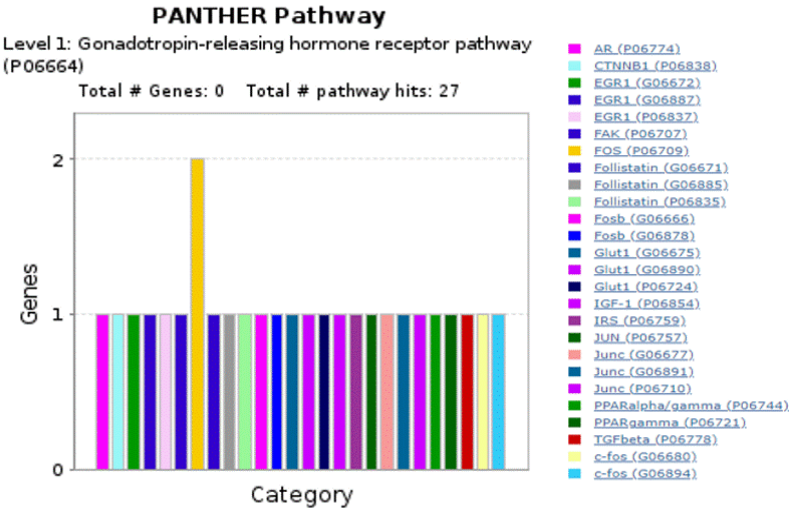

Figure 5: Statistical overrepresentation test analysis of genes involved in gonadotropin hormone-releasing pathway

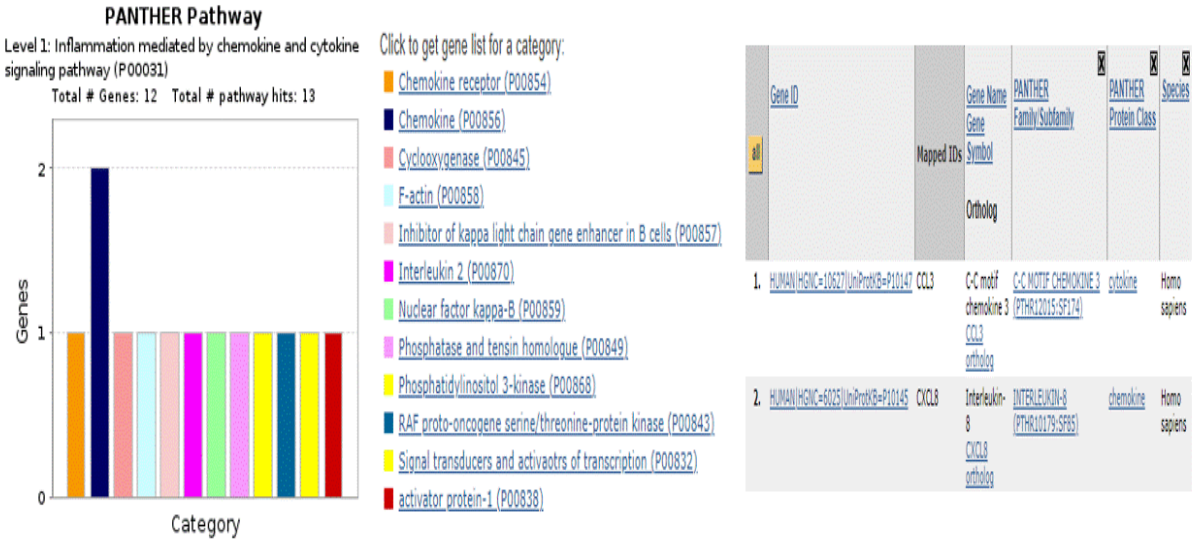

Figure 6: Statistical overrepresentation test analysis of genes involved in inflammation-mediated by chemokine and cytokine signaling pathway

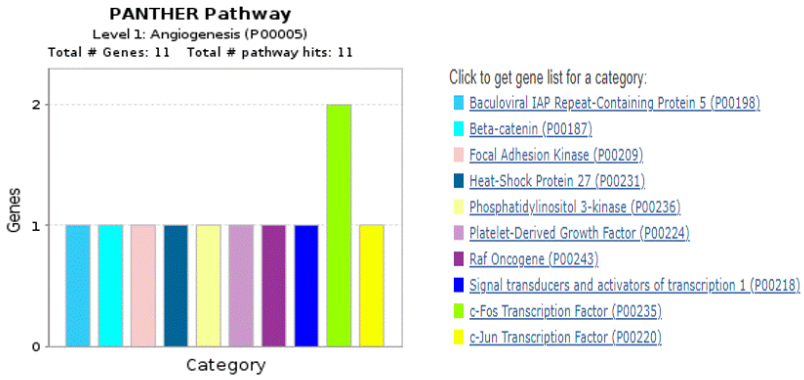

Figure 7: Statistical overrepresentation test analysis of genes involved in Angiogenesis pathway

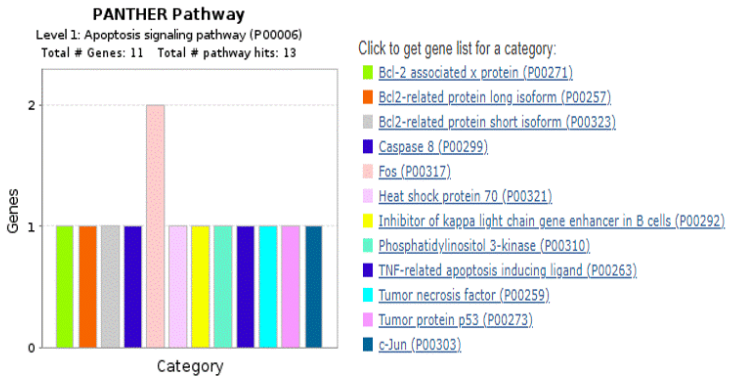

Figure 8: Statistical overrepresentation test analysis of genes involved in the apoptosis pathway

Functional annotation of target genes for expected significant pathways:

Functional annotation of endosulfan-affected target genes in carcinoma hepatocellular shows that 211 genes hit the 17 pathways. Based on fold change, highly upregulated pathways were pyruvate metabolism pathway (34.35 folds), cholesterol biosynthesis pathway (21.8folds), P53 pathway feedback loops-2 (14.82 folds), Insulin/IGF pathway (11.4 folds) and apoptosis signaling pathway (8.81) folds. Whereas based on inference top 5 expected pathways were the Inflammation pathway (2.7), gonadotropin-releasing pathway (2.4), Angiogenesis (1.85), CCKR signaling pathway (1.82), and apoptosis pathway (1.25). Besides, significant pathways were also selected based on FDR value in which the top 5 significant pathways were angiogenesis, CCKR signaling pathway, Insulin pathway, B-cell activation pathway, and Huntington pathway. Results are shown in Table 2.

Table 2: Functional annotation of genes affected by endosulfan toxicity in hepatic cellular carcinoma

	Reference list	Client Text Box Input
Uniquely Mapped IDS:	20595 out of 20595	211 out of 218
Unmapped IDs:	0	0
Multiple mapping information:	0	7

	Homo sapiens (REF)	Client Text Box Input					
PANTHER Pathways	#	#	expected	Fold Enrichment	+/-	raw P value	FDR
Pyruvate metabolism	11	4	.12	34.35	+	1.46E-05	2.71E-04
Cholesterol biosynthesis	13	3	.14	21.80	+	5.74E-04	7.99E-03
p53 pathway feedback loops 2	51	8	.54	14.82	+	1.79E-07	7.45E-06
Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	33	4	.35	11.45	+	5.90E-04	7.58E-03
Insulin/IGF pathway-protein kinase B signaling cascade	39	4	.41	9.69	+	1.05E-03	1.25E-02
Apoptosis signaling pathway	118	11	1.25	8.81	+	1.17E-07	6.50E-06
CCKR signaling map	172	16	1.82	8.79	+	1.49E-10	1.25E-08
Interleukin signaling pathway	86	8	.91	8.79	+	6.55E-06	1.56E-04
p53 pathway	89	8	.94	8.49	+	8.28E-06	1.73E-04
B cell activation	70	5	.74	6.75	+	1.15E-03	1.28E-02
Toll receptor signaling pathway	58	4	.61	6.52	+	4.06E-03	3.77E-02
Angiogenesis	175	11	1.85	5.94	+	4.45E-06	1.24E-04
PDGF signaling pathway	147	9	1.56	5.78	+	4.05E-05	6.16E-04
T cell activation	86	5	.91	5.49	+	2.70E-03	2.65E-02
Gonadotropin-releasing hormone receptor pathway	231	13	2.45	5.32	+	1.92E-06	6.42E-05
Huntington disease	148	7	1.57	4.47	+	1.26E-03	1.32E-02
Inflammation mediated by chemokine and cytokine signaling pathway	255	12	2.70	4.45	+	2.65E-05	4.42E-04
Unclassified	17977	149	190.29	.78	-	4.48E-13	7.48E-11

Discussion:

To our best knowledge, the current study is the first study that assessed the cancer health effect of endosulfan pesticide at the molecular level using an in silico approach. Cancer health effects of endosulfan are specifically studied in hepatocellular carcinoma (HCC). It is a type of Liver cancer that primarily occurs in adults and constitutes the main liver tumor. Among all human cancers, it ranked fifth. The liver is the primary organ that metabolized the chemicals/contaminant and is consequently most affected by toxic metabolites (Ledda *et al.,* 2017). In this context, the cancer health effects of endosulfan were studied in hepatoma cellular carcinoma. Coming to the toxic endosulfan, it is classified under the insecticide organochlorine class of pesticide. It is banned in about 55 countries, yet present in nature under its high soil affinity (Qian *et al.,* 2017). Even in India, it is banned in Kerela state after health damaged was observed in endosulfan applicator farmers (Chlatonand Sridhar, 2006).

For the present study, robust CTD was accessed and definite information about the effect of endosulfan on cancer diseases was extracted. This database is very useful for high throughput studies, provides information about chemical-gene or protein interactions and chemical phenotype relationships in several organisms (Mattingly *et al.,* 2006). Genes for Top 10 cancer diseases were retrieved based on inference score and the total number of

genes affected. Further, this CTD information was used for the development of a biological functional pathway to understanding the toxicity mechanism of endosulfan in HCC with the help of PANTHER software. The PANTHER (**P**rotein **AN**alysis **TH**rough **E**volutionary **R**elationships) is a high-throughput analysis system that classified proteins and their encoding genes into several functions (Muruganujan and Thomas, 2012). In addition, functional annotation of genes was also done. Similarly, in a different study, ToxCast and CompTox databases existing information was used to analyze chemical toxicity. (Richard *et al.,* 2016; Williams *et al.,* 2017). Our finding shows that CCKR and angiogenesis pathways are the most affected pathways in HCC in response to endosulfan exposure. Furthermore, the Fos gene was found potential candidate gene in HCC. Our results are well supported by a recent study where an integrated bioinformatics approach was used to select a potential target gene in liver cancer. Comparative profiling of miRNA and mRNA was done in liver tumors and normal samples using The Cancer Genome Atlas (TCGA) database and 405 differentially expressed mRNA and 223 differentially expressed miRNA were found. Moreover, pathway analysis was also performed and mitogen-activated protein kinases (MAPKs) and G-protein coupled receptor (GPCR) pathway found significantly affected whereas FOS-1 found a significantly affected gene (Hu *et al.,* 2020). In another precedent study Peyre *et al. (*2014) studied the effect of xenobiotics (endosulfan, dioxin, carbaryl, carbendazim, p'p'DDE, and hydroquinone) exposure on cancer cell progression. Under *in vitro* conditions, they found that selected xenobiotics disturb the hepatic homeostasis modulating the cellular process of HepG-2 cells. Among all tested xenobiotics, endosulfan had more carcinogenic potential. Further, in our study angiogenesis pathway was most significantly upregulated. It is supported by the role of angiogenesis in the formation of new blood vessels which supply the cancer cells progression (Yadav *et al.,* 2015). Vascular endothelial growth factor (VEGF) is the most prominent among the angiogenic cytokines and is believed to play a central role in the process of neovascularization in cancer (Kieran *et al.,* 2012). VEGF signaling pathway is already a targeted therapeutic pathway in various cancer diseases (Stacker and Achen, 2013).

Additionally, upregulation of the Fos gene in cancerous tissue is supported by previous studies. Gene C-Fos is a member of Fos family which dimerize with Jun protein to form transcription factor and regulate the growth of the cells (Tulchinsky, 2000). Similarly, Muhammad *et al.* (2017) found involvement of C-FOS in the promotion of cancer increasing cell migration and VEGF expression. VEGF expression also supports the upregulation of the angiogenesis pathway. Besides, cell migration is positively correlated with cancer

metastasis (Friedl and Wolf, 2003). Overall, the present study imparts light on the intact pathways of HCC altered by endosulfan toxicity by the use of a bioinformatics tool. Therefore, we can conclude that the use of innovative informatics technology is not only useful for predicting toxicity it also allows the identification of potential targets, systemically, from already known and published information. However, the use of only bioinformatics tools is not enough to state the toxicity of chemicals and their prospective targets. In-depth, confirmation of present findings under *in-vitro* and *in-vivo* milieus is also required. In the future, more studies are necessary to establish the precise mechanism of endosulfan toxicity in cancer diseases.

Conclusion:

The current study provides a unique in silico approach that can be used to evaluate the toxicity of chemicals in targeted disease. It is a very helpful animal-free method that can give insights into the unknown pathways regulated by the chemical. Though it cannot replace the importance of *in vitro* or *in vivo* testing for chemical toxicity analysis. But it can give specific genes, pathways, and functions which can be targeted for wet-lab studies. From this study, we know angiogenesis pathway and Fos gene are most affected by endosulfan toxicity in HCC which can be focused to know endosulfan or similar chemical toxicity.

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A REVIEW ON ANTIMICROBIAL AGENT BACTERIOCIN: FROM LACTIC ACID BACTERIA (LAB)

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Abstract:

Bacteriocin is the proteinaceous agent produced by Lactic acid bacteria (LAB) Bacteriocin has wide range of antimicrobial activity which makes it important as food preservative, therapeutic agent. Bacteriocins were classified in several classes amongst which class I and class II were well studied. Increasing awareness of the consumers regarding diet and health and growing research regarding LAB enhance its importance as probiotic and a therapeutic agent

Keywords: LAB, Bacteriocin, antimicrobial activity, food preservative, therapeutic agent

Introduction:

Bacteriocins are the proteins possessing the antimicrobial activity. A great number of Gram negative and Gram positive produced bacteriocins (Todorov and Dicks, 2006).The antimicrobial activity of bacteriocin is restricted to the strains of species similar to the producing species (Perez *et al.,* 2014). A wide range of food grade lactic acid bacteria ribosomally synthesized the heat stable bacteriocins which have enormous prospective as food preservative and also targeting multidrug resistant pathogens (Perez *et al.,* 2014).

But an enormous range of bacteriocins which have wide range of antimicrobial activity are also recorded. LAB bacteriocins are naturally tolerant to high thermal stress and pH change over a wide range .Due to number of characteristics like these peptides are colorless, tasteless bacteriocins can be used as antimicrobial agents. In the history of bacteriocin there were no reports showing bacteriocin resistance developed by the target bacteria; indeed even at very low concentration bacteriocins promotes pore formation in the

membrane of target bacteria. However, the proteinaceous bacteriocins can be degraded by proteiolytic enzymes in human body which reduces probability of target bacteria to interact with the degraded fragments whichis the prime basis of development of antibiotic resistance. Bacteriocins are the primary metabolite with simple mechanism of biosynthesis as compared with the antibiotics, hence bacteriocins can be easily submissive by bioengineering to enhance their activity against the target microorganisms.

Lactic acid bacteria bacteriocins are normally considered as food grade as lactic acidbacteria are associated with food fermentation from ancient time.

Lactic acid bacteria (LAB):

Lactic acid bacteria (LAB) are the Gram-positive, non-spore forming rods, cocci and coccobacilli with a DNA composition of less than 35% G+C., non aerobic aerotolerent and capable to fermenting carbohydrates as energy source with lactic acid production (Zacharof and Lovitt, 2012). Although lactic acid can be produced as primary and secondary end product of fermentation by several genera of bacteria, LAB also produce diacetyl, hydrogen peroxide, organic acids and bacteriocin or bacteriocidal proteins during lactic acid fermentation beside lactic acid (Oyetayo *et al.,* 2003; Rodríguez *et al.,* 2003; Holzapfel *et al.,* 2001; Hirano *et al*., 2003). Lactic acid bacteria includes major genera *Bifidobacterium, Enterococcus, Lactobacillus, Streptococcus, Pediococcus, Leucinostoc, Lactosphara, Lactococcus, Melissococcus, Aerococcus, Propionobacterium and Microbacterium*. LAB represent highest % of bacteria that produce probiotic properties (Carr *et al.,* 2002; Metchnikoff, 1908). Amongst the intestinal commensal *Lactobacillus*, *Bifidobacterium* are the predominant members which are commonly studied as probiotic bacteria (Kawai *et al.,* 2001).

Bacteriocins Classification:

The majority of LAB bacteriocins are small (<10 kDa) heat-stable, amphiphilic membranepermeabilizing cationic peptides. The LAB bacteriocins are classified into three major classes, viz. Lantibiotics, Non- lantibiotics and Bacteriocins. However their classification wascontinually revised during the last decade due to the extensive research realized (Rodriguez, 2000; Chen and Hoover, 2003).Most of the bacteriocins shows relatively modest adsorption specificity. The cell wall of Gram positive (+) bacteria allows passage of relatively large molecules. The anionic cell surface polymers like teichoic and lipoteichoic acids, which are part of the cellular wall, are important in the initial

interaction of anionic bacteriocins produced by Gram positive (+) bacteria. Adsorption of lactic Acid Bacteria bacteriocins to it surface and other Gram positive bacteria is pH dependent. It shows significant antimicrobial activity lower pH (pH< 5). There might be amino acid sequence homologies amongst the mature peptides, N- terminal leader regions of the peptide as well as the proteins associated in secretion and processing of bacteriocin amongst the classes of bacteriocins (Cleeveland, 2001).

Class I: Lantibiotics:

The Class-I lantibiotics, are a class of antimicrobial peptides which comprise of unusual polycyclic thioether amino acids and unsaturated amino acids like lanthionine (Lan) or methyllanthionine (MeLan) and dehydroalanine and 2-aminoisobutyric acid respectively. Lantibiotics are further categorized in two types; Type A includes screw shaped, expanded, cationic, amphipatic, flexible molecules with molecular mass between 2 to 4 kDa. Mode of action of lantibiotics is membrane depolarization and pore formation in the membrane of sensitive species. Nisin and lacticin 3147 are the major representatives of this group; nisin produced by *Lactococcus lactis*, active against Gram positive bacteria, such as LAB, *Listeria SP*, *Micrococos SP* and spore forming bacteria like *bacillus SP* and *clostridium SP*. Whereas Type B lantibiotics, are globular in structure, either anionic or they have no net charge with molecular mass ranging between 2 to 3 kDa. Type B lantibiotics show antimicrobial action against target organism through interference in cellular enzymatic reactions (Cleeveland, 2001; Deegan *et al.,* 2006).

Class II - Non-Lantibiotics:

Non-Lantibiotics the Class II bacteriocins are heat stable peptides with molecular mass of < 10 kDa. Non-Lantibiotics peptides lack lanthionine. Non- lantibiotics are categorized in two subclasses. Subclass II a, includes non lantibiotics with an N-terminal consensus sequence Tyr- Gly-Asn-Gly-Val-X-Cys which is ordered into a S- shaped antiparellel ß- sheet that are stabilized by disulfide bond like in pediocin or listeria active bacteriocins (Chen and Hoover, 2003).

Bacteriocins belong to subclass II a show 40%-60% of homology when the corresponding amino acid sequences are aligned. Non-Lantibiotics subclass II a bacteriocins are synthesized with a N terminal leader peptide which is cleaved during process of secretion by proteiolytic action usually after a double glycine residue for example like pediocin PA-1, sakacin A (Daw and Falkiner, 1996). Subclass II b refers to two-peptide bacteriocins it means that requires two peptides to work synergistically in order to have an

antimicrobial activity; these peptides individually show slight or no activity against target organisms. Lactacin F and lactococcin G are members of this group (Paul Ross, 2002; Nes, 2013).

Class III: Bacteriocins:

Class III bacteriocins are mostly high molecular mass heat sensitive peptides of about (>30 kDa). Helveticin I by *Lactobacillus helveticus and* enterolysin produced by *Enterococcus faecium* are the example of this group (Paul Ross, 2002). They show the antimicrobial activity by disruption of cell wall and disorganization of cell membrane.

Table 1: Common bacteriocins produced by Lactic Acid Bacteria

Bacteriocin	Bacteriocin Producing Strain
Plantaricin S ß	Lactobacillus plantarum
Lactacin F	Lactobacillus johnsonii spp.
Lactoccin G	Lactococcus. lactis spp.
Lactocin 705	Lactobacillus. casei spp
Lactococcin MN	Lactococcus lactis var cremoris
Nisin	Lactococcus lactis spp.
Leucocin H	Leuconostoc spp.

Mode of action of bacteriocins:

Biological activity of bacteriocins exerted by its adsorption on the external surface of the target cells through the receptors present on it; followed by translocation in the target cell. At neutral pH the majority of LAB bacteriocins are cationic peptides with presence of lysine, arginine and histidine, hydrophobic in nature with the amino acids viz. alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine and tryptophan and amphiphilic, containing 20 to 60 amino acids (Cotter *et al.,* 2005). These properties bacteriocins play important role during their action on cytoplasmic membrane, where the cationic bacteriocinsbind to negatively charged phospholipids that make up part of the membrane of sensitive cells whereas allocation of the bacteriocins all over the cytoplasmic membrane is supported by its amphiphilic nature of bacteriocins (Thomas *et al.,* 2001). Class I and Class II bacteriocins reveal their antimicrobial activity at acidic and neutral pH, however they are sensitive to proteolytic action of pancreatic and gastric enzymes like

trypsin, chymotrypsin and pepsin, beside this these bacteriocins endure extremes pH, temperature and salinity.

The majority of LAB bacteriocins exert their antimicrobial against the sensitive bacteria through formation of pores in the cell membrane and dissipating the proton motive force. When bacteriocins come in contact with cell membrane of the target cell; bacteriocin bind to the cellsurface of the target cell through N- terminal in the forms a sheet like structure. However the hydrophobic C- terminal into the hydrophobic core of the target- membranes. And pierce cell further binds to the mannose phosphotransferase permease which results in membrane leakage. Immunity proteins which protect the target cells from pediocin-like bacteriocins bind to this bacteriocin-permease complex and stop bacteriocin-induced membrane-leakage. While the two- peptide bacteriocins penetrates through the target cell cytoplasmic membrane as a helix-helix structure; they may also bind the integral membrane proteins (Yusuf and Hamid, 2013).The outer membrane of Gram negative bacteria protect them from the antimicrobial activity of LAB bacteriocins.

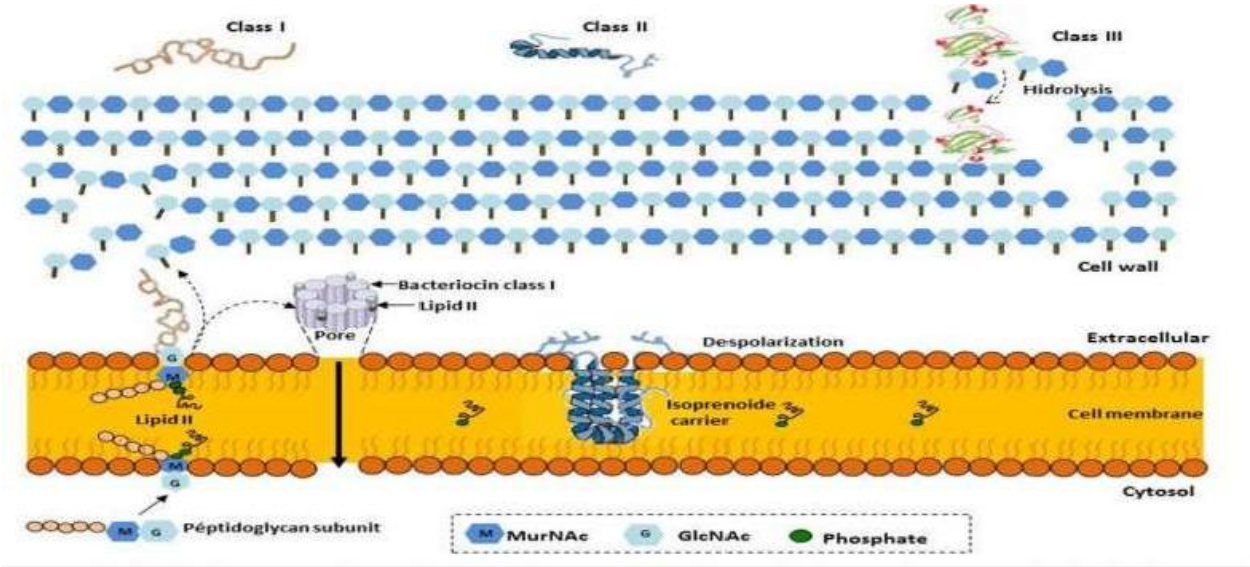

Figure 1: Mode of action of LAB bacteriocins. Adopted from (Álvarez-Cisneros *et al.,* 2011)

Certain Class I bacteriocins shows twofold mode of action, along with pore formation intarget cell membrane it could bind to lipid II; which results into blockage of cell wall synthesis and death of cell. The amphiphilic Class II bacteriocins peptide easily get inserted into the membrane of the target organism and results in depolarization and death of the target cell (Oliveira *et al.,* 2005).

Various factors affect the effectiveness of bacteriocin on target cell like; the load of target cells, concentration of bacteriocin required for destruction of target cell is directly

proportional to its population. Actively growing cells are more sensitive to bacteriocins; whereas the cells which are not actively growing, endospores might show resistance upto certain extent as food processing procedures stimulate germination of these endospores which enhance the bacteriocin effectiveness against the spores. Production of bacteriocin by the organism is associated with its growth, hence the factors (nutrients, growth inhibitors) affecting cell growth also its production and ultimately its effectiveness against the target organism. For example the sausage ingredients and the food additives, with an exception of nitrate considerably inhibit enterocins A and B production by *Enterococcus faecium* CTC492 (Oliveira *et al.,* 2005).

Nisin:

Nisin is the member of Class I lantibiotic, most extensively utilized bacteriocin. It was approved by FDA for its utilization in various products viz. cheese spreads, cheese, heat treated chill storedsoups by 1988. Nisin shows antimicrobial activity against Gram positive food pathogen and spoilage responsible bacteria like *Staphylococcus aureus* and *Listeria monocygenes*. Nisin is a thermostable protein it is stable at 121°C, however extended temperature exposure may abate its thermostability particularly over a pH range 5 -7. It is resistant to several proteases viz. trypsin pepsin, carboxypeptidase etc. but sensitive to α-chymotrypsin. Hence it could be utilized as food additive worldwide as E234 with GRSA status (Cleeveland *et al.,* 2001; Deegan, 2006, Mattick and Hirsch, 1947) designated the name 'Nisin' for the bacteriocin from *Lactococcus lactis* derived "*N i*nhibitor *s*ubstance" as *Lactococcus lactis* formarly classified as Lancefield serological N *Streptococcus* (Dicks *et al.,* 2011). *Nisin* is composed of 34 amino acids including several unusual amino acids as result of post translational modifications. Structure of nisin consists of a lanthionine and four ß-methyllanthionines (Rodriguez *et al.,* 2000). The precursor peptide of nisin was ribosomally synthesized and then subjected to modification. The mature nisin is obtained as result of removal of n-terminal leader sequence. A nanomolar concentration of is capable of showing antimicrobial activity against target organism (Cleeveland, 2001).

Nisin: Mode of action:

Nisin and subgroup Lantibiotics interact with the anionic lipids in Gram positive bacteria membrane and diffuse through it. The formation of pores in the lipid membrane was carried out through interaction of nisin with the Peptidoglycan precursor lipid II it inhibit Peptidoglycan synthesis (Wiedemann (2001). Mostly the amphiphilic of nisin type sub group Lantibiotics owing to its elongated cationic peptides disturb integrity of thee

energy transducing membrane. It was demonstrated that nisin like Lantibiotics stimulates rapid efflux of ions, amino acid or nucleotides, resulted in depolarization of membrane results in instantaneous termination of all biosynthetic processes (Wiedemann, 2001).

Nisin seems to align parallel to the surface of the membrane, the C terminus of nisin inserted in the membrane causes inter monolayer contact of phospholipids, forming pores according to the wedge like model. The ephemeral interruption of the phospholipid of the membrane structure acquired locally during the membrane permiabilization. According to the wedge-like model, the pore formation involves a proton motive force driven by co insertion of lipids and nisin C terminal domains. Such multiple inserted nisin molecules might leads to a great local disturbance of the lipid protein pores. Such structures are inherently not stable owing to the hydrophobic forces, which are the forceful, the rearrangements of the lipids into their usual bilayer organization (Moll, 1998).

Applications of Bacteriocins:

Bacteriocins have been extensively exploited as food preservative in the food industries in the current scenario particularly in dairy, egg, meat and vegetables product industries. It has reported that nisin A and Nisin Z have potent antimicrobial activity against food spoilage and food poisoning causing organisms. Moreover the unique feature of nisin over the other LAB bacteriocins that nisin is has been legitimately utilized and accepted globally in food industry (Moll *et al.,* 1999; Deegan *et al.,* 2006). Various techniques viz thermal treatment like pasteurization, sterilization, use of low pH and water activity environment for example acidification and dehydration or incorporation of preservatives like antibiotic, organic acids viz acetic acid, lactic acid, propionic acid etc or their salts viz sodium acetate, sodium lactate. Beside this these physical chemical preservatives there is increasing requirement for natural microbiologically safe product to ensure the health benefits as the thermal or chemical preservation may lead to deterioration of nutrient (Deegan *et al.,* 2006).

Bacteriocins can be introduced in the food products during their manufacture may be as a starter culture or co starter culture or may be as a already fermented product which contains the desired bacteriocin else as a purified bacteriocin as one of the component during the food processing (Paul Ross, 2002). Bacteriocins are competent against Gram positive bacteria as a food preservative but to be active against Gram negative organisms bacteriocins needs to be used along with the other technologies which facilitate the disruption of cell membrane for its effective antimicrobial activity (Daw and Falkiner, 1996).The non thermal treatment like hydrostatic pressure, pulsed electric field are helpful

since the food functionality and its nutritive quality .These non thermal treatments help in destabilization of cell membrane and consequently obstructing the essential cell functions (Deegan *et al.,* 2006). These non thermal techniques extremely effective along with lower concentration of bacteriocins else alone these techniques might not financially feasible. In addition to inhibit growth of contaminants responsible for food poisoning and spoilage bacteriocins also enhance the food quality and sensory properties like *Enterococcus faecium sp*. FL 3, bacteriocin BacFL31 inhibit the spoilage microorganisms responsible for oxidative rancidity as well as *Listeria monocytogene*s and *salmonella typhimurium* and retained the pH at low. Along with this it also retain the sensory properties like odor, color, texture at suitable levels for long time, thus improve the shelf life of the meat (Turubatovic, 2013).

Bioactive packaging is one of the more application of bacteriocins contributes to improve the shelf-life of food.. Bioactive packaging is method to protect the food from the external contamination during storage and handling. The steady release of bacteriocins from a packaging film on the food surface is more beneficial as spraying of bacteriocin on food products may not be sufficient as its activity may diminish as result of inactivation of interaction with food components. Its low concentration might not sufficient to be against upto interior of the food product. The bioactive packaging films can be used by various methods, the bacteriocins cab be directly added in the biodegradable protein films preparation from soybean, corn through heat press and casting or the bacteriocins can also be coated or adsorbed on the polymer film like methylcellulose, polyethylene, ethylene, vinyl acetate, polypropylene, polyamide, polyester acrylics and polyvinyl chloride (Deegan *et al.,* 2006). Primarily bacteriocins are employed to control food spoilage and related food poisoning however bacteriocins can be utilize as innovative medicinal treatment against pathogens in human body as bacteriocins have different approach of antimicrobial activity over antibiotics (Dicks *et al.,* 2011). Beside this bacteriocins have another benefit over antibiotics that as antibiotics due to their broad spectrum activity inhibit commensal organisms along with the target pathogen while due to comparatively narrow spectrum bacteriocins exhibit its antimicrobial activity against target organism (Riley and Wertz, 2002).

Bacteriocins have desirable properties like high activity even at nanomolar range, high specificity and mechanism of action which prove it to be used as medicine (Van Heel *et al.,* 2011). Multidrug resistance is the major problem in currant scenario which led to investigate an alternative to overcome the infection. As bacteriocins have specific mode of action as that of usual antibiotics, represent them as potential substitute for the antibiotics. Bacteriocins can inhibits multidrug-resistant Pseudomonas aeruginosa,

Klebshiella pneumonia, Acinetobacter spp. (Falagas *et al.,* 2008). Various reports states that bacteriocins also effective for gastric ulcer (kim *et al.,* 2003) and infections of skin (Kang *et al.,* 2009).

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BIODEGRADATION AND BIOREMEDIATION PROCESS

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Abstract:

Biodegradation is the phenomenon of decomposition or biological transformation of organic substances by involving various organisms like plant, bacteria, fungi and algae. In nature, biodegradation makes the conversion of complex organic molecules to simplest one and they are non-toxic in nature. The term biotransformation is used for incomplete biodegradation of organic compounds involving one or a few reactions.It is a complex process which has several steps likely bio-deterioration, depolymerization, assimilation and mineralization etc. Biodegradation and bioremediation process are strongly used to reduce the toxicity of the pollutant and which leads to control the environmental pollution. Such of these interesting facts are described in this chapter.

Keywords: Biodegradation, microorganisms, hydrocarbon, bioremediation, heavy metals.

Introduction:

Pollutant is a substance, evolved from urbanization and industrialization into the environment causes pollution that has undesired effects the usefulness of a resource (Chan *et al.*, 2006). A pollutant may adversely affector damage the living things by changing the growth rate of plant or animal species by interfering with their comfort, facilities and health issues (Sabev *et al.*, 2006).The occurrence of these substances in the environment is one of the largest concerns to science and general public in day to day life which has been recognized as one of the major hazard of the biosphere (Yang *et al.*, 2014; Wan *et al.*, 2020). Nowadays, the modern world is challenged with the contamination of ecosystems with hazardous and toxic xenobiotics. While regulatory steps have been implemented to reduce or eliminate the production and release to the environment of these chemicals (Wan *et al.,* 2020). The industrialization of agriculture, rapid growth in the chemical industry and the

need to produce cheap forms of energy have all caused the continuous release of hazardous chemicals into the biosphere (Sabev *et al.*, 2006).

Millions of natural or synthetic organic chemical substances are present in both terrestrial and aquatic environments. Toxicity and/or persistence of these chemicals determine the polluting principle of the substances (Kowalczyk *et al.*, 2016).Human endeavor to synthesize numerous organic compounds is wrought with the paradox of saving many lives and providing economic benefits to many others while acute and chronic toxicity of some of these chemical substances make many others including plants and animals to suffer (Delacuvellerie *et al.*, 2019). Adverse effects and persistent nature of these chemical substances are beginning to be understood now and these chemical substances not only travel through trade channels but also across the atmosphere. The major pollutant like hydrocarbons, plastics and heavy metals are persistent in the environment and have been associated with mutagenic, teratogenic and carcinogenic effects. (Zhang *et al.*, 2017; Wirnkor *et al.*, 2018). There are various physical and chemical methodologies have been used to convert these pollutants in to non-toxic components which makes more tenuous, high cost with adverse effect to the environment. An alternative biological method could be used to avoid and overcome by these problems (Carolin *et al.*, 2017).

The biological approaches to these pollutants include accumulation and degradation. Biodegradation is the biologically catalyzed reduction in complexity of chemical compounds (Chan *et al.*, 2006). Indeed, biodegradation is the process by which organic substances are broken down into smaller compounds by living microbial organisms (Carolin *et al.*, 2017). When biodegradation is complete, the process is called "mineralization". However, in most cases the term biodegradation is generally used to describe almost any biologically mediated change in a substrate (Yang *et al.*, 2014).The responses of environments with organic pollutants are perceptible from the dwindling degradative abilities of microorganisms compared to other sources like plant (Ghosh and Singh, 2005). Microbial mediated degradation and bioremediation is seen as a cost-effective method for removing the pollutant from the environments (Chan *et al.*, 2006; Yang *et al.*, 2014).

Many bacterial, fungal and algal species have the ability to absorb and/or degrade various chemical constituents like heavy metals and azo dyes (Kowalczyk *et al.*, 2016). Various bacterial species such as *Enterobacter* sp., *Bacillus* sp., *Halobacter* sp., *Aeromonas* sp., and *Pseudomonas* sp. have been used to detoxify a wide range of contaminated chemicals namely phenylamine, benzene-diazonium chloride or phenol (Magnin *et al.*,2019). Similarly, another important microorganism like actinobacterium especially *Streptomyces* sp., is an eminent source for heavy metal as well as rubber degradation (Vidyaand Lali,

2017). Several microorganisms, including fungi, bacteria and yeasts are involved in biodegradation process. Algae and protozoa reports are scanty regarding their involvement in biodegradation (Sethunathan *et al.*, 2004). Biodegradation processes vary greatly, but frequently the final product of the degradation is carbon dioxide. Organic material can be degraded aerobically, with oxygen, or anaerobically, without oxygen (Fritscheand Hofrichter, 2008). By keeping this view, this review will be discussed the implement of microorganism in biodegradation and bioremediation process.

Influence of bacteria in biodegradation process:

Bacteria could be able to bio-convert, degrade or detoxify the pollutants like heavy metals, petroleum hydrocarbons etc. They have the ability to metabolize various organic contaminants and utilizing as an energy source or converting them to non-toxic byproducts (carbon dioxide, water and biomass) (Sabev *et al.*, 2006). Different microbial electron acceptors such as oxygen, nitrate, manganese, iron and sulphate can be involved in the biotransformation of aliphatic and aromatic hydrocarbons. During hydrocarbon degradation, the bacterial strains have been able to synthesize various enzymes such as oxygenases, dioxygenases and monooxygenases etc. Musat *et al.* (2009) reportedthe cultivation of a marine sulphate reducing bacteria on naphthalene supplemented medium and could able to adapt or utilize the compound 2- methylnaphthalene from the medium by the secretion ofmethylating enzymes. In an another study, Jimenez *et al.* (2007) portrayed the biodegradation of n-alkanes from the fuel by using an organism *Rhodococcus* sp., under laboratory conditions. In an another report, the degradation of chemical pesticide chlorpyrifosby an organism *Providencia stuartii* enumerated from agricultural soil and the isolates *Bacillus* sp., *Staphylococcus* sp., and *Stenotrophomonas* sp., from cultivated and uncultivated agricultural soil could able to degrade dichlorodiphenyltrichloroethane (DDT) under laboratory conditions (Kanade *et al.*, 2012).

The degradation of polyethylene by *Phanerochaete* sp., and *Streptomyces* sp., under laboratory conditions were documented by Shah *et al.* (2008). Various researchers like Iiyoshi *et al.* (1998); Kim *et al.* (2005)documented *Streptomyces* sp., was sufficiently convert and detoxify polythene under *in-vitro* condition at the temperature range of 70ºC. In an another study, Vrchotova *et al.* (2013) achieved that the degradation of chlorobenzoic acids (3-CBA) by using the rhizosphere associated microorganisms such as *Pseudomonas aeruginosa* R75 and *Pseudomonas savastanoi* CB35, *Alcaligenes* sp. BR60 under *in-vitro* condition.

Table 1: Influences of bacteria in biodegradation and bioremediation process

Types of Hydrocarbon	Microorganisms	References
Polyethylene	*Brevibacillus borstelensis*	Hadad *et al.* (2005)
	Rhodococcus ruber	Sivan *et al.* (2006)
	Bacillus sphericus; *Bacillus cereus* BF20	Sudhakar *et al.* (2008)
	Staphylococcus epidermis	Chatterjee *et al.* (2010)
	Arthrobacter sp. GMB5; *Pseudomonas* sp. GMB7	Balasubramanian *et al.* (2010)
	Bacillus subtilis H1584	Harshvardhan and Jha (2013)
	Rhodococcus ruber	Santo *et al.* (2013)
	Enterobactera sburiae YT1; *Bacillus* sp. YP1	Yang *et al.* (2014)
	*Achromobacter*sp.,	Kowalczyk *et al.*, 2016
Polystyrene	*Xanthomonas* sp.;	Eisaku *et al.* (2003)
	Microbacterium sp. NA23	Atiq *et al.*(2010)
	Exiguobacterium sp. YT2	Yang *et al.* (2015)
Polypropylene	*Renibacterium salmoninarum* 27BN	Christova *et al.* (2019)
	Ralstonia eutropha H16	Johnston *et al.* (2019)
	Bacillus sp., *Rhodococcus* sp.	Auta *et al.* (2018)
	Yarrowiali polytica 78-003	Mihreteab *et al.* (2019)
Polyether	*Alternaria* sp. PURDK2	Matsumiya *et al.*(2010)
Polyester	*Corynebacterium* sp., BI2; *Pseudomonas aeruginosa*	Kay *et al.* (1991)
Ester	*Pseudomonas chlororaphis*	Gautam *et al.* (2007)
Poly (3-hydroxybutyrate-co-3-hydroxyvalerate	*Actinomadura* sp. AF-555	Shah *et al.* (2010)
Polyether	*Pseudomonas denitrificans, Pseudomonas fluorescens,*	Stepien *et al.* (2017)
Polyethylene Terephthalate	*Pseudomonas putida* GO16,	Kenny *et al.* (2008)
	Thermobifida cellulosilytic	Ribitsch *et al.* (2012)
	Saccharomonos poraviridis	Kawai *et al.* (2014)
	Ideonella sakaiensis	Yoshida *et al.* (2016)
	Thermobifida fusca	Wei *et al.* (2014)
Plasticized Polyvinyl Chloride	*Mycobacterium* sp. NK0301	Nakamiya *et al.* (2005)

Subsequently, they observed that the strain *Pseudomonas aeruginosa* R75 and *Pseudomonas savastanoi* CB35 expressed the highest (74%) removal of 3-CBA from the soil.

In another report, Singh and Fulekar (2010) documented the bioremediation and biodegradation of benzene by *Pseudomonas putida* MHF 7109 enumerated from cow dung. Who also observed the percentage (100, 81 and 65%) of degradation at the concentrations of 50, 100 and 250mg/l of culture within the respective incubation time of 24, 96 and 168h.

In another report, Kathiresan *et al.* (2003) denoted the efficiency of *Pseudomonas* sp., and *Aspergillus glaucus* in degradation of plastics and polythene under shaking conditions for one month. Who also observed that the fungal strain showed more effective degradation of plastics (8.18%) and polythene (28.80%) than the degradation of plastics (7.26%) and polythene (20.54%) by *Pseudomonas* sp. Singh and Fulekar (2010) assessed the biodegradation of petroleum hydrocarbon like toluene and oxylene by using *Pseudomonas putida* MHF 7109 at the concentration of 100 and 50mg/l at the incubation time of 96 and 168 h, respectively. Sharma and Pant (2000) reported the biodegradation of hydrocarbon by using *Rhodococcus* sp., enumerated from crude oil contaminated sea shore sediment soil. They also observed the bacterium could able to degrade the crude oil (50%) at the temperature range of 30ºC and 72 h of incubation. Kang (2013) investigated the bioremediation of cadmium by using a calcite-forming bacteria *Lysinibacillus sphaericus* CH-5 under laboratory condition expressed the highest (99.95%) removal of cadmium for 48 h of incubation.

Influences of fungi in biodegradation process:

Fungi are diverse group of organism, play a significant role in ecosystems, decomposing dead organisms, fallen leaves, feces, and other organic materials. However, recently fungi have obtained substantial attention for their biodegradation and bioremediation efficacy which is characterized to the enzymes they synthesize. In addition, fungi have various significance over bacteria such as fungal hyphae that can penetrate contaminated soil to reach the pollutants (Husaini *et al.*, 2008). Many scientist and researchers successfully worked on the impact of fungi in biodegradation and bioremediation process (Li *et al.*, 2008.). Among them, the ligninolyticfungi have been specifically examined for their extracellular oxido-reductive enzymes highly used in the degradation of many aromatic pollutants (Donnelly and Fletcher, 1995). They are well known to tolerate and detoxify the metal atoms through different mechanisms such as valence transformation, extra and intracellular precipitation and active uptake (Gadd and White, 1993). Many species of fungi can adsorb cadmium, copper, lead, mercury, and zinc into their mycelium and spores. Sometimes the walls of dead fungi bind better than living ones. Some of the fungal genera, namely, *Neosartorya, Amorphoteca*, and *Talaromyces* were

enumerated from petroleum contaminated sediment soil and confirmed to be the efficient strains for hydrocarbon degradation under laboratory conditions (Chaillan *et al.*, 2004).

In an another study, a group of fungi such as, *Aspergillus* sp., *Cephalosporium* sp., and *Pencillium* sp., from the crude oil contaminated sediment soil expressed to be an efficient degrader of hydrocarbons (Singh, 2006). Sharma and Malaviya (2014) reported the bioremediation of heavy metals by using *Fusarium chlamydosporium* under shaking culture technique expressed the reduction of turbidity (64.69%) and COD (71.80%) for 72 h of incubation. Likewise, the biodegradation of monomeric styrene by using *Phanerochaete chrysosporium* KFRI 20742, *Trametes versicolor* KFRI 20251, and *Daldinia concentrica* KFRI 40-1 was examined under laboratory conditions by Lee *et al.* (2005).In an another report, the bioconversion of naphthalan was studied by using various fungi cultures likely Mucor sp., *Fusarium* sp. *Penicillium* sp., and *Cephalosporium* sp., also, the strain *Cladosporium* sp., confirmed to have good aromatic hydrocarbon degrading ability from the contaminated sources (Li *et al.*, 2008) and the hydrocarbon crude oil contaminated sediment soils were employed to test the degradation efficacy of *Pleurotustuber regium* under standard laboratory conditions(Ogbo, and Okhuoya, 2008). Recently in an study, Muhonja *et al.* (2018) documented biodegradability of polyethylene by using various bacteria and fungi isolated from Dandora dumpsite of NairobiKenya.

Table 2: Influences of fungi in biodegradation and bioremediation process

Types of Hydrocarbon	Microorganisms	Reference
Polyethylene	*Aspergillus versicolor* and *Aspergillus*sp	Pramila and Ramesh, (2011)
	Phanerochaete chrysosporium ME-446,	Iiyoshi *et al.* (1998)
	Pseudomonas sp. AKS2	Tribedi and Sil (2013)
	Alcanivorax borkumensis	Delacuvellerie *et al.* (2019)
Low density polythene	*Pseudomonas aeruginosa* (ATCC 15692), *Pseudomonas putida* (KT2440	Kyaw *et al.* (2012)
Polyvinylchloride (PVC)	*Penicillium janthinellum*	Sabev *et al.* (2006)
Polyester	*Aspergillus flavus*	Mathur *et al.* (2011)
Polyester	*Aspergillus tubingensis*	Khan *et al.* (2017)
polyester/polyether	*Penicillium* sp.	Magnin *et al.* (2019)

The researchers Arun *et al.* (2008) and Wang *et al.* (2008) portrayed the effective biodegradation process of pyrene was achieved with *Aspergillus niger*, *Coriolus versicolor*, *Fusarium* sp. and *Trichoderma* sp. through standard procedures. Similarly, Li *et al.* (2005) studied the biodegradation of 1,2,3,4-tetrahydronaphthalene by a marine fungus *Hypoxylonoceanicum*under shaking flask conditions. Sanyal *et al.* (2006) documented the degradation of polyhydroxyalkanoate (PHA) by using various fungal genera such as *Aspergillus, Penicillium, Paecilomyces, Coriolus, Pycnoporus, Pleurotus, Fomitopsis*, and *Daedalea*, in the crude oil contaminated soil and aquatic environments.

4. Influence of Algae in biodegradation process

Walker *et al.* (1975) isolated an algae *Prototheca zopfi* which could be utilized the crude oil and a mixed hydrocarbon as substrate and exhibited extensive degradation of n-alkanes and isoalkanes as well as aromatic hydrocarbons. The exudation of hydrophobic pollutants namely polychlorinated biphenyls was attained by using the brown algae namely *Caepidiumantarcticum* and *Desmarestia* sp. through standard conditions (Lara *et al.*, 1989). Wang *et al.* (2008) reported the biosorption and biodegradation of Nonylphenol by four marine microalgae (*Phaeocystis globosa, Nannochloropsis oculata, Dunaliella salina* and *Platymonas subcordiformis*) through standard laboratory conditions. An another report from Chekroun *et al.* (2014) stated the role of algae in bioremediation of organic pollutants under *in-vtro* conditions. The accumulation of organic pollutants in the ecosystems can cause adverse effect or serious problems to various aquatic organisms. The algae play an important role in controlling and biomonitoring of organic pollutants in aquatic ecosystems (Pavlostathis *et al.* 2001). Liebe and Fock 1992 denoted that *Chlamydomonas reinhardtii* having some adaptation to remove some of the iso-octane-extracted Polyvaromatic hydrocarbon from diesel particulate exhaust. In an another study, Semple *et al.* (1999) reported the biodegradation and bioremediation of aromatic hydrocarbons by various microalgae with the competences of microalgae on aromatic compounds, ranging from simple monocyclic to more complex polycyclic pollutants

In another study, El-Sheekh *et al.* (2009) studied the biodegradation of various dyes by some green algae namely *Elkatothrix viridis*, *Lyngbyalagerlerimi*, *Chlorella vulgaris*, *Nostoclincki*, *Oscillatoria rubescens* and *Volvox aureus* using methyl red, orange II, basic cationic, and basic fuchsin. Pinto *et al.* (2002) documented the microalgae *Ankistrodesmus braunii* and *Scenedesmus quadricauda*c ould able to degrade various phenolic groups likely p-hydroxy benzoic acidc, catechol, p-coumaric acid, hydroxytyrosol, tyrosol, ferulic acid, synaptic acid, vanillic acid andcaffeic acid by about 70% of 400 mg

phenolic compounds mL^{-1} within the incubation of 10 days. The microalgae *Scenedesmus obliquus* GH2, is used to construct an artificial microalgal-bacterial consortium for crude-oil degradation was reported by Tang *et al.* (2010). Recently, Wan et al (2020) attributed the toxicity, biodegradation and nioremediation of organophosphorus pesticide (Trichlorfon) by a freshwater algae *Chlamydomonas reinhardtii.* Who also documented that the growth of *C. reinhardtii* decreased with increasing trichlorfon concentration, and the maximum (51.3%) inhibition ratio was observed at $200mgL^{-1}$ concentration.

Table 3: Influences of algae in biodegradation and bioremediation process

Types of Hydrocarbon	Microorganisms	References
Methyl parathion	*Chlorella vulgaris, Scenedesmus bijugatus, Nostoc linckia, N. muscorum, Oscillatoria animalis*	Megharaj *et al.* (1994)
Tributyltin	*Chlorella vulgaris*	Tsang *et al.* (1999)
Phenanthrene	*S. capricornutum*	Chan *et al.* (2006)
Dimethyl phthalate	*Closterium lunula*	Yan and Pan(2004)
Dibenzofuran	*Ankistrodesmus* sp.	Todd *et al.* (2002)
α-Endosulfan	*Scenedesmus* sp. *Chlorococcum* sp.	Sethunathan *et al.* (2004)

Conclusion:

From this chapter, it could be concluded that the natural resources like plant, bacteria, fungi and algae with their metabolites were effectively detoxify, biodegrade and remove various hydrocarbons, heavy metals and crude oils from the contaminated soil, water and air than the other usage of physical and chemical methods. The usage bioresources for detoxification process of hydrocarbon and heavy metals are easy, natural and cheapest with low cost. They are eco-friendly and highly stable. In future, highly genetically active microorganisms with proper guidelines and regulations should be prepared for bioremediation and treated with oil spill. With the basic prospectus of various studies, this chapter highly authenticated that the bioresouirces like bacteria, fungi and algae are more efficient detoxify the pollutants from the contaminated area.

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SHORTS NOTES ON CLADOCERA: A SENTINEL ORGANISM

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Abstract:

The Cladocera are one of the small group of organism, belongs to crustaceans and it is a representative order in the systematic position. They are generally known as water fleas as they are found in most of the type of waters even in marine habitats. More than 700 species are known to us and many of still unknown because of undescribed. The microorganisms like zooplankton are the integral part of freshwater ecosystem. Among the planktonic communities Cladocera is one of the groups of crustacean organism. They are rich in high nutrient enriched water responded to various physicochemical ecological factors as there is interrelation with changing water environment therefore the group of Cladocera is considered as important freshwater bioindicator of aquatic ecosystem. Now this group is implemented in evaluating the trophic status of many aquatic studies.

Keywords: Cladocera, Bioindicator, Aquatic Ecosystem, Environmental Variable, Pollution.

Introduction:

Sentinel organisms or Indicator species or indicator communities are often used for bio-monitoring. Many of the aquatic organisms are used as indicator species for pollutants including nutrient enrichment (Phillips and Rainbow, 1993). Biochemical, genetic, morphological and physiological changes in certain organisms have been noted as being related to particular environmental stressors and can be used as indicators (Bharti *et al.,* 2013). The absence or presence of indicator organisms or community may reflect environmental conditions. The indicator organisms or sentinel species which accumulate pollutants in their tissues from the surrounding environment are vital bio-monitoring devices (Phillips and rainbow 1993; Kennish 1992).

Zooplanktons are integral component of most of aquatic ecosystem like pools, ponds, lakes, reservoirs and rivers. Zooplankton comprises of four major groups, i.e. Rotifera, Cladocera, Copepoda and Ostracoda. These organisms are highly sensitive organisms responding to varied environmental changes in moderately short periods of time. As they are more flourishes in lentic ecosystem, studies on their dynamics can be very useful tools to understand the status of water bodies (Sampaio *et al.,* 2002; Eskinazi-Santanna, 2013). Past studies revealed that increase in zooplankton biomass is connected to rising level of eutrophication, the higher trophic state will lead to increased resource availability which in turn lead to growth of zooplankton biomass (Serafin-Junior *et al.,* 2010; Bonecker *et al.,* 2007). Cladocera are worldwide in distribution near about all types of geographical areas and all types of aquatic ecosystem including wide range of environmental gradients. A number of mutually interacting factors such as climate change, physical, chemical parameters affect distribution and diversity of zooplankton communities (Neves *et al.,* 2003). They exhibit positive or negative responses to environmental variables and in varied trophic conditions. Because of their adaptability to various habitats, and the relative lack of data available on the various habitat types, it is assume that considerably greater diversity of freshwater Cladocera than currently described (Jeong *et al.,* 2013). As they are found in all types of waters, some of them are also found in profundal zone of large lakes, ground water, caves and mosses of cloud tropical forest (Siney, 2002). Due to its peculiar biological features, they used in experimental studies and used a models for research on environmental stress. In the group of Cladocera, the genus Daphnia receives special attention in various researches due to its wide distribution especially in temperate region (Sarma *et al.,* 2005). The current literature is reviewed to know the distribution, density, their role in ecosystem and bioindicator importance in freshwater ecosystem to describe the relation with several environmental variables.

Cladocera composition:

Cladocera are a group of minute crustaceans ranging from 0.2 to 5.0mm in size. They are also known as water fleas. Cladocera is a order of Branchiopoda subclass of Crustacea subphylum. The total of 11 families' chydoridae is large one. 190 species of Cladocera is reported from India (Raghunathan and Kumar, 2003) in which 18 species are endemic to India. Chydoridae, Daphniidae, Sididae, Macrothricidae, Moinidae, Bosminidae, Polyphemidae, Leptodoridae, Holopedidae and Podonidae are few representative families from the India. Cladocera live in the open water, among the weeds in littoral zone and on the benthic mud. Generally they feed on microalgae, bacteria and detritus with a few

exceptions like the members of Polyphemidae, Cercopagidae and Leptodoridae which are carnivorous while a single genus *Anchistropus* lives parasitically on Hydra. The limnetic genera like *Daphnia, Ceriodaphnia, Moina* and *Diaphanosoma* are planktonic algal-bacterial feeders, the littoral chydorids like *Alona* and *Chydorus* associated with marginal weeds may feed on attached algae or detritus scraped from the substratum. The benthic forms like *Ilyocryptus* and *Leydigia* probably live on detritus or associated bacteria in the bottom mud (Michael and Sharma, 1988). They are relatively tolerant to environmental conditions, which would confirm their opportunistic and potentially invasive nature.

Cladocera are distributed globally and mostly occurred in temporary or permanent freshwater pools, although a few species have colonized marine or brackish habitats (Richter *et al.,* 2007). Cladocera have been used as indicator as well as test organism for estimation of toxicity level of pesticide and other environmental pollutants (Frear and Boyd, 1967; Muirhead-Thompson, 1971; Canton and Adema, 1978; Adema, 1978).

Role of Cladocera:

They plays important role in food web in aquatic ecosystem and helps to reduce the phytoplankton biomass as they are filter feeders and improve the water quality as they consume good amount of phytoplankton (Gonzalez, 2000). Their profusion in vegetated zone found higher than non-vegetated zone (Bonkurt and Guven, 2009) helps to control algal growth by efficient grazing. According to Begon *et al.,* (2007), high environmental heterogeneity favors high diversity of microhabitat, resulting in increase of species number. The littoral region is very rich in microhabitat due to the presence of macrophyte; resulting in high microcurstacen species diversity (Lemly and Dimmick, 1982, Nogueira *et al.,* 2003). Macrophytes presence in the littoral region is probably a controlling feature, since cladoceren richness and diversity could higher in regions with elevated macrophyte diversity than that with scares macrophyte community. Many studies regarding elevated zooplankton diversity with higher the macrophyte diversity have done by Mustumuru-Tundisi *et al.,* (1990), Nunes *et al.,* (1996), Moia-Barbosa *et al.,* (2008). The Genus *Daphnia* is one of popular and considered as important to determine the water quality due to its key role in biomass transfer between phytoplankton and planktivorous fish. The members of Bosminidae constitute an important group of indicators in paleolimnological studies.

Cladocera have peculiar biological features that make their use in ecological studies, those dealing with environmentally induced stress from its many viewpoints like biochemical, molecular, toxicological and physiological etc. These organisms may be establish in extremely fluctuating environments, such as ponds as well as shallow coastal

lagoons (Petrusek, 2002; Santangelo *et al.,* 2008), therefore they became more interesting models for research on environmental stress. The *Daphnia* have a special attention due to its widespread distribution particularly in temperate regions (Sarma *et al.,* 2005). In Daphnia, D. magna have become important model organisms used for various biomedical sciences to ecology (Ebert, 2011; Seda and Petrusek, 2011; Miner *et al.,* 2012).

Cladocera in Aquaculutre:

The fish seeds are habitually fed with rotifer and Cladocera at first followed by artificial pelleted foods later. *Moina* (Cladocera) *Brachionus plicatilis* (rotifer) is generally accepted as a starter for many larval fishes (Tawaratmanikul, 1988). Cladoceran is used as a live feed source in the fish culture because of higher nutritional value and economic feasibility in production in mass number. The fish seeds of *Clarius batrachus, Ompak bimaculatus, Channa stritatus,* exhibit good growth and survivability on Cladocera as live food (Tawaratmaikul, 1988).

The Cladocera practically serves as essential energy sources particularly for larval nutrition towards optimal growth as well as maintenance of metabolism because of higher contents of protein and fats. (Sureshkumar R. 2000). *Moina* and *Daphnia* have been successfully used as larval live food in pond (Qin and Culver, 1996). Cladoceran generally flourish on microscopic organic particle (Bacteria, phytoplankton, Fungi and Protozoan) suspended in water. In aquaculture cow-dung, chicken-dropping, fish-faces, horse-manure, rice-bran and mineral-fertilizer were found to support mass culture is very successful (Punia, 1988, Rottmann *et al.,* 2003).

Relation with Environmental variables:

Cladocerans are important bio-indicators for a wide range of environmental variables (Rumes *et al.,* 2011). Cladocera shows a strong response to environmental factors, such as trophic state, total phosphorus concentration, water depth, temperature, etc. Cladocera species are especially responsive to changes in pH, reflecting their sensitivity to acidification (Edyta Zawisza *et al.,* 2016).

According to Dodson (1991, 1992) the Cladocera population is proscribed by the size of water body and its productivity. The Cladocera are exceptional indicators of environmental changes (Eggerment and Martens, 2011). They are more sensitive towards water pollution than many other aquatic organisms (Brix *et al.,* 2001, Von Der one and Liess 2004). Many of the work done on linkages between environmental variables and

cladoceran community structure, therefore the factors controlling the cladoceren species composition are relatively well known (Leppanen, 2019).

Cladocerens are frequently the target groups of zooplankton studies, only limited reports are available on their ecology, diversity and role in aquatic productivity in freshwater environment in India (Sharma and Sharma, 2009a). Cladocera are one of the important groups for bio-monitoring studies and are an important part of trophic cascades of the aquatic system and highly responsive to pollutants. It can even react to very low concentration of contaminants (Ferdous and Muktadir, 2009, Sharma and Chandrakiran, 2011).

The pH is important to aquatic life because the pH affects physiological functions of aquatic organisms, where the exchange of ions with the water and respiration. The sensitivity of organisms to pH changes can differ considerably in water bodies. The change of pH may be due to anthropogenic NaOH spills. Davis and Ozburn, (1969) revealed that *Daphnia pulex* would not thrive below a pH of 7; however, its potential for reproduction was limited to a fairly narrow range. In acidic water, the crustaceans and fish have suffered retarded growth and skeletal deformity (Haines, 1981).

Numerous studies have attempted to explain the responses of aquatic organisms to different pH. Bulkowski *et al.,* (1985) observed that *Daphnia* species is more sensitive to low pH than *Cyclops* species; however, on pH 3 and 4, the disappearance of *Daphnia* species is recorded. *Chydorus cf. sphaericus* is one of the most widespread species is known for its wide ecological tolerance, especially to pH (Walseng *et al.,* 2003; Belyaeva and Deneke, 2007; Zawisza and Cedro, 2012). According to Sharif (2017), Cladocera showed a moderate positive relationship with air and water temperature and a weak positive relationship with the pH, on the other hand salinity and TDS showed a moderate negative relationship and whereas a weak negative linear relationship with DO.

Daphnia magna is a largest (3mm) in length of typically hard water species and cosmopolitan in distribution. Life span is highly depending upon water temperature which ranges from few weeks to months (MacArthur and Baillie, 1929). It can survive for short period at water temperatures as low as 0° C and as high as 39° (Frear and Boyd, 1967). It can survive at pH between 6 to 9.5 values (Anderson, 1946). Boyd, (1967) observed that the lowest mortality in culture in pH of 7.5. The pH is one of the most interesting ecological properties of dystrophic water bodies. According to Zawisca *et al.,* (2016) in its study observed the most acidic-condition tolerating species like *Alonella exigua*, *Graptoleberis testudinaria*, while *Acroperus harpae* and *Alona nana* showed prominent abundances at high pH. The high relative abundance of these species at pH lowers than 5.5 recommended

a high tolerance to acidic conditions. On the other hand neutral waters preferred by *Bosmina longirostris*, which also increased in abundance with an increase in pH. The abundance of *Alona affinis* appeared to be independent of pH values.

Jha and Barat, (2003) in its studies of polluted lake in Darjeeling where pollutants let into the lake from external sources and the pH of the lake became acidic. It was confirmed by the analysis of other physiochemical parameters and planktons, where *Moina, Daphnia, Bosmina* found most abundantly. Mustaq, (1990) observed that some Cladoceran species can flourish well in polluted waters and hence serves as good biological indicators of water pollution. The *Bosmina longirostris*, dominated the cladoceran community has been noted to tolerate a wider range of salinity than many other cladoceran species (Aladin, 1991; Paturej and Gutkowska, 2015). It has been recorded higher salinity when compared to *Daphnia* (Jeppesen *et al.,* 1994; Boronat *et al.,* 2001; Amsinck *et al.,* 2003).

The *Daphnia* include 30 species is representative of freshwater system except rapid streams, brooks and grossly polluted water (Hutchinson, 1967). Daphnia magna is an important model organism for biomedical studies. Sarma and Nandini, (2006) reviewed eco-toxicological effect of metal organic pollutants and natural toxicants on cladocerens. According to them many studies have considered typically planktonic taxa such as *Daphic magna, D. Pulex, Moina macrocopa, M. micrura and Ceriodaphnia dubia.* It is widely used as the bioassay organism in evaluating the impact of different toxic substances (Sarma and Nandini, 2006). In fact, *D. cucullata*, a species, which nearly vanished from the lake system, is regarded sensitive to elevated salinity within its genus *Daphnia* (Gonvalaves *et al.*, 2007). Increased salinity in mine water has negative impact on freshwater Cladocera population (Elphick *et al.,* 2011, Van dam *et al.,* 2014) changes in salinity also induce shifts in species composition (Aladin 1991). *Bosmina longirostris and Chydorus sphaericus* are reported to tolerate mine water impacted condition (Leppanen, 2018). *B. longirostris* is more sensitive to metal contamination than *Daphnia* (Koivisto *et al.,* 1992; Koivisto and Ketola, 1995; Labaj *et al.,* 2015).

Venkatraman, (2003) reported that *Ceriodaphnia cornuta, Moina micrura, Macrothrix spinosa* and *Chydorus borroisi* can be used as indicators of pollution. Eutrophication can also favor increase of zooplankton diversity in the littoral zones (Lemly and Dimmick 1982). Correlation between increased temperature and abundance of *B. longisostris* has been noted in long term neolimnological research (Adamezuk *et al.,* 2015b). It is predicted that the species will be dominant in an increasing number of eutrophied lakes. *B. longirostris* also tolerate salinity (Aladin, 1991; Jeppesen *et al.,* 1994, Deasley *et*

al., 2012) and show higher resistance to acidification than bigger cladoceran. *B. longirostris* is resistant to strain of *Microsystes aeruginosa* and *Anabaena flosaguae* that have leathel toxic effect on other cladocerans (Fulton, 1988) thus this species can potentially coexist with blue green algal blooms (Jiang *et al.,* 2013). *B. longirostris* is even able to consume them (Fulton, 1988). The volatile fractions were much toxic than the nonvolatile fractions for *Daphnia* (Dorris *et al.,* 1974). A widespread use of insecticides may impair the ability of non-target organisms to survive and reproduce in affected habitats (Wong *et al.,* 1995).

a. Cereodaphnia **b. *Moina species*** **c. *Macrothrix species***

Figure1: Some of indicator species (a,b,c) from lentic ecosystem in Gondia District

Low *Daphnia* percentages linked with very low calcium content in the lake water, an environmental condition that is critical for the development of most species belonging to this genus (Jeziorski *et al.,* 2008; Shapiera *et al.,* 2011). *Alonella excisa* and *Acroperus harpae* are considered as representative of oligotrophic condition, low nutrient and acidic lakes (Fryer, 1968; Whiteside, 1970; Rautio, 1998; Bjerring *et al.,* 2009; Nevalainen *et al.,* 2013; Zawiska *et al.,* 2013). However, these two species are also very successful in soft water lakes because of their low calcium demands (Nevalainen *et al.,* 2013). Phosphorus is an essential nutrient for growth of organisms in aquatic ecosystems (Reynolds 2006; Lampert and Sommer, 2007). Nutrient availability controls the growth of phytoplankton, the productivity of aquatic systems and algae can adjust their metabolism to the available Phosphorus (Rocha *et al.,* 2018). Microalgae are subject to low nutrient concentrations and Phosphorus is maybe the main limiting nutrient (Lampert and Sommer, 2007; Chia *et al.,* 2013; Elser *et al.,* 2013). The overload of this constituent in agricultural fertilizers and in untreated domestic and industrial sewage causes the enrichment phosphorus in aquatic ecosystems, which is one of the main causes of eutrophication (Odum, 2010; Esteves, 2011; Le Moal *et al.,* 2019).

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DIFFERENT ANALYTICAL METHODS FOR THE ESTIMATION OF PESTICIDES IN THE WATER

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Abstract:

The review presents an overview of different Analytical methods by the different analytical instruments for the analysis of pesticide residues in water. The most widely used techniques for the detection of pesticides are different spectroscopic methods as HPLC combined with different detectors, Liquid chromatography with Mass spectroscopy, Gas chromatography technique using various detectors (or) in combination with Mass spectroscopy. This review also focuses on the different pesticides specifically in water by different analytical method and also specific methodologies for sample collection and extraction. Finally the reports of concentration and limits of the pesticides in water of different areas in are reviewed.

Keywords: Pesticides, Water, HPLC, Gas Chromatography, Mass Spectroscopy.

Introduction:

The presences of organic contaminants in the Ecosystem are dueto Contamination from anthropogenic Factors. Despite their numerous merits, pesticides, created by the intensification of agriculture, are considered to be some of the most harmful environmental pollutants. They are not only harmful, but also Movable and capable of bio accumulative. Many pesticides are accumulating in environment especially in surface water and increasing day by day due to physical and chemical properties and their constant usage which inturn affects both drinking water quality and health of human too. Their presence in water, not only for the quality of drinking water and human health, but also for ecosystems, is considered a potential risk. India is one of the world's biggest users of pesticides. Bureau of Indian Standards (BIS) (IS 13428) set limits for residual pesticides at trace levels (sub ppb) like bottled drinking water (IS 14543), natural mineral water (IS

14543), and drinking water (IS 10500). Since pesticides are harmful to health, they have acute or immediate effects from short-term exposure, such as nausea, respiratory irritation, skin rash, vomiting, dizziness and chronic effects that occur long after prolonged exposure to small amounts of pesticides, including cancer, liver, kidney damage, nervous system disorders, immune system damage, their presence in water, affects human health, ecosystem and quality of drinking water too. India is one of the world's biggest users of pesticides. Water needs to be controlled due to the potential risks associated with pesticide use. Therefore, the production of multi-residue analytical methods for the determination of these pesticides in raw water and treated water(or)normal water is becoming increasingly relevant through various spectroscopic methods HPLC combined with different detectors, Liquid chromatography with Mass spectroscopy, Gas chromatography technique using various detectors (or) in combination with Mass spectroscopy. Extensive research has been carried out on the analysis of pesticide residues in water. Some of the common pesticides found in water by using this methods are organochlorine pesticides (aldrin, α, ß, γ, δ,-HCH, DDE, DDT, Heptachlor, dieldrin, α, ß-endosulfan) organophosphorus pesticides (methyl parathion, dimethoate, malathion, phosphamidon, protenotos, chlorpyrifos, parathion, diazon, bensulide) for overall water quality assessments.

1. India is one of the leading agrochemical suppliers and important chemical business. Among several Indian states, the main user of pesticides is Punjab. The presence of chemical residues in Punjab's water and food goods has been well documented. Sneha Rajput et al. planned the current research to mentally establish the level of chemical pollution in lake water in 11 villages in Amritsar (Punjab), India. A fast and synchronous qualitative and quantitative technique has been developed and validated with high sensitivity for pesticides and chemical residues in water samples. The technique represented here by the RP-HPLC may be a novel technique applicable to the simple, quick and accurate detection of pesticides. It was found that 40.02 percent of water samples have been contaminated with multi-residue pesticides. In eighteen. 18 percent samples, carbofuran was the most abundant chemical that was donated (Rajput *et al.,* 2018).
2. Tastaout, developed a study to estimate the extent of subterranean contamination of waters of the bottom water of mostaganem province by organo-chlorinated pesticides, terribly successful analytical techniques square measure essential for their identification and quantification during this work development of a way for analysis of multi-residues of organo-chlorinated pesticides (aldrin, α, ß, γ, δ-HCH, DDE, DDT, Heptachlor, dieldrin, α, ß-endosulfan) in water of well by activity in GC-MS. It's most

tailored attributable to these spectacular performances in term of by selection and sensibility once a stage of extraction of samples in solid part (SPE). (Tastaout, 2018).

3. The current research was conducted by M.Hasanuzzama et.al to observe a total of seven chemical residues listed under organochlorine, organophosphorus and salt pesticides in three different areas (Nagarpur, Dhaleshwari, Ghazikali).Almost 40 water samples analyzed using HPLC-UV detector. Diazinon (organophosphorus pesticides) was detected at a concentration ranging from 4.11 to 257.91 µg/l in 8 water samples, while malathion was detected at a concentration of 84.64 µg/l in only 1 water sample and chlorpyrifos at a concentration of 37.3 µg/l in only 1 water sample. Carbaryl found at minimal quantity below the limit for detection. DDT or its metabolites (DDE and DDD) was Nil. Apart from the Sahabatpur and Dubaria Union fish lake samples, suspected pesticides were above the appropriate level. Necessary pest management schemes like integrated pest management systems should be introduced.(M.Hasanuzzama *et al*).
4. J.casado et.al developed technique for determination of 250 pesticides in water(surface)using solid-phase extraction (SPE), victimization of hydrophilic-lipophilic balanced chemical compound sorbents (HLB) from matrix of sample and detected by reversed-phase liquid activity (LC) - Orbitrap high-resolution mass chemical analysis (HRMS).Using electrospray ionization, quantitative recoveries and limits of quantification (LOQs) were below 5ng per Lfor 204 of the analysed compounds.MCPA (an aryl oxyalkonic acid herbicide) present at the highest concentration, in excess of 130 ng L-11 for 33 different pesticides. 4 river water samples from rural areas within the southwest of England and retrospective analysis of the LC-HRMS chromatograms found bunch of 9 antimicrobials and veterinary medicinal products collectively present in the samples which are processed. (Casado *et al.*, 2018).
5. For the determination of 3 pesticides (abamectin, imidacloprid and b-cyfluthrin) in water Fuad al-rimawi *et al.* developed qualitatively and quantitatively HPLC technique; these pesticides were widely used for square measurement in crop protection in agriculture and could also be leached to the bottom water. RP technique with a C18 column using a mobile component composed of acetonitrile: 1.5 ml/min flow of water (4:1) and U11 detection at 220 nm is employed.As per new strategies that include validation parameters, this technique was valid. The current method showed a decent dimensionality above ppb range for abamectin (1-1000), for imidacloprid (0.5-1000 ppb), and for ß-cyfluthrin(0.4-1000 ppb) with $r^2 > 0.990$. At 3

concentration levels (5,100 and1000 ppb), the percent recovery 97.6- 101.5 percent for three pesticides at intervals. Relative variance of the area of 6 replicates of each pesticide injection at 3 conc. The level (5.0, 100.0, 1000.0 ppb) was less than 1 percent reflecting the strategy's accuracy. This technique is low in the LOQ of three pesticides victimization (1.0, 0.5and0.4 ppb) for three pesticides. (Fuad al-rimawi *et al.*, 2016).

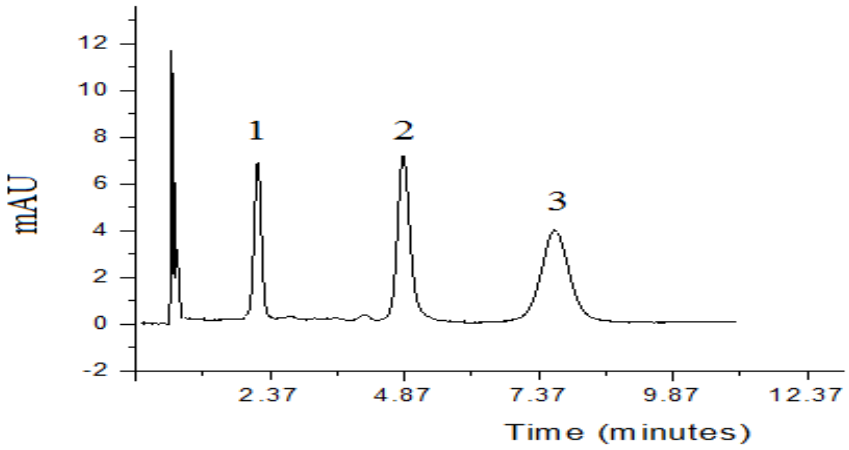

Figure 1: Chromatogram Imidacloprid (1), β-cyfluthrin(2), abamectin(3)

6. Indrajit *et al.* developed and validated and analysed samples of drinking water with residual pesticides using Agilent for routine analysis.According to the Bureau of Indian Standards (BIS), Using Agilent 7000C Triple Quadrupole GC/MS determines lower detection limits in combination with inert sample path and backflushingGC column. For the determination of twenty eight pesticides in water, a quick, fast, economical and economical methodology was established. The approach demonstrates smart sensitivity, accuracy, precision and quick analysis permits. According to BIS specifications the results showed that chemical residues are detected less than most necessary residue levels (MRL). For all pesticides, LOD 5 ng/L and LOQ 10 ng/L were recorded. The approach is applicable and suitable for its use even in an laboratory which is of extremely restricted for estimation of water from surface, drinking, and packaged drinkable pesticides (Indrajit *et al.,* 2016).

7. The SPE disk extraction water exploitation analysis may be a technique developed by Viviane *et al.* that decreases solvent usage, providing a better alternative economically and ecologically. With this rapid extraction technique, 30 pesticides demonstrated smart recoveries by integrating large volume injection and exploitation analyte protectants to boost action resolution (Carbofuran, Propanil ,Carbaryl , Alachlor ,BentazoneDieldrin, Endrin, Endosulfan, Bifenthrin, Boscalid, and Fenvalerate). The technique was introduced to guard the merchandise for 14 brands of bottled mineral

water. None contained higher than the LOQ of any of the target pesticides (Viviane *et al.*, 2015).

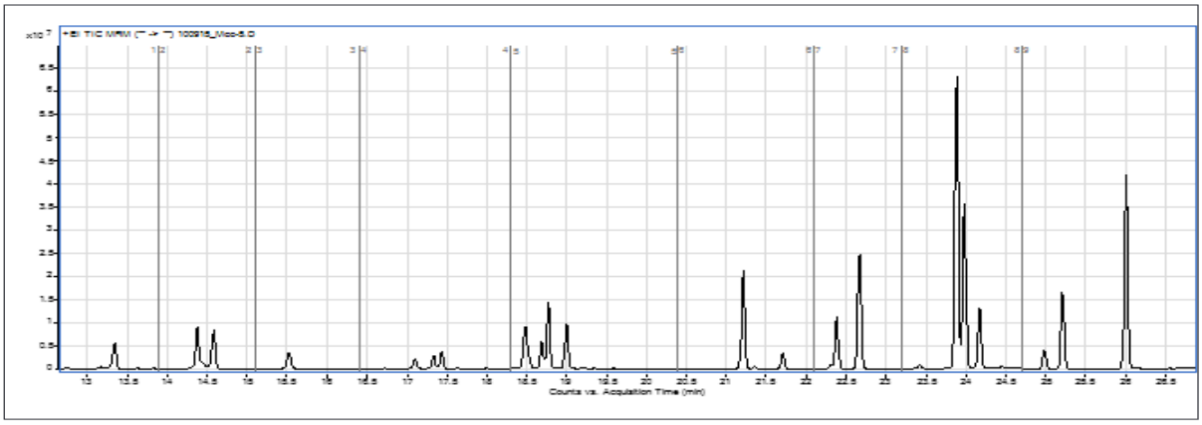

Figure 2: Extracted pesticides standard TIC

8. Twenty organochlorine pesticides were analyzed by Ismail *et al.* (2014) using the SPME-GC-ECD process, obtained from areas Peshawar, KPK, and Pakistan. For all pesticides, this approach showed a smart R^2 values ranging from 0.9933 - 0.9999. A number of concentrations (60-150 mg L-1) were allocated to the Gamma irradiation decomposition of monocrotophos solution and their removal capacity was investigated. For 60 mg per L dose of 1200 Gy, gamma irradiation demonstrated 100 percent degradation. During this research, the dose constants examined ranged from 1.4-10-3 to 3.0-10-3 Gy-11 (radiation chemical yield). Examination of saturated N_2 and N_2O solutions and radical scavengers done and showed that monocrotophos degradation mainly initiated via radicals •OH. NO3-, NH4+ and PO_4^{3-} inorganic by-products were quantitatively calculated by particle behaviour (Ismail *et al.*, 2014).

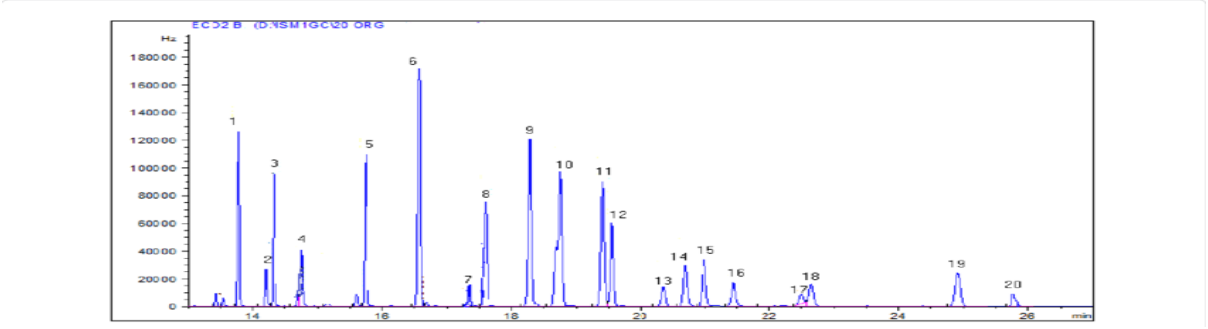

Fig 3.(1) alpha-BHC; (2) β-BHC; (3) γ-BHC; (4) δ-BHC; (5) heptachlor; (6) aldrin; (7) heptachlor epoxide; (8) γ-Chlordane; (9) alpha-Chlordane; (10) endosulfan I; (11) 4,4'-DDE; (12) dieldrin; (13) endrin; (14) endosulfan I; (15) 4,4'-DDD; (16) endrin aldehyde; (17) endrin ketone; (18) 4,4'-DDT; (19) sulfate of endosulfan; (20) methoxychlor

9. The U.S. Geologic Survey (USGS) has developed a system for 119 pesticides identification in support of the National Water Quality Assessment (NAWQA) Program. Via prior prioritization. Accelerated solvent extraction method (ASE®) sediment samples and even interesting compounds isolated by moving extracts by gel-permeation (GPC) high-performance liquid natural action (HPLC).Chromatographically isolated and quantified and identified, by GC-MS of pesticides from sediment-sample extracts. Recoveries at 10 micrograms per unit weight (µg/kg) Ranges 75-102 percent dry weight; RSD found at 3- 13 percent (Michelle *et al.*, 2012).

10. Sonica *et al.*, research study developed a method for determining a specific cluster of pesticides in water (tap and groundwater) using LC ESI MS. Dimethoate, carbaryl, simazine, atrazine, ametryne, tebuthiurone, diurone and weedkiller (pesticides) were isolated with di-chloromethane liquid extraction and analyzed with 1 ml/min gradient extraction on the reverse section column, C-18. Noted 89 - 112 percent recovery study for water (RSD ≤ 10 percent) and from 76 percent to 98 percent for water (RSD ≤ 6 percent).Using selected ion monitoring (SIM) LOQ levels was minimal enough to reach a target level of 0.1 µg per L at 500 fold per concentration under international legislation (Sonica *et al.*, 2004).

11. Due to the poor quality of municipal facilities, bottled water has become a necessity in people's lives as exposure to pesticides through drinking could have possible health effects, Professor HB Mathur *et al.*, set up a study to examine the existence of organochlorine (aldrin, α,β,γ,δ,-HCH, DDE, DDT, Heptachlor, dieldrin, α,β-endosulfan) and organophosphorus pesticides (methyl parathion,dimethoate,malathion,phosphamidon, protenotos, chlorpyrifos, parathion, diazon, bensulide) residues in drinking water marketed by victimization GC Coupled with electron capture detectors showed in Fig 4. in the city and its conterminous room. Looking at the findings, the criteria for acceptable limits for individual and total chemicals recommended by the European Economic Community for Europe, which set quantified limits for residues of pesticides other than BIS, were compared. It sets the most permissible concentration for individual chemicals at 0.1 micrograms per l (µg/l) or 0.0001 milligrams per l (mg/l) and 0.5 micrograms per l (µg/l) or 0.0005 milligrams per l (mg/l) for total permissible chemical residues (Prof HB Mathur *et al.*, 2003).

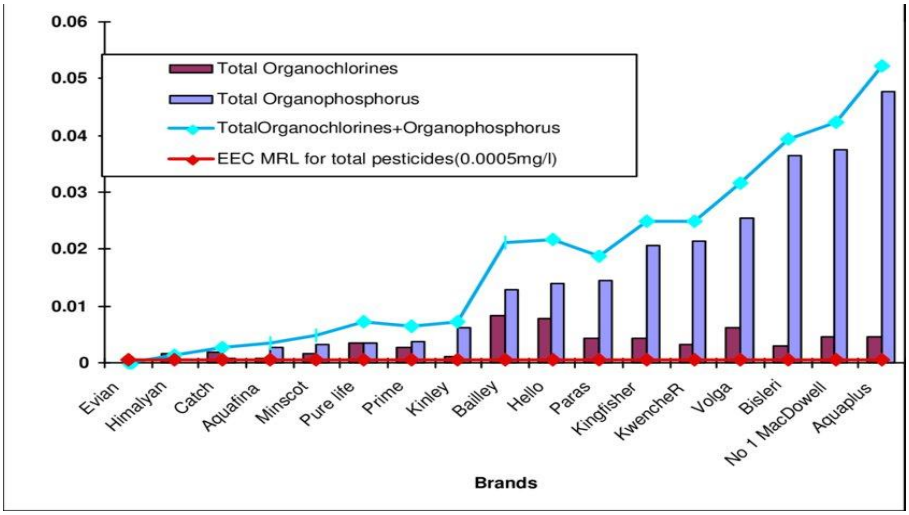

Figure 4: Graph of different pesticides in different brands of water

12. Authors Jian-yinghu *et al.*, through victimization LC/APCI/MS, developed a multi-residue analytical technique for evaluating treated water and its raw water for unstable and polar pesticides or polar pesticides, which are notrelevant for a routine GC/MS procedure. Positive-ion modeDetection of neutral/simple pesticides the LOD ranges at 10-50 mg/l and negative-ion mode for acidic pesticides detected at50- 250 mg/. Unlike Dicamba (500 mg/l) and benazolin (1000 mg/l)Fig 5 Demonstrates unstable and/or polar pesticides 31 in number with neutralor basic pesticides 14 in number and acidic seventeen in number. Through the in-source collision-induced decomposition (CID) process, the structural data was dramatically improved. Extraction recoveries of pesticides were found to be over seventy-two in treated water and sixty-seven in raw water, with the exception of thiophanatemethyl and sethoxydim, have not passed and recovered. Once the multi-residue analytical technique for Niigata's public water treatment plant in Japan was used for research, water which are treated about 8 pesticides and treatment plant of raw water about 10 found (Jian-yinghu *et al.*, 1999).

13. Tuijapihlstrom *et al.* measured the concentration of pesticides in the river using solid phase extraction method. Polymer-divinylstyrene extraction columns were used toobtain samples andethyl acetate used for extraction and injected into columns of capillary gas chromatography which is linked to different detectors. As it is a simple extraction procedure Analysis period is short. Pesticides like Artarzine, tenitrothion, bitenthrin, metazochlor, and entenvalerate are some of them examined. 0.05-0.1µg/l(LOQ)concentration used based on pesticides to be examined. In present study, Samples collected from Sweden agriculture treated water because of their possible

presence in ground and surface water of Swedish, the mean of the recoveries for the system was 85 percent and LOD was found to be 0.01-0.02µg calculated from standard solution S/N ratio 3 (Pihlstrom *et al.*, 1997, Tuija) [13].

14. Joseph P.Romano *et al.* has developed a new analytical method for the study of seven common pesticides present in drinking water using HPLC using solid phase extraction (SPE) off-line for sample preconcentration and showing simultaneous detection of PDA/MS. For HPLC Method optimization, seven popular pesticides (2, 4D, warfarin, dinoseb, atrazine, carbaryl, diuron, linuron) were selected.

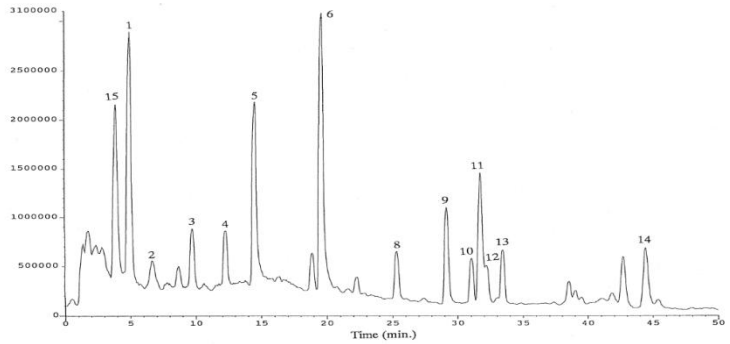

Figure 5: SIM LC/MS chromatogram of 14 neutral/basic pesticides and asulam obtained by analyzing 11 of a treated water sample spiked at the specific 1 mg/l level with 31 pesticides

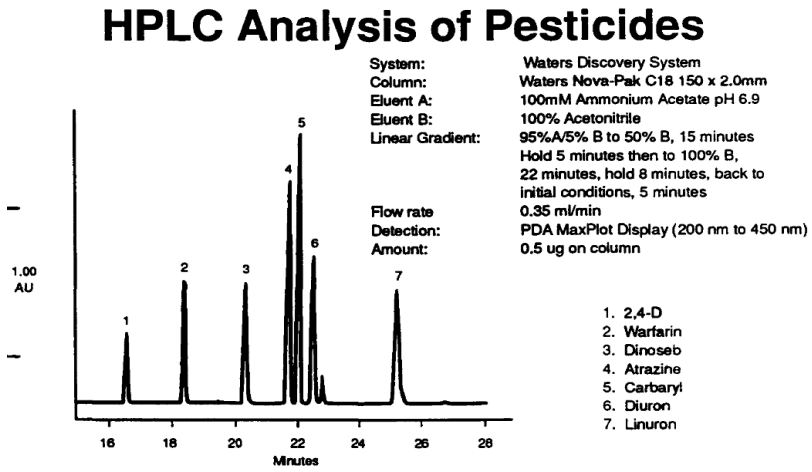

Figure 6: Chromatograph of 7 different pesticides in water by HPLC

Using the water discovery scheme, chromatographic separation was first established. The optimized chromatographic conditions used the column of Nova-Pak C18 narrow bore waters to decrease solvent consumption. A mobile step consisting of a binary linear gradient of ammonium acetate to acetonitrile created the strongest separation of the pesticides in less than 26 minutes the peak is shown in Fig 6 (Joseph p.romano *et al.*, 1995).

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SEDIMENT GEOCHEMISTRY: AS A TOOL IN PRESENT AND PAST LAKE ECOLOGICAL STUDIES

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Abstract:

Lake ecological studies are fast gaining importance throughout the globe in view of fast depletion in quality and quantity of fresh water. The ecological studies can be carried out using water and sediment samples from lake. The lake sediment study is of extreme importance as it, not only provides the past and present ecological conditions of lake but also provides sufficient evidences to predict the possible future ecological changes the lake will be going to experienced. This can be done through multiproxy study of lake sediment. Sediment geochemistry is one such proxy. Sediment geochemistry is used to understand climatic variations, environmental changes, conditions of deposition and possible source of sediments or their provenance.

Introduction:

Lakes are under increasing threat due to the various impacts which often combined, such as a) Nutrient (N and P) enrichment from domestic and agricultural sources, b) Pollution from organic compounds, toxic metals (Pb, Hg, Cd etc.) and radionuclides, c) Acid deposition (S and N) from fossil fuel combustion, d) Accelerated infill from catchment soil erosion, e) Climate change from greenhouse gases etc.

Lake sediments are used in different manners. There is a sophisticated and rapidly growing palaeolimnological studies all over the world. The role of Palaeolimnologists in contemporary discussion on water resources and water quality is immense. There are various applications of palaeolimnology in lake ecosystem research and management studies such as, for providing early warning for future changes, giving information on baseline states and on natural variability, allows calculations of the degree of change over baselines, helps to define sustainable recovery targets i.e. through analogue matching,

enables current trends, directions and rates of change to be defined, enables the causes of change to be explored through hypothesis generation and testing and enables both empirical and process-based models to be evaluated.

Identifying the separate and combined influences of these processes is thus a central challenge for palaeolimnology and a key reason for the use of palaeolimnological techniques in lake management. In this context, lake sediment records enable atmospherically-transported trace pollutants to be modelled not only on to lake surfaces but extrapolated, using lake networks, to all spatial scales. And, for remote regions lake sediment sampling is probably the most powerful method of assessing the impact and extent of air pollution.

In addition, the lake sediment archive can be used to answer other questions concerned more with catchment and atmospheric changes, than lake change i.e. Changes in catchment soil degradation and erosion, export of nutrients from catchment soils and land cover and deposition of atmospherically transported pollutants, especially radionuclides, fly ash, metals and persistent organic compounds.

The sediments deposited at Lake Bottom consist of variety of information that can be used to understand the past conditions of a lake, its watershed, and climate (Meyers and Teranes, 2001). The reconstruction of paleoclimates using lake sediments is very common throughout the world (Bierman *et al.*, 1997; Brown, 1999; McFadden *et al.*, 2005; Schelske and Hodell, 1991, Mullins *et al.*, 2011). The study of the lake sediments and lake water quality forms the basis of limnology. The limnology can be defined as study of both freshwater and saline inland water. It is important to emphasize that limnology correctly encompasses an integration of physical, chemical and biological components of inland aquatic ecosystems (Hammer, 1986). Lakes are mostly fed up by some river or streams and hence the lake consist of autochthonous sediments (sediments derived within the lake) and allochthonous sediments (sediments derived from outside the lake) (Smol, 2008). A stream serves as the major source of allochthonous sediments. The allochothonous sediments carry with them the dissolved chemicals and particulate inorganic and organic matter (OM).The allochothonous sediments on the other hand have with them a significant assemblage of remains of macrophytes, phytoplankton, zooplankton, bacteria, micro organism and aquatic invertebrates, which thrives at that environmental or ecological condition. Hence, the source of autochthonous material includes biological activity and chemical precipitation within the lake. Pollens, dust, aerosols, spheroidal carbonaceous particles and other particulates can also fall directly on the lake and accumulate in sediment, at smaller quantity than stream inputs (Smol, 2008).The geology of the lake watershed, climate, and land use including the anthropogenic activity around the lake and its watershed directly

affect the quality and quantity of material that enters a lake ecosystem (Cohen, 2003).The past changes in the pH, salinity, nutrient status, climatic changes and lake level fluctuations can be inferred by studying the sediment geochemistry in the core extracted from the lakes/reservoirs with the radioactive isotope dating such as ^{137}Cs and ^{210}Pb (Hall and Smol, 1999 and Dixit *et al.*, 1999). Paleolimnology uses the physical and chemical information archived in lake sediments to reconstruct and interpret past environmental conditions over time scales (Fig. 1a and b) (Smol, 2008).

a **b**

Figure 1. a) Scenic View of Bodalkasa Lake, b) Satellite image of the Bodalkasa Lake showing locations of grab samples and core sample, District Gondia, Maharashtra

Review of literature:

To study the record of the present condition and past history of lake, lake sediment surface and core samples have been widely used all over the world. Lake sediments provide an important evidence of the past. Sediment core profiles have also been used to evaluate lake contamination such as heavy metals (Johnson, 1989; Swain *et al.*, 1992 and Sanei *et al.*, 2000). Many workers have worked on various aspects of sediment geochemistry from the lake core sediments (Dean, 1993; Dean, 2002). Cohen (2003) found that fluctuations in lake level and circulation patterns can affect the amount and location of sediment deposition within a lake and physical, chemical and biological processes can alter the sediment once deposited. OM is particularly vulnerable to post-burial diagenesis and can undergo decomposition, reducing total mass and releasing N and P back into the water

column, where it may be re deposited following use by other organisms (Meyers, 1997). Burrowing organisms, chemical dissolution, and compaction are other types of sediment alteration (Engstrom and Wright, 1983). Despite this, the primary signature of the OM is often preserved, maintaining relative differences throughout the sediment column (Meyers and Ishiwatari, 1995).

Methodology:

Grab Sampling:

The surface sediment (grab) samples has to be taken from the same place from where the water samples have to be collected along the maximum length of the lake. The grab sample can be taken using the grab sampler or traditionally by using a heavy stainless steel bucket that has to be thrown in water till it reaches the bottom. The boat should be then slightly moved forward, so that the bucket gets drag at the bottom and sediments could be collected in the bucket. The sediment samples from bucket were then collected by using mug and spatula which should be later on kept in a polythene zip-lock bag with proper labels by permanent marker.

Core Sampling:

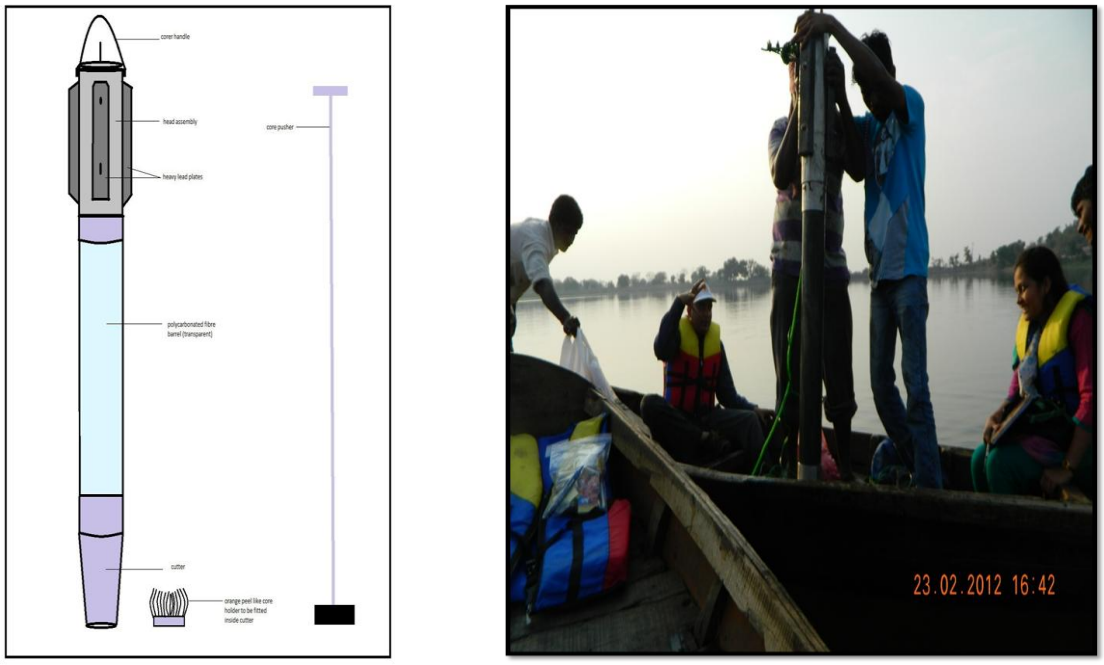

Figure 2: Schematic diagram of gravity corer and original photograph of core sampling on boat at Bodalkasa lake

Core sampling is the most important part of the paleolimnological study. Coring can be carried out using a Gravity corer that is made up of various parts i.e., Head assembly,

barrel, orange peel part, cutter and core pusher (Fig. 2). Head assembly is the heaviest part of the instrument. It is made up of stainless steel and it is loaded with 4 lead plates of approx 25 kg to create the necessary force and pressure for taking sediment core. Barrel is attached to the head assembly. The sediment core is collected in the barrel. When the corer gets collided with the bottom of lake, it penetrates in to the sediments and helps to get core of desired size and shape. After assembling the different parts of gravity corer it is tied with the high strength wired rope. Then it is kept in vertical position at the side of the boat and then released. Due to free fall under the influence of gravity and high load, it moves towards the lake bottom at high speed and penetrate into the sediments at the bottom of lake. After few minutes the gravity corer should be pulled up with the help of wired rope. After pulling it on boat, corer should be kept in vertical position. Then, the head assembly and core blade has to be detached from the assembly and the barrel top and bottom were tightly closed with the PVC caps. The barrel has to be kept in vertical position for 6 to 7 days before cutting the core sample. When the sample will be sufficiently dried so that it could not be mixed after laying the barrel in horizontal position, the sediment core has to be pushed out from the barrel using core pusher on a plastic coated graph paper with suitably marked top and bottom and with graduated scale. Then the sediment core was vertically cut into two equal halves and core profile was drawn with details and photographed. These halves had to be further sub sectioned then and cut at an interval of 0.5 cm or 1 cm along the length of the core. One half of the core was retained as archives while another half was used for geochemical analysis.

a) Sample preparation and Analysis for Major and Trace element (XRF): For X-Ray Flourescence analysis, the grab and core sediment samples must be crushed and powdered to -170 mesh size that can be transformed to pellets using hand pellet press. These pellets will be then directly used for XRF analysis. The major oxides such as Na_2O, MgO, Al_2O_3, SiO_2, P_2O_5, K_2O, CaO, TiO_2, MnO, and Fe_2O_3 and minor elements such as Rb, V, Ba, Sc, Ni, Cr, Cu, Zn, Ga, U, Nb, Zr, Sr, Y, Pb, Th and Co can be analyzed using XRF having suitable caliberation and accepted standards.
b) Sample preparation and Analysis for REE using ICPMS: The sediment samples are also to be analyzed for La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu using ICPMS (Model: Perkin Elmer SCIE x Elan DRCe). For analysing the sediment samples by ICPMS the sediment samples were first transformed to aliquots. For preparing aliquots 0.04 gm of sample needs to be taken in a teflon crucible and moistened with doubled distilled water. 10 ml of acid mixture (HF and $HClO_4$in 3 :1) has to be added to it and should be heated on a hot plate for two hours with lid on it.

After acid digestion the lid has to be removed and the contents should be evaporated at 200° C untill a crystalline paste results. 10 ml of acid mixture have to be added again and left to get evaporated to incipient dryness. Then 2ml of $HClO_4$ acid should be added and contents should again let to get evaporated till dryness. 10 ml of HNO_3 acid is then added, warmed gently to get a clear solution. It is then made up to 100 ml volume with double distilled water.

Results:

The results obtained from XRF and ICPMS analysis has to be systematically presented in tabular form. The results of major elements will be in percentage while that for Trace and REE will be in ppm. These results have to be normalized with crustal abundance of these elements to know the anamoly in their concentration in the lake sediments. The results have to be further subjected to various statistical analysis like correlation coefficient and regression analysis. The results of the grab samples should be graphically represented in the form of bar diagrams, whereas the results of the core samples have to be represented as vertical profiles using various softwares. Hierarchic dendograms have to be prepared to know the affinity of one element with the other and also their possible source. The graphical representation of concentration of various elements and their oxides ease in understanding the trend of change in deposition of these elements during the history of lake and their possible source. It will also help in interpretation of the climatic changes which the lake experienced during the past and also its comparison with the present.

Discussion:

The geochemical analysis must be carried out to know the source and variation in the sediment input and to compare present day input of sediments with those of the past. The quantity and quality of geochemical variables helps to understand which watershed activities have the greatest impact on the lake. The variables aluminum, potassium and titanium are indicative of detrital aluminosilicate materials and thus changes in their profiles points changes in soil erosion rates (Warrier and Shankar, 2009; Veena *et al.*, 2014). Potassium is found in both soils and synthetic fertilizers. Therefore, its profile will reflect changes both from soil erosion and the addition of commercial fertilizers in the watershed. The trophic status or the nutrient status can be determined by the concentration of phosphorous and nitrogen since phosphorus and nitrogen are important for plant growth, especially algae and aquatic plants, whereas the lake productivity is reflected

in the profiles of organic matter. The organic matter determination includes a number of elements, especially carbon (Garrison and Laliberte, 2010).

Table 1: Selected chemical indicators of watershed or in lake processes (Garrison and Laliberte, 2010)

Process	Chemical Variable
Soil erosion	aluminum, potassium, titanium
Synthetic fertilizer	Potassium
Urban	zinc, copper
Ore smelting	zinc, cadmium, copper
Nutrients	phosphorus, nitrogen
Lake productivity	Organic matter

The intensity of the impact on the lakes of different watershed can be analyzed using geochemical variables (Table 1; Garrison, 2000a, b;2003, 2005a, 2005b, 2006a, b, 2008; Garrison and Laliberte, 2007, 2010). The chemical elements titanium (Ti) and aluminum (Al) are derived from detrital aluminosilicates and thereby the fluctuations in their profiles will suggest the transformation in rate of soil erosion (Garrison and Laliberte, 2010). Phosphorous and nitrogen are the important nutrient for the growth of aquatic plants algae. Thus, the organic matter profiles will indicate the lake productivity in general. The synthetic fertilizers and soils contain the potassium (K). Thus, the soil erosion and the input of commercial fertilizers in the watershed can be distinguished from the variation in the profile of potassium (K). The urban runoff is mostly accompanied with zinc (Zn) as it is a component of galvanized roofs and tires (Garrison and Laliberte, 2007). The addition from smelting of lead-zinc-ores is seen from zinc (Zn) profile of the cores (Dean, 2002). The use of soil modifications for the development of amenities is reflected from the calcium(Ca) profiles and the changes in the oxygen levels in the bottom waters is indicated by the manganese (Mn) profiles (Garrison, 2008). The calculation of the rate of accumulation of some geochemical elements of both the lakes was done by adding the geochemical concentrations with the rate of sedimentation. The elemental deposition in the lakes through time would be ascertained from the accumulation rate. The accumulation rate gives information about the impact of watershed processes on the lake ecosystem (Garrison and Laliberte, 2007, 2010). The accumulation rate can be calculated using the absolute dating techniques like ^{210}Pb and ^{137}Cs. To distinguish the anthropogenic inputs other than mineral sediments the selected geochemical elements from the both the lakes

were normalized to aluminum (Al). Thus, the factors which influence the lakes in addition to sediment/soil input from the watershed are known.

Case Study: Bodalkasa Lake:

The Bodhalkasa Lake (Fig. 1a) falls between latitudes 21° 2' to 21° 22' N and longitude 80° 01' to 80° 03' E. It has a circumference of 30 kms. It has a small embankment of 500 m long and 8m high. The average depth of the lake is 8 m. The overflow empties into the Bodhalkasa river and finally in to the Wainganga River.

Geological setting and lithological observation:

Geologically, the study area comprises of the Amgaon Gneissic Complex, the Tirodi Gnessic Complex, the Bailadila Group, the Nandangaon Group, the Dongargarh Granite, the Sakoli Group, the Sausar Group and the Khairagarh Group.The rocks of the Vindhyan Supergroup equivalent (Neoproterozoic, 1600-900 M. Y.) and the Gondwana Supergroup (Permocarboniferous 215-275 M. Y.) occur as isolated outcrops overlying the rocks of the Amgon Gneissic Complex in the southern part. The Bodalkasa lake is geologically surrounded by meta gabbros, cherty quartzite, tuffaceous phyllites, carbonaceous phyllites, quartzites, gritty quartzites, conglomerates and granitic gneiss (DRM, 2000)

Results and Discussion:

The surface sediments of the Bodhalkasa Lake clearly show the abundance of SiO_2, Al_2O_3 and Fe_2O_3, MgO, K_2O, TiO_2, CaO and Cr_2O_3 and ZrO_2 during the Pre and Post monsoon seasons with their varying concentrations. The elements which are increased in their content during the post monsoon season are SiO_2, TiO_2, CaO, ZrO_2 and Cr_2O_3. The enhanced concentration of TiO_2 and SiO_2 supports the increased soil erosion in the watershed of the Bodhalkasa Lake. The Cr_2O_3, NiO and ZrO_2 rich soils, which are formed from the weathering of rocks associated with these metals, is one of the reasons behind increased content of these elements in the surface sediments (grab) of the Bodhalkasa Lake. Concentrations of CaO and TiO_2 in this lake are increased during post monsoon season. The heavy metal concentrations particularly, Cr_2O_3, NiO, ZnO and ZrO_2 are also enhanced during the post monsoon season. The enriched values of these heavy metals again indicate the accelerated rate of soil erosion which contains their higher concentration. This again suggests the existence of Cr_2O_3 and ZnO associated rocks of the Sakoli Group in the watershed and Tirodi gneisses (Chawade and Naik, 2003). The enrichment of Cr_2O_3 may also be the result of the weathering of the Pre-Sakoli rocks particularly meta - ultramafic rocks with chromite pods (Chawade and Naik, 2003).

Conclusion:

a) The elements which are increased in their content during the post monsoon season are SiO_2, TiO_2, CaO, ZrO_2 and Cr_2O_3. The enhanced concentration of TiO_2 and SiO_2 supports the increased soil erosion in the watershed of the Bodhalkasa Lake.

b) The heavy metal concentrations particularly Cr_2O_3, NiO, ZnO and ZrO_2 are enhanced during the post monsoon season. The enriched values of these heavy metals again indicate the accelerated rate of soil erosion which contains their higher concentration. The sediment cores of the Bodhalkasa Lake encompasses historical accumulation of metal concentrations covering approximately 138 years by assuming the constant accumulation rate down the core.

c) The comparison of the correlation coefficient of elements in the Bodhalkasa Lake and the polluted 10 lakes in the Vidharbha point that the level of pollution in the Bodhalkasa Lake for Ti, Al, Fe, P, Cu, Zn, and Mn are lower than the polluted lakes of the urban lakes of the Vidharbha Region.

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A REVIEW ON SIGNIFICANCE OF DIATOMS IN LAKE ECOLOGICAL STUDIES

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Abstract:

Lake ecological studies can be achieved using various proxies among which using diatom are very significant. Diatoms are very much in use as indicators of environmental conditions in lakes because of their sensitivity to limnological variables as nutrient concentration, pH, conductivity and their extraordinary preservation in fossil deposits, diatoms are powerful indicators of environmental changes in aquatic ecosystem. The change in the abundance of any particular species from one season to another can be used to interpret the affinity of species to that particular water quality condition. Similarly, the sudden disappearance or appearance of any diatom species or variation in their abundance during the past can also be satisfactorily used to interpret the past ecological condition of the lake.

Introduction:

Diatoms in particular are useful ecological indicators because they are found in abundance in most lotic ecosystems. The great number of diatom species provides multiple, sensitive indicators of environmental change and the specific conditions of their habitat. Diatom species are differentially adapted to a wide range of ecological conditions. Because diatoms are sensitive to water chemistry and lake depth (Haworth, 1972), diatom fossils are an important source of information about environmental change in lakes. Paleoecologists have reconstructed past pH and salt concentration using the environmental preferences of diatom taxa (Hustedt, 1937) used a surface-sample to reconstruct the post aquatic environment showed that surface sample diatom assemblages are good indicators of

modern lake environment. This study is to reconstruct the regional climatic changes of Holocene times. Paleolemnological techniques, using diatom assemblages as biomonitors of aquatic change, provide an effective approach to supply missing historical data (Battarbee *et al.,* 1990: Dixit *et al.,* 1992a, Charles *et al.,* 1994: Dixit and smol 1994). Sedimentary diatom assemblages have been used successfully to evaluate water quality trends resulting from Lake Acidification and concentrations of dissolved organic carbon (DOC), Lake Eutrophication, salinity associated with climatic changes (Anderson *et al.,* 1993, Christic and Smol 1993: Hall and Smol 1993).

Diatoms (Class-Bacillariophyceae) have been widely used as indicators of environmental conditions in lakes (Hall and Smol, 1999). Diatoms are microscopic, single celled algae that build complex, Beautiful cell walls of silica. These tiny algae range between 2µm to 500µm in length or diameter. Because of their sensitivity to limnological variables as nutrient concentration, pH, conductivity and their extraordinary preservation in fossil deposits, diatoms are powerful indicators of environmental changes in aquatic ecosystem (John, 2008). The diatoms are abundant in the lakes, rivers and other fresh water bodies. Several studies have indicated that the distribution of such sensitive diatoms taxa in the surface sediments can be correlated to know the trophic status of the water bodies (Fritz *et al.*, 1993; Hall and Smol, 1999). The Diatoms can also be used to develop a long term series data on the trophic status of the water bodies and also on its recovery. The pre disturbance water chemistry can also be analyzed from the fossil diatoms of sediment core as the target for the rehabilitation of the lake (Hall and Smol, 1999). The past changes in the pH, salinity, nutrient status, climatic changes and lake level fluctuation can be inferred by studying the sediment geochemistry and diatoms in the core extracted from the reservoirs with the radioactive isotope dating such as ^{137}Cs and ^{210}Pb (Kumar *et al.,* 2007). Paleolimnology uses the physical, chemical and biological information archived in lake sediments to reconstruct and interpret past environmental conditions over many time scales (Smol, 2008).

Methodology:

Diatoms for ecological studies can be retrieved from water samples as well as sediment samples. The methodology involves a) field work for sample collection (both water and surface samples), b) Processing/Sample preparation for extraction of diatoms, making diatom slides and study the diatom slides under biological microscope followed by microphotography, identification and counting. A systematic sampling of water sample from the lakes has to be carried out during two seasons pre-monsoon and post-monsoon.

The surface sediment or grab sediments from the lake surface bottom has to be collected from 3-6 different parts of the lake along its maximum length and from the same position from where the water samples were collected. In case if paleoecological condition of the lake is to be studied a vertical core sediment sample has to collected with the help of pvc pipe (when lake is almost dried during summer season) or with the help of gravity corer if lake is holding enough water throughout the year. The core sediment samples collected thus has to be vertically cut and divided into two equal halves and photographed. These halves would then have to be further sub sectioned and cut at an interval of 1cm. These sub sectioned sediment samples has to be processed for preparing diatom slide. The microphotography and identification of diatom taxa were done to generate diatom inferred tropic status.

Maceration and Analysis for Diatom:

5 gm of sediment sample obtained through cone quartering was taken in a beaker for isolating siliceous matter especially diatom. 10 ml of HCL (10%) has to be added to the sample to remove carbonate. After dissolution of carbonate, the samples should be washed with distilled water several times (2 to 4 times at 4 hours of intervals). After washing the samples with distill water 10 ml of H_2O_2 (Hydrogen peroxide) acid should be added to remove organic material. The samples could also be boiled for 1 -3 minute to quicken the process and then distilled water is added to wash the H_2O_2. The acidified sample have to be repeatedly treated with distilled water till all the traces of H_2O_2 were removed completely (Battarbee, 1986). For making a diatom slide, the prepared or macerated samples have to be taken in a dropper and put on a cover slip of 22mm x 40mm and spread by needle and allowed to dry naturally. After drying, the cover slips have to be mounted on the glass slides using DPX mountant and should be dried again for few days. After the slides were perfectly dried the slides have to be observed under biological research microscope at 20x, 40x, 63xand 100x using oil immersion and photographed. Calibrated scale has to be given on one side of the diatom microphotograph to know the size of the diatom. After microphotography the plates of diatoms were prepared followed by identification up to the species level.

Results:

The diatom must be identified up to species level or at least up to generic level from the plates that would be made after diatom microphotography. A systematic description and systematic classification has to be made for making interpretation and before reaching

to final conclusion about the ecological condition that the diatom species recovered thrives in.

Systematic description:

A Systematic Description of Diatom has to be written which deals with the taxonomy of diatoms recovered from surface and core sediments respectively. The widely employed scheme of classification is based on the similarity of phenetic characteristics proposed by Round *et al.*(1990). Taxonomic work done by Mann (1999) can also be considered for classification. Identification of the species can be based on the comparison with www.environment_agency.gov. uk; John (1986, 2010, 2014); Gandhi (1998); Sarode and Kamat (1984), along with various research publications.

Systematic classification:

The systematic of diatoms has been entirely depending upon the characteristics of frustules i. e. size, shape, structure, symmetry and nature of raphae, density of striae etc. The classification proposed by Round *et al.* (1990) adopted by the International Journal of Diatom Research i. e. the official journal of the International Society of Diatom Researchers and the same could be followed to make systematic classification. The nomenclature of some of the specimens may be kept open up to generic level whose species explanation if not match with any other earlier report.

The systematic classifications of diatoms proposed by John (2014) may serve as important way of classification (Fig. 1).

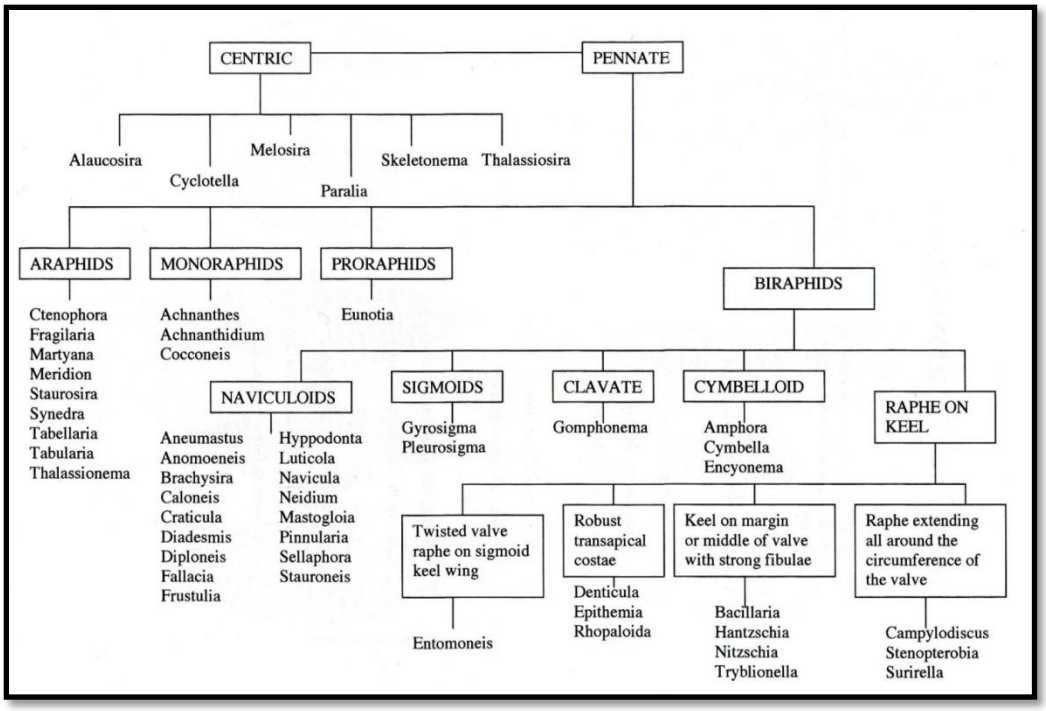

Figure 1: Showing the Systematic Classification of diatom (John, 2014)

After writing the systematic description and classification of diatoms, the counting of species has to be carried out to know the abundance of species. The results so obtained after counting has to be represented graphically in Excel specifically for grab sediment samples. To analyze the change in species variation during past a vertical profile has to be drawn in Coniss.

Discussion:

The results obtained after diatom counting has to be converted in to percentage variation. The ratio of planktonic and benthic taxa (P/B) (Centric: Pennate) has to be calculated then. The most abundant species obtained from the grab sediment samples has to be compared with the physicochemical quality of water samples obtained from same location or from same lake during the pre-monsoon or post monsoon season. This will indicate as which species thrives best under which specific environment and their sensitivity to particular pH, conductivity, alkalinity and temperature condition. It could also help us to know the pollution level of the lake and their present trophic status.

To study or to interpret the past ecological or paleoenvironmental condition a stratigraphic profile of the biotic indicators, Hill's N2, benthic and planktonic diatom percentage and means linear diameter (MLD) of diatom for the lakes has to be drawn. The mean linear diameter can be made by classifying the MLD into four different classes as <10µm, 10 -25 µm, 25-50 µm and >50 µm. The analysis of the effective number of taxa in each sample is considered as diatom diversity (Hill, 1973). The diatom diversity for each sample i.e. Hill's N2 for the lakes can be calculated using the program C2 1.5 (Juggins, 2007). The substantial change in the species composition can be distinguished by program CONISS.

Case Study: Navegaonbandh Lake

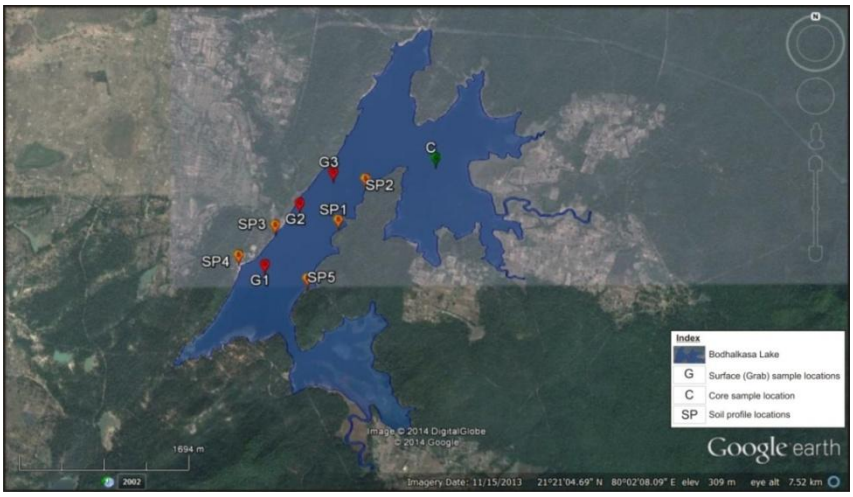

Figure 2: Satellite image of Navegaon band Lake Showing Sample location (Image after Google Earth)

The Navegaon Lake lies between latitude 20°53' to 20°56'N and longitude 80°06' to 80°09'E. It has a circumference of 27 kms and a water surface of about 20 sq. kms. The average depth of the lake is about 12 m increasing at place to 30 m. The catchment area is about 90 sq. kms.

Geological setting and lithological observation:

Geologically, the study area comprises of the Amgaon Gneissic Complex, the Tirodi Gnessic Complex, the Bailadila Group, the Nandangaon Group, the Dongargarh Granite, the Sakoli Group, the Sausar Group and the Khairagarh Group. The rocks of the Vindhyan Supergroup equivalent (Neoproterozoic, 1600-900 M. Y.) and the Gondwana Supergroup (Permocarboniferous 215-275 M. Y.) occur as isolated outcrops overlying the rocks of the Amgaon Gneissic Complex in the southern part. The Navegaon Bandh lake is geologically surrounded by granitic gneiss, pockets of laterites, quartz, phyllites, brecciated quartz vein, phyllite, pelitic schist, basic andesite and epidiorite (DRM, 2000)

Results and Discussion

The surface (grab) sediments collected during the Pre and Post monsoon seasons were investigated for understanding the diatom diversity of the Navegaon Bandh lakes and the related water quality was compared with the relatively most abundant species. The surface sediment of the Navegaon Bandh Lake revealed maximum abundance of *Rhopalodia gibberula, Rhopalodia musculus, Gomphonema parvalum, Gomphonema undulatum* and *Rhopalodia* sp. The average values of physicochemical parameters which support the dominance of above diatoms in the Navegaon Bandh Lake during the Pre-monsoon and post monsoon season are shown in Table 1.The surface sediments study of the Navegaon Bandh Lake revealed the presence of 37 diatom species belonging to 20 genera in pre-monsoon samples out of these 5 species are centric and 32 species are pennate. Post monsoon samples reveal presence of 39 diatom species belonging to 18 genera, out of these 06 species are centric and 33 species are pennate. The abundant diatom species are *Rhopalodia musculus kutz.* and *Gomphonema undulatum* Her. (Pre-monsoon) and *Rhopalodia musculus kutz. and Nitzschia palea Kutz* (Post-monsoon).

The core sediment of the Navegaon Bandh Lake includes 62 species of diatoms belonging to 24 genera. Out of these species 08 are centric and 54 pennate diatoms (07 araphid, 01 monoraphid, 01 proraphid and 45 biraphid). The abundant diatom species are *Rhopalodia musculus, Rhopalodia gibberula, Aulacoserira granulate and Gomphonema undulatum* (Plate 1).

Table 1: Most abundant diatom species in the Navegaon Bandh Lake surface sediments and their preferred range of physicochemical parameter

Season	Most abundant Diatom species	Physicochemical parameter													
		pH	EC µmhos/cm	Alk mg $CaCO_3$/L	Cl mg/l	SO_4 mg/l	Ca mg/l	Mg mg/l	NO_3 mg/l	TP mg/l	Fe mg/l	Na mg/l	K mg/l	Al mg/l	Si mg/l
Pre-Monsoon	*Rhopalodia gibberula*	7.75	104.00	69.00	2.50	2.18	15.00	3.90	0.17	0.02	0.15	1.65	0.15	0.05	5.36
	Rhopalodia musculus														
	Gomphonema undulatum														
Post-Monsoon	*Gomphonema parvalum*	7.48	118.75	69.00	2.13	1.18	11.20	4.15	0.20	0.03	0.25	2.18	0.35	0.04	4.48

The core sediment of the Navegaon Bandh Lake includes 62 species of diatoms belonging to 24 genera. Out of these species 08 are centric and 54 pennate diatoms (07 araphid, 01 monoraphid, 01 proraphid and 45 biraphid). The abundant diatom species are *Rhopalodia musculus, Rhopalodia gibberula, Aulacoserira granulate and Gomphonema undulatum* (Plate 1).

The Navegaon Bandh Lake, four diatom zones and ten sub-zones were identified for major species abundance using constrained cluster analysis. A total of 62 diatom taxa were found in the Navegaon Bandh Lake core of which 20 attained a maximum abundance of >1% in at least one sample. Diatom assemblages were categorized as 1) Planktonic diatoms, such as *Aulacoseira granulata* Ehrenberg (4.4 - 100%), *Discostella stelligera* Cleve and Grun (6.3 – 14.9%), *Aulacoseira distans* Ehrenberg (1.0- 13%) and *Stephanodiscus niagarae* Hakansson and Hickel (1.9 - 15.4%) with benthic diatoms such as *Rhopalodia musculus* Mullar (8.3 – 100%), *Rhopalodia gibberula* Mullar (2.9-50%),*Gomphonema undulatum* Kutzing (2.5 – 70%), *Fragillaria rumpens* Kutzing (2.9 – 18.6%), *Nitzschia palea* Smith (5 – 57.1%), *Encyonema minutum* Mann, *Gomphonema parvalum* Kutzing (2.5 – 37.5%), *Navicula cryptocephala* Kutzing (0.6 – 50%) and *Eunotia bilunaris* (Ehrenberg) Mills (1.4 – 9%). The planktonic diatoms were dominated by *A. granulata*, whereas benthic diatoms were dominated by *R. musculus*, *R. gibberula* and *G. undulatum*.

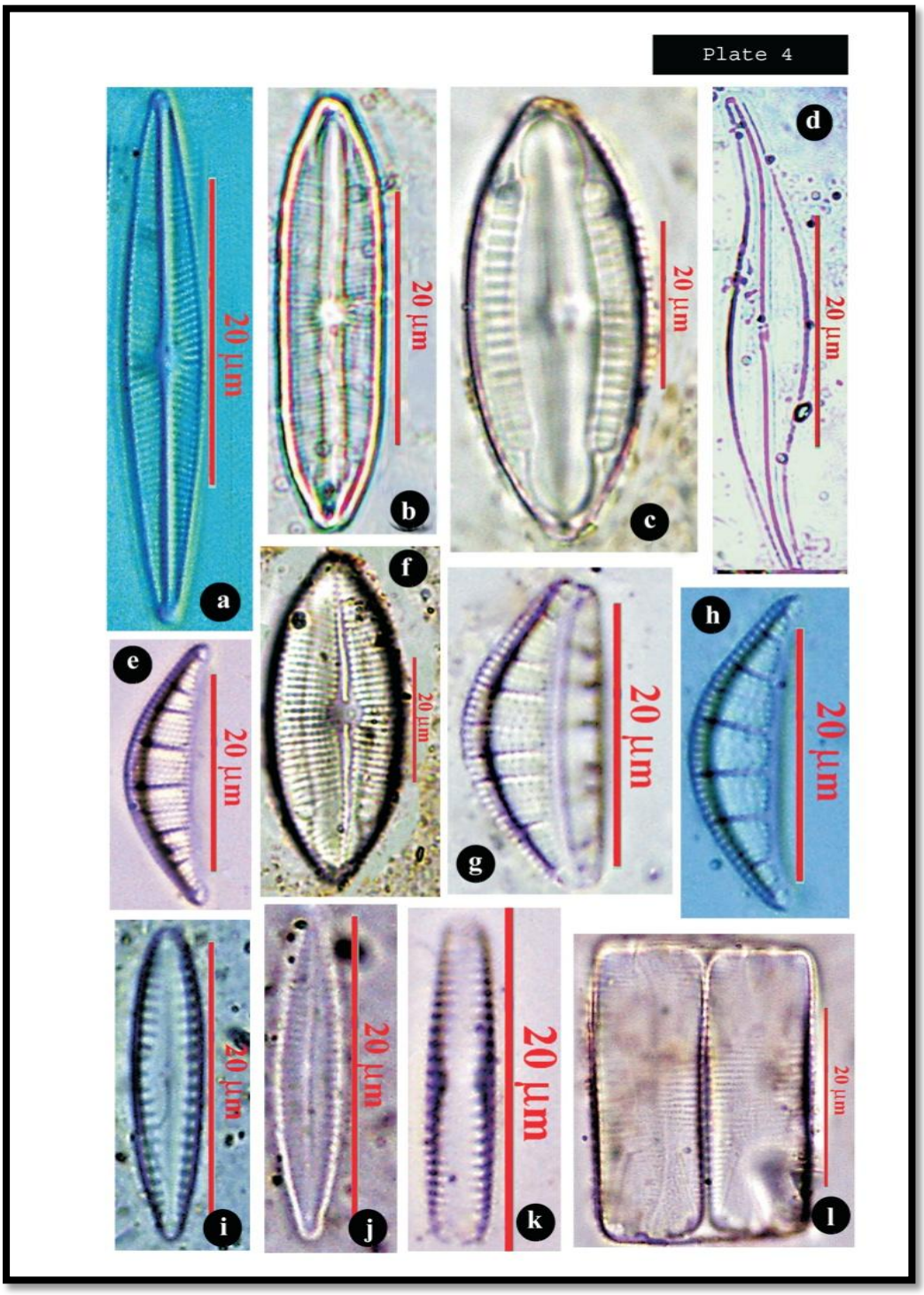

Plate 1. a) ***Navicula radiosa (Valve view);*** **b)** ***Mastogloia brauni (Valve view);*** **c)** ***Mastogloia smithii (Valve view);*** **d)** ***Gyrosigma spencerili (Valve view);*** **e)** ***Rhopalodia gibberula (Valve view);*** **f)** ***Mastogloia elliptica (Valve view);*** **g)** ***Rhopalodia musculus (Valve view);*** **h)** ***Rhopalodia gibberula (Valve view);*** **i)** ***Gomphonema clevei (Valve view);*** **j)** ***Nitzschia palea (Valve view);*** **k)** ***Gomphonema undulatum (Girdle View);*** **l)** ***Eunotia bilunaris (Girdle View)***

A. granulata was abundant during ~ 1901-1980 A.D. and progressively decreased on the top of the core (~1983 and above) whereas in contrast *R. musculus* was dominated in the core, where *A. granulata* was decreased. *Synedra ulna* Ehrenberg (2.7-88.9%),

Cocconeis placentula Grunow (4.5-100%), *Diploneis ovalis* Hilse (1844) (2.7-83.3%), *Amphora ovalis* Kutzing (2.7-30%), *Epithemia adnata* Kutzing (1.8-60%), *Cymbella affines* Kutzing, 1844 (1.1 - 62.5%), *Cymbella lanceolata* Ehrenberg (1.8-40%) and *Rhopalodia gibba* Mullar (1.8-66.7%).

The planktonic diatoms were dominated by *A. granulata* and *S. minutulus* and benthic diatoms were dominated by *C. placentula*, *A. ovalis* and *D. ovalis*. The abundance of *S. niagarae* abruptly decreased in the core up to 40 cms below the lake bed (~1964 AD) and above it up to 1979 AD except minor presence at ~1980 AD and further appeared in minor concentrations after ~2008 AD. Whereas, *S. minutulus* appeared at 65 cm (~1899AD) and continued up to 60 cm (~1914 AD). Diatoms of the Navegaon Bandh Lake show considerable change of the centric diatom *A. granulata* at ~ 1901 AD and remained more or less constant till ~ 1961 AD (Juare, 2016). A major shift in speciation was seen at ~ 1983 AD with preponderance of the benthic species *R. musculus* and continued till ~ 2006 AD. The abrupt disappearance of *R. gibberula* was observed during this period (Fig. 2). The significant shift of diatoms from benthic to planktonic diatom assemblages have been assigned to climate warming (Chen *et al.*, 2014). The small size and fast growing planktonic and diatoms in the sediment cores indicate longer and stronger thermal stratification (Smol *et al.*, 2005; Rühland *et al.*, 2010; Chen *et al.*, 2014).

Conclusion:

a) The Navegaon Bandh Lake had mesotrophic, meso euryhaline and alkali biontic waters. The increased comparative salinity may be due to longer dry period / less rainfall prevailed in the watershed and swampy conditions of the lake.
b) During ~1862 – 1901 AD, *R. musculus reached to maximum with decrease in A. granulata, indicating again the mesotrophic, meso-euryhaline, alkalibiontic waters. This also points less rainfall/ dry period. The presence of highly eutrophic waters was observed during ~1902 – 1961 AD in the core, where R. musculus declined drastically.*

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RESTORATION OF DEGRADED AGRICULTURAL LAND

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Abstract:

Land degradation alludes to alteration to the biological, physical, and chemical environment of soil rendering disadvantageous to the soil, soil organisms, vegetation, and ecosystem. The rebuilding of agricultural land is significant for the manageability of agribusiness and ecosystem. The land is under colossal tension because of the ever-expanding population, therefore, resulting in developing interest in food, fiber, and shelter. Land debasement, normally linked with soil disintegration, consistently brings about a decline or complete loss of land productivity and delivers on-site and off-site contamination to soil and water. Agricultural land is being weakened because of various anthropogenic and natural activities operating over the years. The fundamental driver of disintegration land is exhaustive cultivation, overgrazing, deforestation, mining, pollution and agricultural malpractices. Land productivity can be recovered by adopting various physical, chemical, and biological strategies. A consortium of biological methods can play an effective role in restoring the productivity of land and concurrently maintaining the sustainability of the ecosystem.

Keywords: Agribusiness, ecosystem, land productivity, exhaustive cultivation

Introduction:

Land a non-renewable source is central to all primary production systems. Pastures and crops are the two most extensive forms of land use which occupy 25% and 12% of the total global land surface (Ramankutty and Foley, 1999; Anser *et al.*, 2004). So for the agricultural land is concerned the per capita availability of land has abated 0.48 hectare in 1951 to 0.16 hectare in 1991 and is likely to decline further to 0.08 hectare by 2035 (Yadav, 2000, Nagaraja, 2009).

Fertile lands are the key means for agricultural production. Man-induced environment stresses change the pattern of nutrient cycling, primary productivity, and biodiversity which lowers land's capacity to support desirable vegetation. Limitations of

highly productive land in the world cause the demand and need for restoring soil quality and their return to agricultural use. The issues linked to maintaining the quality of the land, and especially the potential fertility of agricultural land can be categorized into two groups: soil degradation and loss of soil fertility due to improper unsustainable agricultural practices and land disturbance caused by mining, construction, and other types of negative anthropogenic activities. Natural components of degradation processes are mainly associated with the manifestation of erosion, and they are compensated by self-ecosystems. In developing nations like India due to the high population rate, there is extensive pressure on agricultural land. In lieu of high productivity to feed the growing population and the maximum economic returns, excluding environmental factors, the continuous use of high-yielding varieties, prolific use of chemical fertilizers and pesticides have rendered the land unproductive.

It is quite interesting that out of the total 238.05-million-hectare geographical area of India, about 143.0 Mhectare is net cultivated area (MOEF 2009). Of this 40% is irrigated and the remaining 60% is rain-fed. This area is under different states of land degradation due to intensive agricultural practices and subject to wind and water erosion. In developing and underdeveloped nations where economic stability is largely dependent on agriculture, there is a dire need to improve productivity per unit of land.

Sources of land degradation:

The initial step in ecosystem rehabilitation projects is to identify the primarysources of land degradation and then address them properly.Degradation may occur through different physical, chemical, and biological factors which are directly or indirectly are induced by human activities.Various sources which result in the degradation of land are natural, social, and anthropogenic.

Natural sources: Natural sources of land degradation include earthquakes, tsunamis, droughts, avalanches, landslides, volcanic eruptions, floods, tornadoes, and wildfires.

Social sources:

The underlying social causes of soil degradation are land shortage, the decline in per capita land availability, economic pressure on land, land tenancy, poverty, and population increase.

Anthropogenic sources:

Anthropogenic sources include poor agricultural practices such as improper use of land, shifting cultivation, fertilizers and pesticides overuse, overgrazing, poor crop rotation,

poor water management, inadequate organic matter inputs, and other activities such as deforestation, industrialization, and mining.

Types of land degradation:

Various types of land degradation which finally result in loss of biological agricultural productivity are wind erosion, water erosion, dryland salinity, soil acidification, waterlogging, and soil structure decline.

a) Wind erosion:

When soil is devoid of vegetation it is left exposed to air which blows away the top fertile soil leaving behind poor-quality sub-soil.

b) Water erosion:

Water erosion is caused by the detachment and transport of soil by rainfall, runoff, melting snow or ice, and irrigation which carries along with precious topsoil thus threatening the productivity of agricultural land.

c) Soil acidification:

Excess use of fertilizers, pesticides, acid rain, growing certain plants, certain animal wastes make the soil acidic and render it unfit for cultivation of crops and pastures.

d) Irrigation and dryland salinity:

Irrigation water used for crops and pastures seeps down to the water table with its dissolved salts when rises again kill the crops and other plants which are not adapted to heavy salty zones.Dryland salinity is the development of salt in surface soil in non-irrigated areas, ordinarily in light of rising groundwater tables. Groundwater leaks to the surface, carrying salt with it.

e) Soil structure decline:

Different types of organic matter, mineral particles, nutrients, air, and water make the soil structure. Poor agricultural practices make the organic matter and mineral particles decline subsequently hence upsetting the soil structure.

Restoration strategies:

Restoration through agricultural practices:

a) Crop residue cover:

Wind and water erosion can be reduced by goodcrop management practices. Retention of crop residues after harvesting is an effective anti-erosion measure. This technique manages optimum nutrient levels, water conservation and prevents overheating of soil.

b) Inter-cropping:

The impact of flash floods is reduced with thesoil cover by the fast-growing legumes such as cowpeas andbeans early in the season along with slow-growing cottonor maize crop. This exercise reduces soil erosion andhelps the agricultural land to regenerate since it considerably checks soil erosion and being leguminous crops, add nutrients to the soil.

c) Crop rotation:

The act of growing a series of dissimilar types of crops in the same space in consecutive seasons is crop rotation for advantages such as avoiding pathogen and pest growth that occurs when one species is continuously cultivated. Crop rotation balances the nutrient demand of various crops by circumventing nutrient depletionThe replenishment of nitrogen with the use of green manure and legumes in succession with cereals and other crops is a customary part of crop rotation. Soil structure and fertility by alternating shallow-rooted and deep-rooted plants can likewise be improved by crop rotation.

d) Cover crops:

The gap between the harvesting of a crop and sowing of the next crop can be effectively utilized to restore the soil quality by growing cover crops. The use of cover crops on a bare field is an age-old practice and dates back to theancient civilizations in Greece, Rome, China, and others (Lamb *et al.*, 2005): The cover crops manage soil erosion, soil fertility, soil quality, assist in weed and pest management and biodiversity in agroecosystems. The most efficaciouscover crops are grasses and legumes but may be comprised of other green plants.

e) Shelterbelts:

Shelterbelts are a cheap and long-term option to reduce wind erosion on farms and shade to livestock. Shelterbelts act as windbreakers**.**they also function to protect valuableagricultural land and irrigation canals from creeping sands, improve the microclimate and increase livestockyields. Field explorations in arid zones show that crop productionmay be enhanced by as much as 300% while the increase inaverage years is often 30 to 50%.The farmers at large are, however, disinclined to sacrifice cultivatedland for shelterbelts. The species used are mostly *Eucalyptus*, *Casuarina* sp., *Populus,* and *Prosopis* whichare easier to establish and can withstand adverse climatic conditions. Shelterbelts also provide essential habitats for native wildlife.

f) Strip Cropping:

Strip cropping is a strategy for farming, utilized when a slope is excessively steep or excessively long, or otherwise when one doesn't have an elective technique for forestalling soil erosion. Strip cropping uses alternates strips of closely planted crops like wheat, hay, or

other small grains with strips of row crops, like corn, soybeans, or cotton.Strip cropping assists with halting soil disintegration by making regular dams for water, assisting with safeguarding the strength of the soil. At the point when segments of soil are sufficiently able to hinder water from traveling through them, the more fragile soil can't wash away like it ordinarily would. Along these lines, farmland stays fruitful any longer.

g) Contour farming practices:

Cultivation across the slope rather than up and down in hilly terrain to conserve rainwater and to minimize soil losses from surface erosion is called contour farming. These objectives can be accomplished by crop rows, furrows, wheel tracks across the slope, all of which act as a repository to gather rainwater. Grass barrier strips of *Pennisetumpurpureum* or other fodder grasses which grow well in marginal land are planted along the contour. Plant residues are placed in lines along the contour for the construction of bunds. These bunds slow down the runoff and trap the eroded soil.

h) Agroforestry:

Agroforestry is the management and integration of trees, crops, and livestock on the same plot of land and can be an elemental part of productive agriculture.Trees decline the magnitude of splash erosion by lessening the raindrops' impacts on the soil. They regulate soil temperature by shading the soil thus reducing water evaporation. They also curtail wind erosion by acting as windbreaks. Additionally, they play a significant role in nutrient recycling in the deep soil; leguminous trees fix nitrogen that benefits food crops.

Restoration through soil management practices:

Optimum soil management practices aim to provide favorable conditions for plant growth through improved nutrient availability and the physical environment of soil.

a) Use of appropriate tillage methods:

Tillage is soil groundwork for agriculture by mechanical agitation such as digging, stirring, overturning, etc. The tillage method affects soil characteristics such as aeration, temperature and, water use, and soil structure.Tillage practices ought to be adopted by keeping in mind that; the soil is neither too fine nor powdery, and it breaks up the hardpan if necessary. The main objective of tillage is to upgradethe physicalconditions of soil for better crop production. Besides, it ensures timely seedbed preparation, planting, and weeds control.

b) Administering organic manures and mineral fertilizers:

The applicationof manure and fertilizers is fundamental in the restorationof agricultural land. Manure and fertilizers provide essentialplant nutrients in the soil for

better crop yield. Essentialplant supplements such asnitrogen, phosphorus, potassium, andsulfur required by plants are provided by inorganicfertilizers. There is no substitute for inorganic fertilizers thereforecoordinated use of organic and inorganic fertilizers shouldbe adopted. Farmyard manure, green manure, and composts are the main sources of organic fertilizers. Grande *et al.* (2005) revealedthat manuring can reduce water runoff by 70 – 90% andsediment loss by 80 – 95% as a result of increased organic mattercontent. A study carried out in West Africa by Yamoah *et al.* (2002) revealed that a blend of crop residues and fertilizerreestablished the degraded agricultural land in this way led to the highestmillet grain and straw yields production.

c) Soil conservation using physical measures:

Physical measures in the restoration of eroded land are permanent features designed to arrest the uncontrolled surface run-off, erosion, and conserve water. Some of the physical techniques used for the rehabilitation of agricultural land are:

i) Cut-off channels:

Cut-off channels are made across the slope to block the surface overflow conveying it securely to an outlet like a channel or stream. Their primary intention is the protection of cultivated land from uncontrolled overflow, and to redirect water from crevasse heads.

ii) Retention ditches:

These are made along the contours to catch and hold approaching spillover water and hold it until it saturates the ground. They are substitutes to cutoff channels when there is no channel to release the water close by. Retention ditches are used for water harvesting in semiarid territories.

Restoration of ecosystem services on agricultural land:

There is a scope of potential outcomes to switch the negative environmentalimpacts on agricultural land by adopting biodiversity-based agriculture and the use of traditional farm practices which can enhance agricultural production.

a) Exercising biodiversity-based agricultural practices:

Conservation of existing biodiversity in agricultural landscapesand the adoption of biodiversity-based practices have been proposedas methods of improving the sustainability of agriculturalproduction through greater reliance on ecological goods and services with less damaging effects on environmental qualityand biodiversity (Jackson *et al.*, 2007): Biodiversity-based agriculture incorporates the variety and variability of animals, plants and micro-organisms, at the genetic, species, and ecosystem levels, which are important to support key elements of the agroecosystem, its structure, and processes.

The differentelements of agricultural biodiversity can be distinguished as:

1) Genetic resources for food and agriculture:

- Plant genetic resources, including crops, wild plants harvested and managed for food, trees on farms, and pastures.
- Animal genetic resources, including domesticated animals, wild animals, farmed fish, and other aquatic file forms,
- Microbial and fungal genetic resources.

2) Elements of biodiversity that sustain ecosystem services upon which agriculture is based:

These include a diverse range of organisms that contribute, at various levelsof nutrient cycling, pest and disease control, pollination, pollution, maintenance of the hydrological cycle, erosion control, and climate management and carbon sequestration.

3) Abiotic factors:

It includes local climatic, chemical and physical factors and functions of ecosystems, which have a deciding impact on agricultural biodiversity.

4) Socio-economic and cultural dimensions:

It includes traditional and local knowledge of agricultural biodiversity, cultural factors, and participatory processes, as well as tourism associated with agricultural landscapes.Agricultural biodiversity isto a great extentmolded and maintained by human exercises and management practices, and a large number of people depend on agricultural biodiversity for sustainable livelihoods.

b) Learning from customary farming practices:

Customary farming portrays practices that developed through humanhistory to produce an assortment of agricultural goods, largely forlocal use. Traditional farming perseveres in many regions ofthe world, particularly in developing countries, but also in some developed countries, where such practices are remnants or havebeen re-introduced to meet explicit needs. Traditional farming methods are extremely diverse and eco-friendly by their nature: on-farm cycling of nutrients and resources,the development of local varieties and breeds, high spatial andtemporal structural diversity, use of local pollination and pestcontrol services, and successful utilization of local environmental heterogeneity (Altieri, 2004). Traditional farming has been appeared to have many environmental and societal advantages, including enhancement of soilcarbon sequestration and nutrient cycling,

limiting soil erosion, effective water use, and conservation of crop genetic diversity, as well as providing resources for endangered species (Badalucco *et al.*, 2010).

c) Intensive agriculture to organicagriculture:

The advantages of organic farming to the environment incorporate less pollution by fertilizers, herbicides, and pesticides, increases in biodiversity, upgrade of soil carbon sequestration and nutrients, enhancement of natural pest control,and protection of the genetic diversity of local varieties of domestic plants and animals (Gabriel *et al.*, 2010). There has been a significant expansion of organic farmland areas in the world, mainly in developed countries. The demand for healthy and environmentally friendly food and subsidies to producers of organic food and fiber has supported this exercise. However, organic farming comprises a little fraction of the farming activity.

In 2018, Sikkim in the North of India received the Gold Award of the UN backed 'Future Policy Award' for being the first 100% organic state in the world. The policy emphasizes soil fertility and increases biodiversity at the field and landscape level. The transition to sustainable food and agriculture systems is crucial for a sustainable future. Both Sikkim and Bhutan show with their 100% organic goals that such a transition is possible. Sikkim, Bhutan, and other Himalayan states arepracticing organic farming and agroecology as a functional pathway for accomplishingsustainable development goals. "They show that achieving land degradation neutrality is no longer a pipe dream but can become reality." WFC (World Future Council, a Germany-based environmental NGO) Executive Director, Alexandra Wandel said.

Reclamation through bioenergy plantation:

Bioenergy is quite possibly the main potential source of rural development guaranteeing economically viable energy supply and environment and soil security in developing countries. Farmers have the choice to convert their food crops to fuel crops anticipatingexceptional returns but at the same time generating a scenario where food production may decline, subsequently, the bioenergy plantation is constantly connected with food security. Bioenergy plantation combined with legitimate equilibrium with food crops could be an effective measure for the restoration of degraded land. Most bioenergy plants like *Jatropha*, *Simmondsia chinensis*, *Pongamia pinnata*, *Moringa oleifera*, *Ricinus communis* can withstand in poor soils and semi-parched environments and thus build payment to re-establish degraded ecosystems. It is reported that *Jatropha* can easily grow

on heavy metal contaminated soil by adding biofertilizers to the soil and degraded land can be converted to arable land in few years (Kumar *et al.*, 2008).

Reclamation through forestry practices:

Forestry practices are the most ideal approachto arrest the degradation of soil. Afforestation programs are advantageous to regain soil quality, alleviate adverse climate changes, provide a range of forest products, and provide a livelihood to poor people in developing and underdeveloped nations. Selection of suitable plant species, choice of suitable plantation technology, reasonable inputs, and effective organization in afforestation programs are very critical to accomplish the particular objective. Exploration is needed to identify the source and kind of land degradation before adopting the specific corrective measure. For reclamation of saline soil-plant species that can withstand high salt content and waterlogging conditions ought to be selected for planting. The plant species *Acacia nilotica*, *Casuarina equisetifolia,* and *Eucalyptus tereticornis* are accounted for to bring down soil pH from 10.5 to 9.5 in five years(Gill and Abrol, 1986; Grewal and Abrol, 1986): For acidic soils, the plantation of *Michelia oblonga*, *Alnus nepalensis*, *Parkia javanica*, *Parkia facataria*, was recommended by Dhyani *et al.* (1995) as these species are profoundly adapted to acidic soil. The tree species like *Prosopis juliflora*, *Achras japota*, *Acacia nilotica,* and *Tamarix articulate* can withstand more than pH10.0 hence, are suggested for recovery of alkali soils (Hasan and Alam, 2006). The knowledge of tree characteristics is a vital segment of the restoration process.

Restoration through medicinal plants:

India is blessed with a rich wealth of medicinal and aromatic plants. In any case, regardless of the rich legacy of the knowledge on utilization of plant drugs, little consideration has been paid to cultivate them on large scale in the country. About 400 plants used in the regular production of Ayurveda, Unani and Siddha drugs less than 20% are cultivated in our country (Anon, 1997). Due to the growing interest of the public, the demand for medicinal plants in the international market is keepingexpanding. To meet out this demand degraded land used for the cultivation of medicinal plants can tackle two purposes. The demonstration trial carried out at CIMAP and NBRI, research stations, Lucknow have shown promising possibilities of growing some of the aromatic plants on sodic soils. Among aromatic grasses, Vetiver(*Vetiveriazizanioides*) Palmorosa(*Cymbopogon martini*), lemon grass(*Cymbopogon flexuosus*)are the potential crops for cultivation on sodic soils (Singh, 1997): *Acacia auriculiformis*, *Acacia nilotica*, *Azadirachta indica*, *Bauhinia*

purpurea, *Butea superba*, *Dalbergia sissoo*, *Gmelina arborea*, *Eucalyptus eretocorus*, *Madhuca indica*, *Saraca asoka*, *Tamarindus indicus*, *Terminalia arjuna, Aegle marmelos, Carica papaya*, *Terminalia bellerica*, *Terminalia arjuna*,etc. (Ghosh, 1997) are among the recommended plant species for restoration of degraded land in Raniganj coalfields, West Bengal. Studies conducted by Das *et al.* (2009) have explored the potential of *Albizia procera* and *Leucaena leucocephala* to bring about improvement in the soil properties as reflected by the changes in pH, EC (Electrical conductivity) organic carbon, available nitrogen, phosphorus, and potassium. Higher available N, P O, and K as well as higher organic carbon percentage, were noted under the canopy of *Albiziaprocera*followed by *Leucaena leucocephala*. The pH and EC were minimal under *Albiziaprocera*and changes were observed from 8.7 to 7.7 and 0.76 to 0.40 dS/m (Deci Siemens per meter), respectively in the span of 12 years. *Albizia procera* delivered maximum litter fall followed by *Leucaena leucocephala* plantation with high nutrient returns.

Thus, the plantation of herbal plants on degraded land will assist with saving the diversity of herbal plants and simultaneously will help to minimize the pressure on croplands and reclaim the degraded land.

Conclusion:

Restoration of eroded agricultural land can be achieved through several agronomic and biological strategies. Beyond scientific and technical research, an increase in such restoration projects is needed if we want to halt man and nature-induced agricultural degradation. We need a widespread expansion of agricultural management based on ecological knowledge, biodiversity-based agricultural practices, organic farming, agroforestry framework, learning from customary practices, and particular ecosystem services, and conversion of some agricultural land into natural ecosystems such as forests and pastures. Financial support, public awareness, education, and training of all stakeholders, particularly farmers, are necessary to accomplish such objectives. Restoration actions can act as an engine ofthe economy and a source of green employment, so the restoration of degraded farmland areas can generate multifaceted benefits.

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A REVIEW ON USE OF MAGNETIC SUSCEPTIBILITY AND PARTICLE SIZE ANALYSIS FOR PAST LAKE ECOLOGICAL STUDIES

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Abstract:

The chemical and physical characteristics of lake sediments can provide us with information about sources of organic matter, lake productivity and a record of previous climatic fluctuations. To study the climatic fluctuations magnetic susceptibility and particle size analysis are very important tools. High magnetic susceptibility values and moderate concentration of clay indicates high rainfall or wet period during that particular time while low magnetic susceptibility value with high clay concentration indicate low rainfall or dry period.

Introduction:

The last few decades of 21st century are marked by scarcity of natural resources and increase in all sorts of pollution which has affected the Earth's climate among the natural resources the fresh water is considered as one major basic resource. The fresh water is depleting fast due to increase in demand with the rise in population, irregular climatic cycles affecting the normal rainfall pattern or reduced rainfalls, increase in industrialization causing pollutionof the rivers and lakes and global warming leading to melting of glaciers etc. The situation is even worst in developing countries like India having agriculture as a major occupation and depends largely on rainfalls as a source of fresh water. But, during last few decades the climate change has affected the normal rainfall pattern of India leading to deficient rains in many parts of India, affecting the societal well being and major cause of farmers suicides in central India. Hence, the climatic models to forecast future trends of climate have direct societal relevance, as rainfall prediction is very important for agriculture and water management.

As water crisis is focused globally in view of climate change issues and pollution monitoring, there is an urgent need to generate a long term data on climatic models, on pollution of lakes and rivers and their recovery. Hence, the economic planners are in search of climatic models to forecast the future trends of climate. To predict the future we need to understand the past with fine time resolution. The reliability of such models depends upon the length of the time data. Such models can be designed or generated using lake sediments and using various proxies from the lake sediments.

The value of lake sediments as paleoenvironmental archives is widely recognized for studies of sedimentary records and long-term environmental change (Smol 2002). The significance of lake study lies in the fact that they have continuous and greater sedimentation rates and therefore are able to preserve regional or even global variations in the Earth's climate history at high resolution (Meyers, 2003).The chemical and physical characteristics of lake sediments can provide us with information about sources of organic matter, lake productivity and a record of previous climatic fluctuations (Meyers, 1997; Smol, 2008). Climate change is highly considered as an important factor of lake ecosystems (George and Harris, 1985; Adrian *et al.*, 1995; IPCC, 2001; Williamson *et al.*, 2009).The awareness about the level of impact of climate change and anthropogenic activity on lakes is highly incomplete and this gap widens when the lake is situated in the populated catchments in tropical countries (MEA, 2005).The lake's normal ecological path and trophic status have been strongly affected by local anthropogenic activities like agriculture, fisheries, recreation etc. since last few decades (Jeppesen *et al.*, 2007). To forecast the effects of climate change because of global warming in view of various local anthropogenic disturbances is therefore complex, either because the climate signal is oppressed by other impacts or because climate and other environmental pressures may interact, creating unexpected ecological responses (Schindler, 2001; Leavitt *et al.*, 2009; Smol, 2010; Battarbee *et al.*, 2012). Hence, different proxies like sediment geochemistry, particle size analysis, magnetic susceptibility have now beenwidely used to study the evidences of climatic change and to evaluate the climatic models.

Magnetic Susceptibility:

Magnetic susceptibility is a measure of the degree of magnetization of a materialin response to an applied magnetic field (Nowaczyk, 2001). The characteristicsof magnetic minerals, i.e. their concentration, mineralogyand grain size in sediments, can be studied bymaking mineral magnetic measurements, which yieldlarge quantities of environmental data rapidly and nondestructively (Evans and Heller, 2003).

Particle size analysis:

The interpretation of sediment samples has often been based on a number of classical statistical describers of particle size data, such as the mean, median, mode and sorting etc. However, grain size is an indicator used to infer changes in the past environment of the lake and its watershed, and also of the sediment transport capacity of the surrounding watershed (Beierle *et al.*, 2002). Sediment size is typically broken up into these categories: clay (<0.002 mm in diameter), silt (0.002-0.06 mm), sand (0.06-2.0 mm), and gravel (>2.0 mm).Fine-grained sediments in an aquatic environment may aggregate into larger, porous aggregates commonly called flocs (Van Rijn, 1993; Roberts *et al.*, 1998 and Kim *et al.*, 2005). These sediments are cohesive by definition (Hayter andPakala, 1989; Paterson, 1997) and their composition and structure are temporally very changeable.

Review of literature:

Magnetic susceptibility:

Magnetic susceptibility is a measure of magnetic mineralogy and is widely used as a proxy for variations in lithostratigraphy (Peck *et al.*, 1994).Thompson and Oldfield (1986) and Thompson *et al.* (1980) have studied the environmental applications of magnetic measurements. Warrier and Shankar (2009) have worked on chemical evidences for the use of magnetic susceptibility as a proxy for paleorainfall in the tropics. Bloemendal and Menocal (1989) have worked on evidence for a change in the periodicity of tropical climate cycles at 2.4 Myr from whole-core magnetic susceptibility measurements. Maher and Thompson (1992) studied the reconstruction of paleorainfall from Pedogenic magnetic susceptibility variations in the Chinese Loess and Paleosols. Wolfe *et al.* (2006), have reconstructed multi-century flood histories from oxbow lake sediments, Peace-Athabasca Delta, Canada. Vigliotti*et al.*, (1999), has found in most other studies that, grain-size distribution has a direct relationship to magnetic susceptibility because the carrier mineral that retains the remnant magnetic intensity has a specific grain-size distribution and reflects a particular particle size. Petrovsky *et al.* (2000) have used low-field magnetic susceptibility as a proxy method of estimating increased pollution of different environment systems. Bityukova *et al.* (1999) used magnetic susceptibility as an indicator of environmental pollution of soils in Tallin. Charlesworth and Lees (1997) have worked on the use of mineral magnetic measurements in polluted urban lakes and deposited dusts of Coventry. Dearing *et al.* (1986) have studied movement of top soil by magnetic measurements.Wang *et al.* (2008) have investigated themaganetic signature of environmental change from the pleistocene lacustrine sediments.

Particle size analysis:

Particle Size Distribution (PSD) and carbonate content are two of the most frequently used parameters for describing lake sediments. The particle size analysis or the grain size analysis of lake sediments is carried out to know the overall energy level of the lake environment and also to determine the changes in lake level and large storm events (Noren *et al.*, 2002; Parris *et al.*, 2009). The clastic sediments are generally classified as sand, silt and clay depending on the grain size. The sand particle ranges in size from 62.5μm to 2mm, silt particle range in size from 3.9 μm to 62.5 μm and clay particle less than 3.9 μm (Wentworth, 1922). Clays suspended in the water column will only be deposited into sediments under very calm conditions, such as under the cover of iced, where wave action is eliminated (Koff, 2012).Lake sediments are commonly used to infer climate variation through clay mineral assemblages, clay mineral preservation, grain-size, and sediment structures (Chamley 1989; Gale and Hoare 1991; Ariztegui *et al.*, 2001; Yuretich *et al.*, 1999).

Grain-size variations in lake sediments reflect changes in the processes and energy of sediment transport. Particle sizes are closely linked to turbulence, wave energy, and proximity to shoreline; increased grain sizes generally correspond to higher energy conditions of sediment production or transport, whereas decreased grain sizes indicate lower energies (Cohen *et al.*, 1997). Grain-size fluctuations in sediment through time, particularly increases in sand sizes, may reflect low-level lake stands related to periods of dryer and warmer climate. Conversely, decreased in sand content may reflect periods of wet and cold climates (Alin and Cohen, 2003).In large lacustrine environments, depositional patterns are controlled by a number of transport mechanisms including aeolian, turbid underflows, fluvial inflows, and lake ice-rafted debris (Last, 2001). These depositional mechanisms can be revealed by particle- size distribution (Gale and Hoare, 1991). Further, the analysis of sedimentary units sheds light on depositional environments and can be extended to climatic interpretations. For example, shifts between laminated and non-laminated sequences can be linked (in the right settings) to globally significant changes (Behl, 1995). A critical study concerning the sensitivity of Northeastern Asia to global climatic change was carried out by Anderson *et al.*, (2002) and Hu and Shemesh, (2003). The changes in lake level can cause the redistribution of grain sizes. The lower lake levels cause shoreline regression, causing coarser sediments to be deposited over finer sediments. Similarly, higher lake levels may deposit finer sediments on top of coarser ones.

Methodology:

Core Sampling:

For past ecological study of the lake a core sample has to be collected from the lake of interested using a PVC pipe or Gravity corer. Different types of gravity corer are available for past lake ecological study. Sometimes core can also be retrieved by drilling in the deepest part of lake. After the core sample has been recovered it should be vertically cut into two equal halves and core profile was drawn with details and photographed. These halves had to be further sub sectioned then and cut at an interval of 1 cm along the length of the core. One half of the core was retained as archives while another half was used for magnetic susceptibility and particle size analysis.

Sample preparation and analysis for magnetic susceptibility:

For magnetic susceptibility analysis the sediment samples powdered to -170 meshes were directly used. A Bartington magnetic susceptibility meter (Model MS-2B) can be used to measure the magnetic susceptibility. The susceptibility is mostly measured in six directions at low and high frequency.

Sample preparation and analysis for particle size:

The Particle size analysis is analytical technique by which distribution of size in a sample of particulate material is measured. Partical size analysis ranges from the historical sieves to modern automated light scattering instruments. Laser Particle size analysis consist of measuring the size of particles (powder, suspension and emulsion) using the diffraction and diffusion of a laser beam. During the laser diffraction measurement, particle is passed through a focused laser beam. These particle scattered light is then measured by series of photosensitive detectors. The map of scattering intensity verses angle is the primary source of informationused to calculate the particle size. Shepard (1954) classification system for sand, slit and clay for triangular plot is highly adopted for the classification of particles into sand, silt and clay.

Results:

The results obtained from magnetic susceptibility and particle size analysis has to be systematically presented in tabular form. The results of particle size analysis will be obtained in in percentage of sand, silt and clay concentration. Whereas the results obtained for magnetic susceptibility will be in χlf .The results have to be further subjected to various statistical analysis and have to be plotted graphically for better understanding. The results

of the particle size analysis can be plotted in triangular diagram using tridraw software while the magnetic susceptibility results can be plotted in form of vertical profile using excel or C2 software. For better interpretation a comparative graphical plot of sand, silt, clay and magnetic susceptibility χlf values can be made.

Discussion:

The proxies like magnetic susceptibility (Warrier and Shankar, 2009; Cheng-long *et al.*, 2000; Dearing, 1999; Maher and Taylor, 1988; Maher and Thompson, 1992) and grain size analysis i.e. sand, silt and clay ratios (Veena *et al.*, 2014; Kashiwaya *et al.*, 1988; Thompson and Morton, 1979) are used widely to study the paleoecological and paleoenvironmental interpretation in different lakes. The distribution of sand, silt and clay in the end member classification was proposed by Trefethen (1950).Magnetic Susceptibility (χ_{lf}) and Particle Size Analysis proxies are used to study the paleorainfall and paleoclimates. The magnetic susceptibility values particularly χ_{lf} are compared with sand-silt-clay ratio.The triangular plot of sand-silt-clay distribution (Modified after Shepard, 1954) in the core sediment of the Lake could be used to indicate dry, flooding or regular rainfall pattern. The process of the pedogenesis involves the development and preservation of very fine grained magnetic minerals (i.e. magnetite), which is mainly dependent upon two prime parameters such as temperature and precipitation (rainfall) (Warrier and Shankar, 2009; Maher and Taylor, 1988 and Dearing *et al.*, 1996).The watershed of the lakes, present in the tropical region mostly have nearly stable temperature during past few centuries to some millennia. Therefore, the amount of rainfall in the catchment area of the lakes controls the development of pedogenic magnetite particularly in the upper soil horizons.

Case Study: Ghodajhari Lake

The Ghodajhari Lake of the Nagbhid Taluka of the Chandrapur District falls in the eastern part of the Maharashtra state covering an area of about 5.366 sq. km. The study area is included in the latitude N19^{o} 5'to 20^{o} 35$^{'}$ and longitude 79^{o}6' to 79^{o}53'E. The catchment area is about 90.65 sq.kms. The Ghodajhari Lake is a part of the Godavari Basin and the Wainganga sub-basin. It has a circumference of approximately 30-32 kms. It has a small embankment of 731.1 m. long, 3.6 m width and 20.04 m high. The average depth of the lake is 13.5 m (Fig. 1).

Figure 1: Satellite image of the Ghodajhari Lake showing locations of sample studied (C:Core, G:Grab sediments and water samples and SP: Soil Profiles)

Geological setting and lithological observation:

The Ghodajhari Lake of the Nagbhid Taluka of the Chandrapur District falls in the eastern part of the Maharashtra state covering an area of about 2.5 sq. km. The study area included in the latitude N19° 5'to 20° 35' and longitude 79°6' to 79°53'E. It is surrounded by the sandstones of the Neoproterozoic age belonging to the VindhyanSupergroup and granite gneiss with magmatic gneiss (crystalline and older metamorphic) and DeccanTtrap Basalt (DRM, 2000). The VindhyanSupergroup comprises of red, pinkish red to light red, fine to medium grained, hard compact sandstone of Neo Proterozoic age (1600-570 my.) These sedimentary rocks were originally deposited during the Neoproterozoic in a tectonic basin, in shallow marine environment and commonly found in the most part of the central India (Singh *et al.*, 2009; Paikaray *et al.*; 2008). The maximum area around the lake is surrounded by sandstones of the VindhyanSupergroup. The youngest formation studied in the study area is laterite of Recent to sub recent age (<1my) (Ravindra Kumar, 1991). The small patches of laterites are observed nearby the lake (DRM, GSI, 2001)

Results and Discussion:

The distributions of sand-silt-clay in a sedimentary core of the Ghodajhari Lake as per triangular classification (Modified after Shepard, 1954) show predominance of silty sand to sand (Fig. 2).

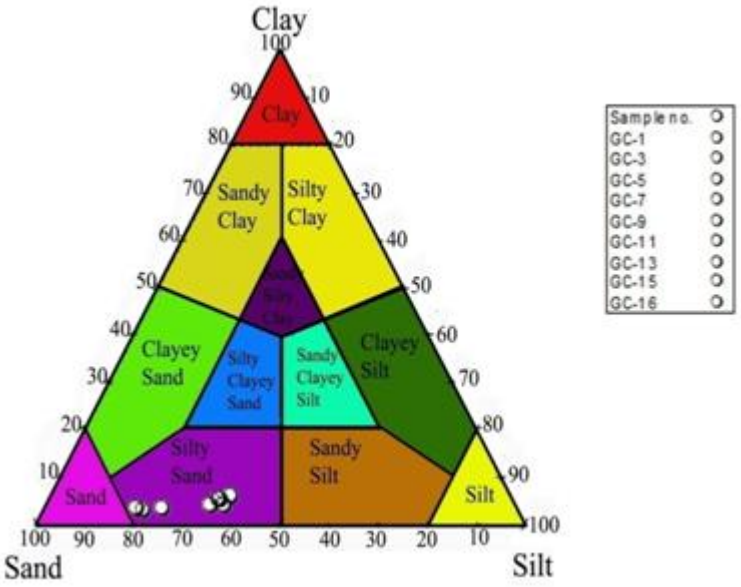

Figure 2: Distribution of sand, silt and clay in the sediment cores of Ghodajhari Lake

The sand-silt-clay distribution with depth in a sediment core of the Ghodajhari Lake was correlated with magnetic susceptibility values i.e.χ_{lf} to understand the fluctuations in rainfall of the region. There are five distinct units of rainfall patterns with depth in core i.e. (I) ~1924-1942 AD: this period is represented by low sand, high silt, high clay content, and high χ_{lf} values, which indicates high rainfall i. e wet period.(II) ~1942-1957 AD. During this period sand content was low, silt and clay concentrations were moderate, with low (decreasing) χ_{lf} content. This indicates moderate to low rainfall i.e. moderately wet period. (III) ~1957-1971 AD: This period shows moderately low sand silt content with moderate-high clay and silt content, and low χ_{lf} values which suggest moderately low rainfall i.e. moderately dry period.(IV) ~1971-1996 AD: During this period sand percentage shows increasing trend (high) with decreasing trend of silt and clay content (low) and very low χ_{lf} values. Thus moderate sand, low silt and clay content with low χ_{lf} indicate moderate low rainfall i.e. dry period. (V) ~1996-2012 AD: this period is represented by high sand, extremely low silt, low clay content and moderate to high χ_{lf}. Thus, this period suggest moderate to high rainfall i.e. moderately wet period (Fig. 3).

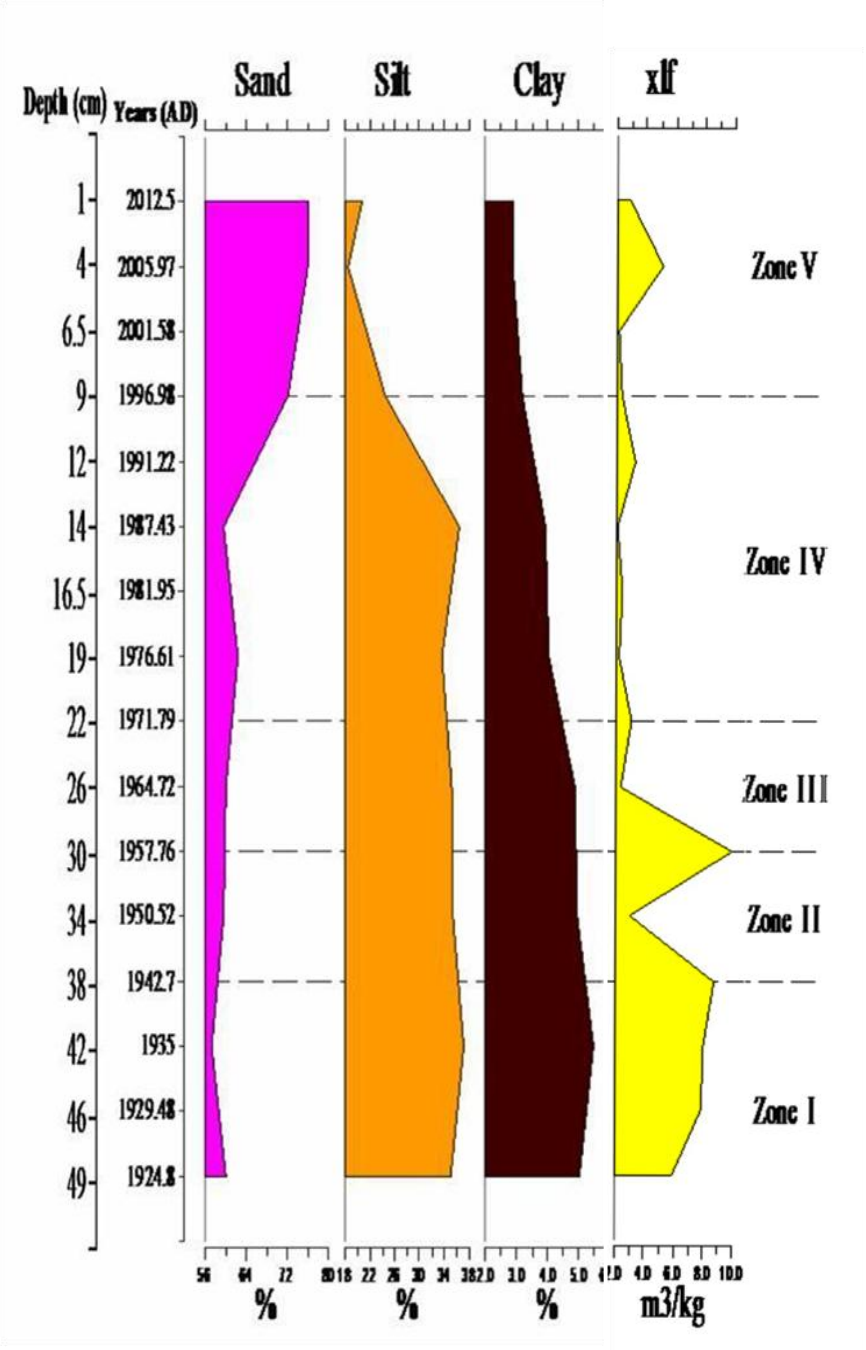

Figure 3: Profile of Particle Size Analysis and Magnetic susceptibility in sediment core of the Ghodajhari Lake

Conclusion:

The triangular plot of sand-silt-clay distribution in the core sediment of the Ghodajhari Lake shows abundance of silty sand to sand. The Ghodajhari Lake during these years shows a little abundance of sand with sudden peak in its silt concentration, low clay concentration, and moderate value of $\chi_{lf,}$ sand-silt-clay ratio and magnetic susceptibility indicate high rainfall i. e wet period.

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ORGANIC FARMING AND SOIL MICROBIOTA

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Abstract:

The popularity of organically grown food is increasing day by day due to its benefits on health and nutrition. It is cultivation without application of chemical fertilizers, synthetic pesticides, genetically modified organisms, growth hormones and antibiotics. Organic farming protects the environment and has greater impact on an agroecosystem function and soil microbial communities. Microbial biodiversity and their composition have significantly improved. Organic amendments not only improved the content of carbon, nitrogen phosphorus but also increase phosphate solubilizers in the soil. In addition, clinically important genera like Mycobacterium, Staphylococcus, Neisseria, Treponema etc. were completely absent in the soil. Beside enhancing soil fertility and microbial diversity, organic practices have impact on soil born pathogens. In this concept an active soil microbiota plays an important role in nutrient cycling, pest and disease control. Organic farming has positive effect on soil health and quality of microbial community. In summary, overall organic farming enhances total microbial abundance and activity in organic soil on global scale.

Keywords: Organic farming, microbial biodiversity

Introduction:

There is a great need for agricultural systems that are capable of producing enough food while coping with changing climatic conditions, which do not further increase the exploitation and degradation of Earth's limited resources. A possible option is eco-functional organic farming, an approach whichbased on making optimal use of internal natural resources and processes that improve agricultural productivity and minimizing negative environmental impacts such as loss of biodiversity, nutrient leakage and soil degradation.Organic farming is a technique involves the cultivation of plants and rearing of animals in natural ways (Fig. 1). The process involves the use of biological materials, avoiding synthetic substances to maintain soil fertility and ecological balance thereby

minimizing pollution and wastage. In other words, organic farming is a farming method that involves growing and nurturing of farms in organic reservoir instead of supplying nutrients through addition of synthetic fertilizers frequently. Genetically modified organisms are also not permitted. It believes on crop rotation, green manure, organic waste, biological pest control, mineral and rock additives. In organic farming plants are provided with macro and micronutrients that causes fertility of soil. Ideally, organic farming systems are designed to improve soil fertility to achieve following goals;

- Improvement of physical condition of soil, so that soil supports healthy plants and soil dwelling organisms.
- Increase the use of water and nutrients effectively by increasing biological fixation of nutrients and their retention.
- Maintenance of soil buffering capacity.

The holistic intention of organic farming is to manage organic and inorganic nutrients of soil and prevent their loss by retaining them in the form that can be accessed by crops. The process also enhanced symbiotic association between plants and their microbial flora, organic matter and physical environment. In this system some soil fertility management practices used that determines the cycling and availability of nutrients in the soil.

These practices are:

- Use of organic residues as soil amendments.
- Use of nitrogen fixation as major source of nitrogen.
- Plant species are diversified in space.
- Rotation of crops results active plant growth.

In this concept highly active microbial community break down organic matter into plant available nutrients. Conventionally managed systems are wellprofited from abundance of microorganism which are involved in nutrient retention (Wagg *et al.,* 2014) and soil structure improvement (Rillig and Mummey, 2016) which might positively influence nutrient efficiency but also water dynamics. Recovery of N in crop plants is usually less than 50% worldwide (Fageria and Baligar, 2005). Nowadays, approximately one per cent of the world's farmland is organically managed. North America has the biggest retail sales of organically farmed products though harbours less than 7% of the total worldwide organic production area scaled (Ifoam, 2015)

Figure 1: Organic farming, Biofertilizers and their uses in Agriculture

Principles of organic farming:

Organic agriculture grows and develops with principles which can improve organic agriculture for the world.

Principals of organic farming are as follows:

Health: Concerned with the health of the ecosystem, people, and communities.

Ecology: The correct balance inbetween ecosystem and environment or .

Fairness: Good human relationships and quality of life.

Care: The present and future environment consideration

Types of Organic Farming:

Organic farming is of twotypes: -

(a) Pure organic farming – In pure organic farming, every synthetic fertilizer is avoided. In the process of pure farming, fertilizer and pesticides obtain from natural sources.

(b) Integrated organic farming – Integrated organic farming consists of integrated nutrients management and integrated pest management.

Techniques of Organic farming:

In India, organic farming is practiced by following techniques:

Soil management: Soil management is basic technique of organic farming in India. After cultivation, soil loses its nutrients, and its fertilizer goes down. The process in which soil is recharging with all the necessary nutrients called soil management (Fig.2). Organic

farming uses natural ways to increase the fertility of the soil. It uses bacteria, available in animal waste. The bacteria help in making the soil more productive and fertile.

Figure 2: Soil management in organic farming

Weed management: One of the important aim of organic farming's is to remove the weeds, which are the unwanted plant, growing with the crop. Weeds sticking with nutrients of the soil and affect the production of the crops.

There are two techniques which give a solution to the weed.

Moving or cutting – In this process, cut the weed (Fig. 3)

Mulching – In this process, farmers use a plastic film or plant to residue on the soil's surface to block the weed's growth.

Figure 3: Weed management

Figure 4: Crop diversity

Figure 5: Chemical management

Figure 6: Pest control

Crop diversity: According to this technique, different crops can cultivate together to meet the growing demand for crops (Fig. 4). Agricultural farms contain useful and harmful organisms that affect farms. To save crops and soil, the growth of organisms needs to be controlled.

Chemical Management in Farming: In this process, natural or fewer chemicals, herbicides, and pesticides used to protect soil and crops (Fig.5). Proper maintenance is required throughout the area to control other organisms.

Biological Pest Control: In this method, use living organisms to control pests with or without the use of chemicals (Fig. 6)

Advantages of Organic Farming:

- Organic farming in India is very economical, it uses no expensive fertilizers, pesticides, HYV seeds for the plantation of crops. It has no expenses.
- With the use of cheaper and local inputs, a farmer can earn a good return on investment.
- There is a huge demand for organic products in India and worldwide and can earn more income through export.
- Organic products are more nutritional, tasty, and good for health to chemical and fertilizer utilized products.
- In India, organic farming is very environment friendly, it does not use fertilizers and chemicals.

Disadvantages of Organic Farming:

- Organic farming in India has fewer choices, and off-season crops are limited.
- Organic agricultural products are low in the early years. Farmers find it difficult to accommodate mass production.
- The main disadvantage of organic farming is the lack of marketing of the products and Inadequate

The role of microorganism with organic agriculture:

Soil microbial biomass is composed of eukaryotic (fungi, yeasts, protozoa and algae), and prokaryotic (eubacteria, actinomycetes and archaea) organisms, (Fig. 7) whose populations vary from soil to soil (Shannon *et al.,* 2002). Many microorganisms in soil synthesized urease enzymes which play important role in enrichment of soil by the degrading organic nitrogen (Hasan, 2000). One of the important factors that determine status of soil microbs is the type and amount of organic material that enters the soil ecosystem. The majority of soil microorganisms is heterotrophic and requires both carbon and energy sources (Shannon *et al.,* 2002). Microorganisms play a fundamental role in making soil fertile by binding of soil aggregates by hyphae and by theexternal secretions (Andrade *et al.,* 1998). There are many examples in which soil microbial systems help ecosystem health and stability.

Antagonistic and antibiotic microorganisms:

There are many reports suggested that soil organisms might be antagonistic to plant pathogens, pests and weeds. Number of species of fungi living on nematodes (Jatala, 1986), and many fungi are hyperparasites of other fungi (Adams, 1990). These activities not only influence the general nutrition, health, and vigour of higher plants (which also affects disease susceptibility), but they also determine the competitive behaviour of root-infecting fungi and their microbial antagonists (Curl, 1988). Streptomycesis effective and persistent soil saprophytes that is associated with plant roots and producers of antibiotics and hydrolytic enzymes. Samac *et al.* (2003) reported that they contribute to an integrated disease management system that includes alfalfa and other crops such as potato, maize and soybeans as they colonize plants and decrease damage from the pathogens.Rhizobacteria, plant growth- promoting rhizobacteria (PGPR), can produce antibiotics and hormones that suppressthe growth of plant pathogens andcompeting with pathogens for resources.

Mycorrhizal microbes:

Mycorrhizal fungi (mycorrhizal fungi and bacteria that live on and near the roots) provide several benefits to the host plant, includes fast growth (Thompson *et al.,* 1994), better nutrition, drought resistance (Parke *et al.,* 1983), and provideprotection from pathogens (Cooper and Grandison, 1986). Mycorrhizal fungi can be broadly categorised into two groups: vesicular arbuscular mycorrhizas (vam or am) and ecto-mycorrhizas (em), which vary widely in structure and function (Harrier, 2001). In agriculture the most significant function of both em and vam fungi is in optimising phosphate uptake by the plant. As uptake of phosphate by plants occurs at faster rate than the movement of phosphate in the soil, depletion of phosphate can develop at the root surface (Mosse, 1986). Mycorrhizal hyphae can make them available to the plant (Mosse, 1986). This is most significant in low p soils, as would be the case in many organic, farming systems. The addition of p fertiliser can increase p availability to plants but it suppresses mycorrhiza.

Mycorrhizal fungi are also control root pathogens by (Adams, 1990) improving nutrient acquisition by host plant; (Andrade *et al.,* 1998) supressingpathogens at root infection sites and within the rhizosphere; (Azcon- Aguilar and Barea, 1996) inducing structural changes in the root thus creating physical barriers to pathogen entry; (Bailey and Duczek, 1996) antagonistic substances produced for root pathogens; and (Bailey and Lazarovits, 2003), activate defence mechanisms of plant. Over last two decades, mycorrhizal fungi and other microorganisms has received much attention in inducing resistance to plant pathogens in number of crops (Azcon- Aguilar and Barea, 1996) and it is much beneficial to organic farming. Interactions between plants, mycorrhizal fungi and soil

bacteria appear to occur in an ordered manner between compatible species. The presence of mycorrhizalspecies in soil can affect the persistence and activities of native bacteria and vice versa (Andrade *et al.,* 1998).

Combinations of a range useful bacteria and fungi have used to improve plant growth and health. In addition, they also used to achieve concomitant improvements in soil (Caravaca *et al.,* 2002). Mycorrhizal fungi provide protection to the plant against uptake of heavy metals by adsorption on hyphae and by fungal metabolism (Gadd, 1983). Agricultural practices can have major short- or long-term impacts on mycorrhizal fungi, as well as on other soil microorganisms. Scullion *et al.* (1998) found that white clover only benefited from mycorrhizal infection in a low-fertility (organically- managed) soil. Furthermore, inoculate from organic soils were more effective in both achieving mycorrhizal infection and in allowing more efficient p uptake by the crop. Application of composts may enhance beneficial soil microorganisms. As the active microbialpopulation increases, soils capacity to utilize carbon, nutrients and energy is increased and thus these resources might not be available for soil-borne pathogens (Sullivan, 2001).

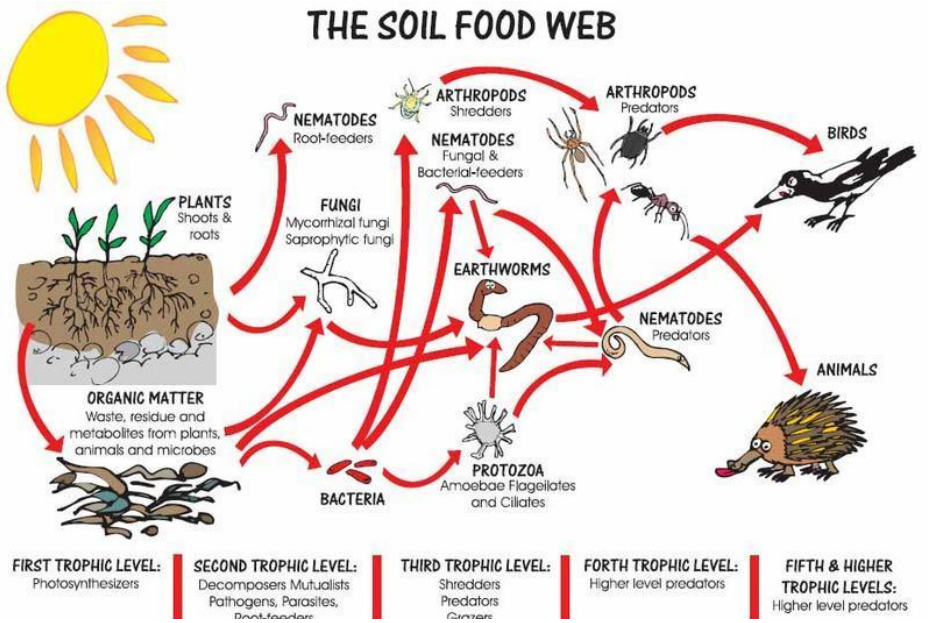

Figure 7: Soil microbial biomass

The size and structure of the soil biomass is influenced by carbon content of the soil that depends on soil management practices. Application of organic manure increases carbon availability to microorganisms. Schjonning *et al.* (2002) found that, carbon in microbial biomass was higher in organicpractices than in conventionally managed dairy farm soils. Soil microbial activity is increased by carbon that released from residues of crop. The installation of the residue in soil can cause disposition of a pathogen from its preferred

place, thusreduce the survival ability of pathogen (Bailey and Lazarovits, 2003). Therefore, soil microbial biomass changes as a consequence of switching to organic land management (Shannon *et al.,* 2002). Total microbial activity of soil increases with application of organic matterthat led to suppress the pathogens by increasing competition for nutrients. Croprotation is most successful to control the pathogens that require living host tissues, or those pathogens with low saprophytic survival ability (Bailey and Duczek, 1996). These microorganisms involved in the decomposition of organic matter thus supply nutrients to crops, detoxify contaminated soil and increase plant growth by production of phytohormones (Fig. 8).

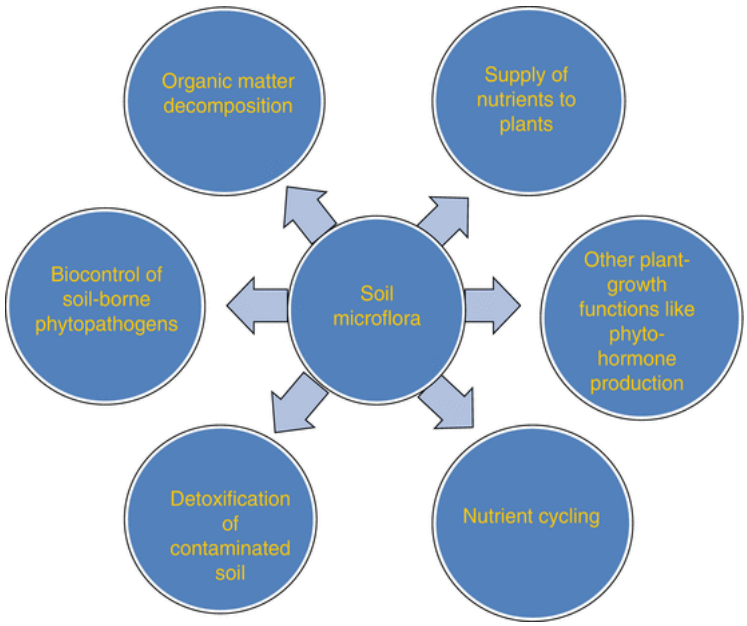

Figure 8: Benefits of Soil microflora in organic agriculture

Microbes as biofertilizers:

Organic farming is increasing the production of pollutant-free crops. It involves the use of biofertilizers and biopesticides which increases the nutrient quality of the crop and controls any kind of pest and pathogen.Biofertilizers are nothing but the microorganisms that add nutrient quality of the soil. Bacteria, fungi, and algae are some of the beneficial microorganisms that help in improving the fertility of the soil.

Biofertilizers are classified as:

- Free-living nitrogen-fixing bacteria like Azotobacter, and Rhodospirillum.
- Free-living nitrogen-fixing Cyanobacteria like Anabaena, and Nostoc.
- Loose association of nitrogen-fixing bacteria like Azospirillum.
- Symbiotic nitrogen-fixing bacteria like Rhizobium, and Frankia

The following microorganisms are used as biofertilizers:

- **Rhizobium:** They form root nodules in leguminous plants and fix the atmospheric nitrogen into an organic form. Rhizobium also has no negative effect on soil quality and improves the quality, nutrient content, and growth of the plant.
- **Azotobacter:** These are free-living nitrogen fixers found in all types of upland crops. These not only fix nitrogen but also provide certain antibiotics and growth substances to the plant.
- **Azospirillum:** Unlike Azotobacter, these can be used in wetland areas. They are found inside the roots of the plant (non-free-living) where they fix the atmospheric nitrogen.
- **Blue-green algae:** These are free-living nitrogen-fixing Cyanobacteria that are present only in wet and marshy lands. However, they do not survive in acidic soil.
- **Mycorrhiza:** It is a symbiotic association between the fungi and the roots of a plant. The mycorrhizal fungi play an important role in binding the soil together and improve the activity of the microbes. The fungi draw water and nutrients from the soil thereby increasing the plant productivity. It also helps the plant to survive under various environmental stresses.

Advantages from microorganism for organic agriculture:

Organic fertilizer:

There are a lot of microorganisms in fertilizer which are interested for doing research and developing them such as microbiological biotechnology used for organic agriculture and microorganism used for organic fertilizer because they can improve better soil to be suitable for growing sustainable plants.

Organic fertilizer can give nitrogen.

Group of microorganisms	**Kind and name**
Microorganisms can fix nitrogen	
Free living	A.Blue green algae (anabena, nostoc etc.) B.Bacteria (*azotobacter, azospirillum pseudomonas*)
Living with other plants	A.Blue green algae (azolla-anabena azollae B. Bacteria rhizobium)

Organic fertilizer can melt phosphate

Group of microorganisms	Kind and name
Microorganisms can melt phosphate	
Free living	A.Bacteria *(Bacillus sp., Scherichia freundii, Pseudomonas*) B.Fungus *(Aspergillus sp., Penicilium sp., Fusarium oxysporum*)
Living with other plants	A. Fungus (Mycorrhizal fungi)

Microorganism can decompose organic matter.

A group of this microorganism can use for producing compost and extract.

Kind of microorganism	Kind and name
Microorganisms produce compost	*Trichoderma viride, chaetomium abuanse, myrothecium roridum, aspergillus niger, a. Terreus, cellulomonas., cytophaga sp., bacilluss sp. etc.*

Microorganism can protect pest and get rid of unwanted weed

Kind of Microorganism	Kind and Name
1. Microorganism can get rid of insects destroy weed	*A. Virus (DNA viruses, RNA viruses)* *B. Bacteria (Bacillus thuringgiensis, B. popilliae, B. lentimorbus, B.sphaerius etc.)* *C. Fungus (Entomophthora, Masospora, Cordyceps, Aschersonia etc.)* *D. Protozoa (Nosema Locstae, N. bombycis etc.)* *E. Nematode (Neosplectona, Carpocasae, Romanomernos culicivorax, etc.)*
2. Microorganism can control weed diseases.	*A. Bacteroal Pathogens Control (Agrobacteroum radiobacter, Pseudomonas fluorescens, Streptomyces scabiobies etc.)* *B. Fungal Pathogens Conteol (Peniophora giganta, Pseudomonas fluorescens etc.)* *C. Nematode Pathogens Control (Bacillus Penetrain, Nematophthora gymophilla etc.)*
3. Microorganism can control unwanted weed.	*Cerco sporarodmanoo, Celletor trichumglocoaporipeds, Punccinia chroudrillina etc.*

Microorganism can produce antibiotic substances:

- it can protect diseases and pests.

Kind of Microorganism	Kind and Name
1. Fungus can produce antibiotic substances.	*Cyclohexinide (streptomyces griseus) blasticidins (s. Griseo chromogenes) polyozins (s. Cacaoi)*
2. Bacteria can produce antibiotic substances.	*Streptomycin (s. Griseus) oxytetracycline (s. Viridi faciens)*

Microorganism can have advantages with others

Microbes have been considered as the key drivers in the agroecosystem. A number of soil microbes could promote plant growth by producing plant hormones and increase nutrient availability (e.g., excrete phosphatase), producing antibiotics andimprove the quality and productivity of plants (Bacon *et al.,* 2015). Soil microflora and its benefits in agriculture production system receiving increasing attention recently. Many scientists investigated thatsoil microbs could reflect ecosystem processes such as crop productivity (Ding *et al.,* 2018), the regulation of decomposition (Hartmann *et al.,* 2015), nutrient cycling (Wagg *et al.,* ,2014) and protection against soil-borne pathogens (Van bruggen, *et al.,* 2006). Several studies have shown that agricultural practices have significantimpact on soil microbial communities and composition, including the tillage regime (Degrune, *et al.,* 2015), fertilization (Ye *et al.,* 2018), monoculture (Xiong *et al.,* 2015), crop residue management (Jiménez-bueno *et al.,* 2016) and plant protection schemes (Bünemann *et al.,* 2006). Hence, the soil microbial community could be shifted to a positive organization for plan.

Carr (2016), used compost to introduce microbes that would protect seedlings against damping off disease caused by a Pythium species.Nebert found that seed disinfection and inoculation as a way to prevent seed-borne diseases, particularly Fusarium (Nebert *et al.,* 2016); Zinati examined that use of compost extract can inhibit weed seed germination and reduce weed competition (Zinati, 2015). These studies open the new area of researchthat explore the beneficial role microbes in organic plant production.

In competitive world, one organism formulatesan environment that isundesirable for another organism, effectively prohibited the second organism from becoming fixed without directly killing it. A well seen example of this is creation of film on a root surface to prevent pathogens from infecting the plant.The mycorrhizae make nutrients biologically available can also antagonistic for a number of plant pathogens (Azcón-Aguilar and Barea, 1997). Microorganism like Streptomyces produces natural antibiotics, which became a commercial

source. These antibioticssuppress different pathogens in the soil the same way they do in people and animals.Specific microorganisms are known to protect seeds and seedlings from various diseases. For instance, various Bacillus, Trichoderma and Pseudomonas species protect the roots of plants from infectious diseases (Trabelsi and Mhamdi, 2013). These organisms can be introduced by inoculation of the soil.

Summary:

Organic farming significantly improved nutrient level of soil and increased microbial mass and diversity as compared to conventional farming. Differential microbial taxa analysis suggested that organic cultivation-enriched diverse bacterial linkages related to plant to a range of soil nutrient parameters. Furthermore, the microbial community composition was significantly correlated to the soil environment. It has been observed that organic farming improved the carbon and nutrient content of soil and sustainability of beneficial microorganisms. The excess nutrients and zn content may negatively affect the soil health and the microbial community in terms of their sustainable agricultural development.

Soil microorganisms play pivotal role in health and sustainability of soil. Organic matter indirectly provides energy to soil microorganisms that enhance the structure and stability of soil thus affecting the density and diversity of microorganisms. Thus, after harvesting it is important to replenish soil with organic matter. In ecological farming systems, organic matter is maintained by mixed farming, rotations, recycling, compost and green manures. However, the diversity of soil microorganisms is well appreciated now a days, from number of studies it is evident that, beside mycorrhizal fungi, other soil organisms not only play an active role in suppressing pathogens but also mediate the activity of beneficial organisms.

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PHYLLOPLANE MICROFLORA AS FOLIAR BIOCONTROL AGENTS AGAINST LEAF SPOT OF *CENTELLA ASIATICA* (MANDOOKPARNI)

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Abstract:

Phylloplane microflora (fungi) presented on the surface of leaf were screened and selected for the evaluation of their potential against *Colletotrichum gleosporioides* causing leaf spot disease of *Centella asiatica* (Mandookparni). *In vitro* studies conducted to evaluate the efficacy of phylloplane fungi. *Trichoderma harzianum* ISO-1, *T. harzianum* ISO-2 and *T. piluliferum* caused maximum inhibition of test pathogen followed *Aspergillus niger* and *Penicillium sublateritium* whereas *P. frequentans* and *Cladosporium cladosporioides* showed minimum antagonistic efficacy.

Keywords: Biocontrol, Antagonistic efficacy, Phylloplane fungi, *Centella asiatica*

Introduction:

The term 'phyllosphere' was proposed for the environment provided by the wet leaf surface and enabling microbial development (Last, 1955; Ruinen, 1956).The phylloplane is a natural habitat on the leaf surface which represent a heterogenous population comprising of both pathogen and nonpathogen (Mukerji, 1973, Mishra and Tiwari, 1976). According to Lindow (2006) the aerial habitat influenced by plants is termed phyllosphere and inhabitate are called epiphytes.

Centella asiatica (L.) Urban (Syn. *Centella coriacea* Nannfd., *Hydrocotyle asiatica* L., *Hydrocotyle lunata* Lam., and *Trisanthus cochinchinensis* Lour.) is a tropical medicinal plant from Apiaceae family native to Southeast Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia as well as South Africa and Madagascar. *C. asiatica*, commonly known as "Gotu kola, Asiatic pennywort, Indian pennywort, Indian water navelwort, wild violet, and tiger herb" in English. The leaves, which are edible, are in yellowish-green color, thin, alternate with long petioles, and quite characteristic reniform,

orbicular, or oblong-elliptic shapes with seven veins. The plant grows horizontally through its green to red stolones which combine to each other and roots in underground.

Around the world, there are some microorganisms commercialized to inhibit phytopathogens. *Trichoderma* is one of the most studied biocontrol agents against fungal phytopathogens. Mainly, the species of *Trichoderma harzianum*, *Trichoderma asperellum*, and *Trichoderma virens* has been reported as BCA (Joshi *et al*., 2016).

Mycoparasitism depends on of the microbial enzyme system, which includes a large array of enzymes. Depending on the *Trichoderma* system nature, it is possible to produce 5 to 7 individual chitinases and 1 to 7b-1, 3-glucanases and these enzymes can act complementarily (Markovich and Kononova, 2003). Other authors also reported that the influence on the microorganism developmentand inhibition it is associated with antibiotic production, as well as, volatile compounds (Ghisalberti *et al*., 1991).

Materials and Methods:

Isolation of leaf pathogen:

Leaves of *C. asiatica* infected with *C. gloeosporioides* were collected from Non Wood Forest Products Division Nursery, Forest Research Institute, Dehradun, and Uttarakhand. Isolation of pure culture of fungal pathogen was done by taking a portion of leaf which is containing brown spot was surface sterilized with 0.1% mercuric chloride for 1 min, followed by rinsing with three changes of sterilized distilled water and was placed on potato dextrose agar medium in Petri plates. The plates were incubated in a B.O.D. incubator at 25±1°C for mycelial growth.

Isolation of phylloplane fungi:

Phylloplane fungi were isolated from healthy leaves of *C. asiatica* through leaf washing technique (Dickinson, 1967, Aneja, 2003) and identified with standard monographs (Ellis, 1971) and expertise available. To study their antagonistic properties pure cultures were maintainedon potato dextrose agar medium at 4°C in a refrigerator.

***In vitro* colony interaction (Dual culture technique):**

Sterilized potato dextrose agar medium was poured aseptically into sterilized Petridishes of 7c media. Dual inoculation of the pathogen and an antagonist was setup. Culture discs of 5mm diameter were cut from the periphery of the actively growing colonies using a sterilized cork borer. Disc of test fungus was placed aseptically at the edge of the Petri plate. These plates were incubated at 25±1ºC for 3 days. Mycelial disc (5 mm) of antagonist was inoculated on opposite side of Petriplate three days after the pathogen to adjust for the slow growth rate of the pathogens. Paired cultures were again incubated at

25±1°C for 6-9 days and observed periodically. Then antagonistic fungi were tested against *C. gloeosporioides*. Each set was made in 3 replicates.

Antagonistic behaviour was measured quantitatively by calculating the area. Graph paper was used to measure the area of the antagonists, test pathogen species and inhibition zone inthe Petri plate. Antagonistic efficacy for each antagonist against the pathogen was worked out according to the following formula (Ojha, 2000):

Antagonistic efficacy = b+c-a

Where,

A = % of area of test pathogen sp. with antagonist in the same Petriplate (cm^2)

b = % of area of antagonist, and

c= % area of inhibition zone between antagonist and pathogen or overgrowth of antagonist over test fungus

Results and Discussion:

Potential antagonist stried were identified based on their cultural and microscopic characteristics as *Trichoderma harzianum* Rifai ISO-1 and ISO-2, *T. piluliferum* Webster and Rifai, *Aspergillus nigervan* Tieghem, *Penicillium sublateritium* Biourge, *P. herquei* Bainier and Sartory, *P. frequentans* Westling, *P. tardum* Thom, *P. citreo-viride* Biourge and *Cladosporium cladosporioides* (Fresen.) de Vries.

Antagonism between colonies of *Colletotrichum gloeosporioides* and *phylloplane* fungi:

Mycelial growth measurement of *C. gloeosporioides* and the tenantagonists towards each other on Potato Dextrose Agar on the tenth day after inoculation and percent inhibition of *C. gloeosporioides* are summarized in Table 1. A significant interaction was exhibited by *Trichoderma* isolates in which growth of *C. gloeosporioides* was affected as the antagonistic fungi grew over the colony of *C. gloeosporioides* and completely inhibited its growth. After inoculation and percent inhibition of *C. gloeosporioides* are summarized in Table 1. A significant interaction was exhibited by *Trichoderma* isolates in which growth of *C. gloeosporioides* was affected as the antagonistic fungi grew over the colony of *C. gloeosporioides* and completely inhibited its growth.

Trichoderma harzianum ISO-1, *T. harzianum* ISO-2 and *T. piluliferum* inhibited the growth of *C. gloeosporioides* by (90.00%) and exhibit maximum efficacy followed by *A. niger* (62.97%), *P. sublateritium* (61.02%) and *P. herquei* (42.19%) were found to be significant, in inhibiting the growth of C. gloeosporioides, except *P. tardum* (36.91%) and *P. citreo-viride* (34.90%) and C. cladosporioides (30.97%). However, it was found that the minimum

antagonistic efficacy was shown by *P. frequentans* (29.15%) showed the minimum antagonistic efficacy against *C. gloeosporioides* (Fig.1).

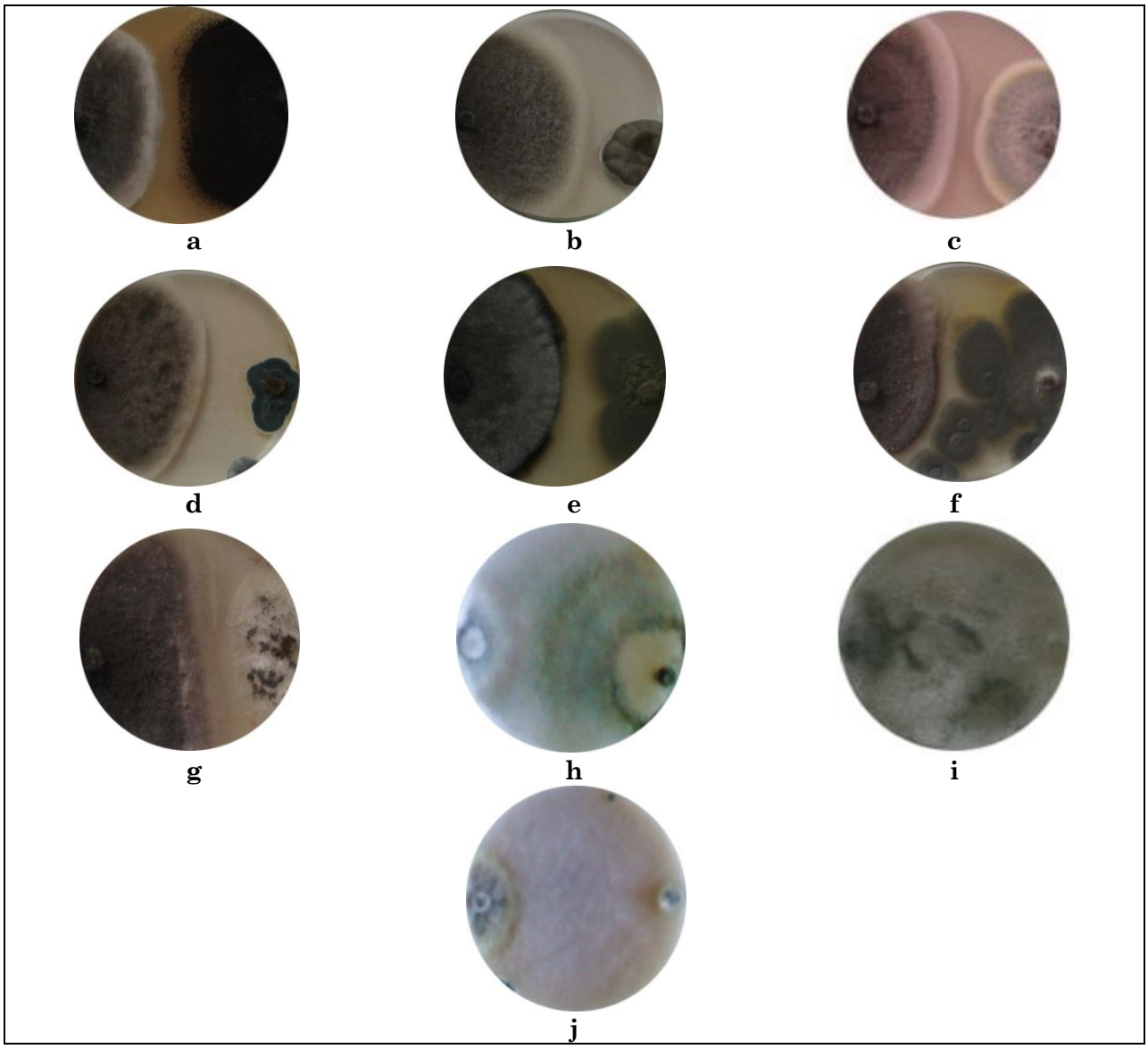

Figure 1: a-j. Screening of antagonistic fungi against *C. gloeosporioides*
a) A. niger, b) C. cladosporioides, c) P. citreo-viride, d) P. frequentans, e) P. herquei, f) P. sublateritium, g) P. tardum, h) T. harzianum ISO-1, i) T. harzianum ISO-2, j) T. piluliferum

Antagonistic fungi proved effective in inhibiting the fungal growth (Rai and Singh, 1980). Several workers have been reported that the use of *Trichoderma* species against number of plant pathogenic fungi (Harman, 2006, El-Mougy *et al.,* 2007). Antagonistic activity of *Trichoderma* species have been reported against *Colletotrichum gloeosporioides*

(Svetlana *et al.,* 2010). Dual culture interaction for *A. niger* leads to the formation of zone of inhibition which was similar to the findings of Santha Kumari (2002), who reported that the isolates A1 and A2 of A.niger were found effective in inhibiting the growth of *C. gloeosporioides* causing anthracnose of black pepper under *in vitro* condition. Reynaldo *et al.* (2018) analyzed the growth inhibition of *Colletotrichum gloeosporioides* by *Trichoderma* species.

Table 1: Antagonistic efficacy of antagonist isolates against *C. gloeosporioides*

Sr. No.	Antagonists	Percent Antagonistic efficacy (Mean ± S.D.)
1	*A. niger*	62.97(79.33)±0.83
2	*C. cladosporioides*	30.97(33.65)±5.56
3	*P. citreo-viride*	34.90(32.77)±2.12
4	*P. frequentans*	29.15(23.73)±0.56
5	*P. herquei*	42.19(45.10)±0.42
6	*P. sublateritium*	61.02(76.43)±2.96
7	*P. tardum*	36.91(36.07)±0.88
8	*T. harzianum ISO-1*	90.00(100)±0.00
9	*T. harzianum ISO-2*	90.00(100)±0.00
10	*T. plililiferum*	90.00(100)±0.00
Mean		57.08
SEM±		1.24
CD at 5%		3.65

*Original values are given in parentheses

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FISH HANDLING AND PRESERVATION

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Introduction:

Fish is one of the vital protein sources that need a special handling (Eyo, 2004). The fish spoilage occurs very rapidly on account of the high temperatures that drives the bacterial, enzymatic activities and also adds to oxidation of fat present in the fish. Due to improper handling, more than 40% of the harvested fish are wasted in African countries. To minimize these losses, proper handling, processing methods and suitable preservation techniques must be employed (Bate and Bendall, 2010). The ultimate of fish processing and preservation is to make it fit for consumption and satisfy consumer demands. The process starts right before fishing expedition, and continues till the fish is consumed or processed in oil (Karube *et al.*, 2001). Fish spoilage starts as early as it is taken out of the water. Hence extra care is required to during the process of synthesis, catching, handling and storage. A spoiled fish is certainly unfit for consumption (Gopakumar, 2000). A mishandled fish is not only unfit, but loose the value due to off-flavors, mushed texture, and a bad color, which eventually refrains the consumer for buying (Brut, 2003). A good service comes with a good product, which ultimately leads to a good seller buyer relation (Nelson *et al.*, 2004).

Spoilage of the fish commence with the formation of enzymatic and complex bacterial or chemical changes which start during netting/hooking of the fish (Brut, 2003). The process starts after the mortality of the fish. Warm climates are favorable for fish spoilage. Fish's gut is a rich enzyme source, that helps live fish to digest own food (Lima Dos Santos et al, 2011). As the fish dies, these enzymes start to digest the stomach. Subsequently these enzymes begin to act upon the flesh of the fish and digest it. As a result the fish become very soft and its smell becomes more distinguished. There are numerous bacteria present naturally on skin, gills, intestine of the fish (Karube *et al.*, 2001). Though

these bacteria are harmless to the living fish, but they rapidly multiply, and after 3 to 4 days they consume the flesh of the fish which is well preserved in the ice as it begin to soften due to enzymatic digestion. The bacterial load contained by the fish is the function of its health, the environment, and on the method by which it was caught. A healthy fish derived from the clean water, has better containment then fish from dirty pond. The enzymatic digestion as well as bacterial decomposition employs chemical changes which gives the familiar spoilage odors (Putro, 2005). Chemical reaction of oxygen with oil results in rancid odors as well as taste. The purpose of fish processing and preservation is to minimize or slow down the rate of enzymatic action, bacterial growth, and chemical action of these substances , and to maintain the condition of flesh of the fish nearly same as that of fresh one(Bate and Bendall, 2010).

Fish freshness:

Freshness of fish is normally assessed by the appearance, smell/odor and the texture (Karube *et al.*, 2001). The assessment is a function of senses; they are called sensory/organoleptic. Following are the factors to be looked for the freshness.

1. The basic fish appearance includes eyes, surface scales, gills and the extent of looseness or tightness of its flesh.
2. Gill's odor and also bell cavity.
3. Checking the presence of any discoloration at the side of backbone.
4. Checking the presence of death stiffening.
5. Belly walls appearance(Bate and Bendall, 2010)

Fish preservation methods:

Long time Preservation:

Chilling:

Keeping the fish cool is the first and the foremost method to preserve the fish. Chilled fish relatively lasts longer than the uncooled fish, though both of them will ultimately spoil over a period time (Tawari and Abowei, 2011). This is done by covering the caught fish in the ice layers. Generally ice preservation is not an effective method for long time preservation, as the melting of ice causes water causes altering of flavor by mixing with the nutrient flesh. However for short term preservation, ice can be employed where transport to nearby local markets has to be done. The autolytic activities need to be checked with the lower temperature (FAO, 2007). The trained panel tastes are usually not able to

distinguish the properly iced fish kept for six to seven days than the fresh flesh. Storage life extension can be done by antibiotics addition to the ice. Ice functions in following two ways:

1. By reducing the temperature, it slows down the bacterial growth rate.
2. It flushes away the bacterial stuff as it melt. Hence the melt water must be kept away from fish.

Chilling

Salting

Drying

Long time Preservation:

1. Salting:

There are a plenty of salts choice available for curing the fish. But in any outskirt places, where there is no option other than what is available, the salt that is available has to be used for the very own purpose. The two prime techniques of salting namely wet salting or dry salting (FAO, 2005).

a. Wet Salting:

It employs to keep the fish for a long time in brine solution. The process requires a watertight container such as barrel, drum etc. The brine solution is made by taking clean water and salt in the ratio 4:1. In case of coarse salt, grounding has to be done first (Tys and Peters 2009). After this a uniform mixture is made by stirring the mixture with a stirrer. The next step depends upon the type of fish to be salted. Generally it is good to first chop off the head, and clean the fish. For large fish, it must first cut open and the backbone must first be taken off. Heavy amour scales fish should be scaled. Slashes are made on the places where there is thick flesh, as it helps in salted brine to penetrate the fish. For very large fish, thin fillet cut are made. After the preparation of fish according to the required size, it is cleaned and put into the brine solution (FAO, 2008). A layer or matting is put over it to cover the fish completely with the brine. The salted fish is kept for long durations in dark or any shady place (Leistner and Gould, 2002).

b. Dry Salting:

This method employs salting of fish, but its juices, brine and the slime are allowed to flush away. This process can be done on mats, boxed etc. In any condition, the brine which is formed by the juices of the fish and salt must flush away. The fish and the salt must be taken in the 2:1 (Kauffeld *et al.*, 2005). Fish layers are separated by salt layers. It is useful techniques when there is no availability of containers. This technique is used to salt flying fishes in opened fishing boats whilst at sea, and fish are kept whole. It is also possible to flush the salt away by soaking in fresh water before the use (FAO, 2005).

2. Drying:

The small and the thin fish can be dried in the sun if given sufficient time to dry out. If these conditions are not achieved then fish should be put in brine for one full night, and dried next morning (Deepchill, 2010). In case of rainy weather, one should wait for the time weather has cleared. In the other case, it is essential to wash the salt from the fish by making it soak in fresh water for few hours before drying. This also depends upon the taste required and on the fish curing purpose (Huss. 2009). Small fish generally can be dried in the sun either by suspending, or putting them onto the mats. In case of rains, avoid getting them contact with rainwater and transfer them to a shady place .In case placed on mats, they must be turned over and around every couple of hours in order to make them dry quickly. For large fish, hanging the fish is considered better. For dry salted fish, they must first be cleaned. It takes around 3 days for the fish to dry. If the large percentage of fish has dried and kept for some time, it is piled in the dark place, off the ground and on wooden planks. It is then covered with a mat. The next day fish should again be put in sun for couple of hours and they kept away.

3. Smoking:

The major 3 methods of smoking are:

(a) Smoking and roasting; (b) Hot smoking; (c) Long smoking.

a. Smoking and Roasting:

It is the simple preservation method, for either direct consumption after cure or within next 12 hours. This technique can keep the product fit for another 12 hours (Kauffeld *et al.*, 2005). Here an unsalted fish is taken and put over the wooden husk fire. The fish must be turned over and around in every five minutes. The fish is ready for consumption in half an hour. By employing this way the fish can be preserved in the open fish boats, where the

smoking is done in a half- drum. Salted fish is also smoked by this technique, but it is generally used in cases of instantaneous consumption or to local nearby market.

b. Hot Smoking:

This method is employed in case of immediate consumption or storing the fish for maximum two days. Small fish are salted first for 1 hour. After the salting they are put onto the iron grids and dried in a windy atmosphere for half an hour. It is essential to have an oil drum for making smoking stove. The top portion of the container is chopped off and holes are drawn 0.5 feet below rim for placing spits. For controlling the fire a small opening is provided. This opening is covered with a flat plate of steel. The fire out of dry husk is initiated, and once it is fully developed, it is regulated such that it becomes flameless (Tys and Pieters, 2009). Fishes can then be put over those spits. During the time smoking is carried out, the top portion of the drum must be blanketed with sacks, and also the fire opening must also be closed. Continuous observation of fire front must be done. The fish takes about 45 minutes to 1 hour to get completely roasted. The completion of process is reflected by the color of the fish which generally turns to golden yellow. In case of a big fish (over 2 feet), it is divided into two parts, on either side of the backbone. Each part consisting of the fish is tightened between flat sticks or bamboo logs. These parts are then placed over the racks built at a height of around 4 feet above the ground. Plenty of split fish can be put next to one another in series.

A fire is then lit beneath the rack. Number depends upon the quantity of the fishes that have to be smoked. The fire arrangements should be so made that it is soft and slow for the first half an hour, followed by intense one for next one hour. Then the fire is again brought to low intensity for next 5-6 hours (only smoking) (Alasalvar *et al.*, 2011).

After the above process, fish is ready to be transported and will certainly be in a very satisfactory condition for 3 days in tropical condition. This method is employed in Celebs for skipjack and other tunas (Ananoui *et al.*, 2007).

c. Long Smoking:

For long time storage, of over two months fish can be preserved by the method of smoking provided the fish must not be oily.

A closed shed made out of any local material is used. The shape and size of the shed to use depends on the quantity of fish that have to be smoked. The minimum height should be 6 feet. Racks are provided to hang fish and lay them. Using the spits for hanging the fish is often the best method, but can also be laid on loose matting. Fish can be hung at least 3

feet above the bottom to the roof (Deepchill, 2010). The fish preservation is affected by the smoke, very slow burning is preferred which can extend to over 48 hours. After this the flesh is completely dried. If it is required to transport to other island, they must be packaged in bunch of dry leaves and along with bamboo. This way the fish can be dispatched over to long distances (Idachaba, 2001).

Smoking

Canning

4. Fish canning

In this process, fish is subjected to heat treatment in closed tin or aluminum containers till it is completely sterilized (Isachaba, 2001). The heat treatment should be carried out such that all the bacteria and spores are destroyed. The nutrition value must remain unaltered and suitable for consumption. Canned food can be preserved for a longer period of over years without refrigeration by storing them in an airtight container (Leistner and Gould, 2002). The fish that has to be canned must be handled hygienically to prevent microbial growth on fish. The fish of poor quality will ultimately produce canned fish product of bad odor and flavor (Brut 2003).

Demerits of fish preservation:

1. As a result of chilling, denaturation of the flesh occurs. This is mainly because ice crystals affect adversely to muscles. The cell wall burst out, deformation of flesh results in loss of flavor as well as taste. Texture loss and dehydration of the flesh also take place (FAO, 2008).
2. Lack of hygienic measures during the process of washing, evisceration and washing would cause more damage to the preserved material and a rapid rise of bacterial population would occur.
3. The nutritional value is compromised as a result of drying, it also cause loss of weight and reduction in digestibility of the flesh.

4. Over salting provides the gateway for bacteria which are salt tolerant, which cause pink eye spoilage of the flesh.
5. Rancidity of the fat enhances due to smoking as a result of which digestibility reduces.
6. Loss of protein takes place due to salting by 1 to 5 %and 8 to 30% in case of smoking.
7. Loss of essential vitamins such as vitamin B1, C, panthotenic acid and pteroxylglutamic acid take place (FAO, 2005).

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ANALYSIS ON CLIMATE CHANGE THROUGH AN ANIMAL BEHAVIOUR AND PREDICTIONS

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Abstract:

Animals have very specific responses to climate change. They respond to changes through adaptation, migration and if not, death occurs. Animals behaviour accordingly also consist of prediction analysis like Earthquake, natural storms etc. Behavioural thermoregulation provides adjustment when and where an animal is active reprieving from warm temperature as well as floods, snow falls, stronger storms etc. This present paper deals with effects of climate change on various regime of animal behaviour.

Keywords: Adaptations, Migration, Thermoregulation, Prediction, Animal behaviour.

Introduction:

Climate change is a natural event that significant and direct effect on animals as they are the major driver of the process of speciation and extinction. In general, climate change affects animals and birdlife in various different ways. Birds lay their eggs earlier than usual in the year, plants bloom earlier and sometimes mammals come out of their hibernation state. Climate change is projected to effect individual organisms, populations, species distributions and ecosystem composition and functions both directly and indirectly changing the intensity and frequency of disturbances like wild fire and severe storms (IPCC, 2002).

The effects of climate changes are

- Rising maximum temperature
- Rising minimum temperature
- Rising sea levels
- Higher ocean temperature
- Heavy rain and hail
- Shrinking glaciers
- Thawing permafrost

The first recorded reference to animals predicting earthquake is found in ancient Greece from 373 BC. Underground animal rats and weasels, Cats, Elephants, Flamingo, Dogs, Bees, Bats, Snakes, Horses, Cows, and even centipede abandoned their normal environments giving strong predictions through their abnormal behaviour.Reports of similar activity have been recorded throughtout history. Anecdotal in nature, these reports seems to indicate the many species adapt abnormal behaviour prior to earthquake or any other natural disaster occurance. Mammals, fish, birds and insects have been seen showing strange behaviour weeks ahead with activity intensifying closer to the time of the event. Animal sense even a small change in nature as well as climate and that influence their behaviour. Detailed observation of surroundings can be applied to knowledge acquisition in traditional Native Knowledge and western science. Although the applications of knowledge may vary, it is understood that observation must take place repeatedly over time in order to build a knowledge base that can support pattern recognizition, inferring and predictions. In traditional Native Knowledge, observation of surroundings including animal behaviour often leads to inferences and predictions about weather conditions, natural disaster, storms and sometimes earthquakes.

Observation:

Every organism has a distinct set of preferences or requirements , a niche and biodiversity has been tied to the diversity of animals niches. These include or is affected by temperature, aridity, resource availability, habitat requirements, enemies, soil characteristics, competitors and pollinators. Since the factors that compose a niche can be complex and interconnected, the niches of many animals are bound to be affected by climate change. It is mostly observed that animals can react to climate change in only three ways: They move, adapt or die. Animals that do not adapt to changing environments are in danger. Climate change is significantly disrupting organisms and ecosystems on land and in water. Animals are not shifting their range and altering the timing of key life stages, there exhibits differences in their sex ratio, heat tolerance and others in their bodies. Out of which some change may help a particular species to adapt while others could speed its demise.

Humans and wild animals always face new challenges for survival due to climate change. These are rising Sea levels, Melting glaciers, Global warming, intense drought, Storms, Heatwaves etc. These effects survival as it destroys places they live and is havoc on their livelihoods. There are evidences that animals, plants and birds are being affected by climate change and global warming in both their distribution and behaviour. In future if

use of green gas emission is not reduced the climate change occurring could cause a quarter of land animals, birdlife and plants to become extinct. In animals climate change affects their reproduction, migration, plants blooming, hibernation, egg laying capacity etc. Although it is always thought till no species has extinct exclusively because of climate change, but many migratory and non-migratory species are expected to become extinct in the coming near future.

Droughts, floods or changes in precipitation and global warming considered as direct effect of climate change influence the quality and amount of vegetation present in particular region , in addition to the soil fertility and plant diversity. Sometimes these direct effects of climate change have negative impacts and an indirect effect in animals throughout.

It may be:

- Extinction or declines in population
- Increased competition for remaining resources
- Increased foraging difficulty
- Migration
- Changes in phenology
- Reduced livestock production
- Evolutionary thrive

Results and Conclusion:

Unusual behaviour is very difficult to define, and determining, if there is a characteristic behaviour is not simple, clear-cut process, although there are some distinct patterns which have emerged. For example, an intense fear that makes some animals cry or bark for hours and others flee in panic has been reported often. Some animals appear agitated, excited, nervous, over aggressive, or seems to be trying to burrow or hide, restlessness , heightened sensitivity to mild stimulation, vocal responses, a tendency for burrowing, premature termination of hibernation and leaving their normal habitats. Although majority of accounts pertain regularly to dogs and cats there are many other types of animals in wild or domestic to predict or unusual behaviour.

- Catfish are reputed to become agitated.
- Snakes have been observed to leave their underground places.
- Bees have been seen evacuating their hive in a panic, minutes before any natural calamities and not returning back until fifteen minutes ended.
- Mice appears to be dazed and allow them to easily be captured by hand.

- Homing pigeons take much longer to navigate to their destination.
- Hens laying fewer eggs, or no eggs at all.
- Pigs aggressively try to bite one another
- Even creatures like millipedes, leeches, squids and ants have been reported to exhibit abnormal behaviour.

These strange behaviours generally occur anywhere from moments to week in advance of any natural disaster giving predictions to human beings. A number of theories have been proposed to explain this phenomenon, and the precursory signals that the animals are picking out might be. Some suggests high degree of sensitivity of animals, some suggest fluctuations in earth's magnetic field or magnetite found in brain of animals or sensitive to changes in electric field gradient of the atmosphere.It is believed that species in regions with very consistent climate will have the harder time adapting to climate change. It is also observed that tropical species have less diversity in their genes to deal with changing conditions they have less climate variability.

In conclusion we will need a mix of adaptation and mitigation measures to meet the challenge of climate change, but this is hampered by a lack of information on the costs and benefits of adaptation. It is therefore, essential to develop a portfolio of strategies that includes, technological development with mitigation and adaptation with research on impacts of climate change. But analysis of the benefits of various mixes of strategy is severely restricted at present by lack of information on the potential costs of impacts by lack of comparable information on the damage that could be avoided by adaptation, and especially by lack of understanding of how these impacts will vary under different socio-eeconomic development pathways. It is important to fill these gaps in our knowledge to conclude animal behaviour through their signalling predictions or unusual behaviour.

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ETHNOMEDICINAL PLANTS USED TO TREAT DIABETES AMONG TRIBES IN KHANDWA DISTRICT, INDIA

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Abstract:

The present paper deals with 19 ethnomedicinal plants species used to cure Diabetes in Khandwa district of Madhya Pradesh. Ethnomedicinal information was recorded during the extensive field surrey carried out during 2016-2020. The information covers botanical names, vernacular names (Tribal names T.N.) family and mode of usages.

Keywords: East Nimar; Ethnobotany; India; Madhya Pradesh; Tribes; diabetes.

Introduction:

East Nimar is situated in the South West corner of Madhya Pradesh. It lies between 21^0 05' and 22^0 25' N Latitude and between 75^0 57' and 77^0 13' E Longitude and 304 M above sea level. Madhya Pradesh State has the largest population of tribals in the country. The present work mainly covers the tribal villages situated at the foothills of Satpuda inhabited by Korku, Gond and Nihal tribes of East Nimar. The tribes are original inhabitants of this region. There are about six more tribes like Bharia-Bhumia, Bhil, Bhunjia, Oraon, Pradhan and Pardhi residing in this region. These three tribes were selected due to the following reason.

Field observation on plants, the Tribal Names (T.N.) and information, their uses were recorded in the field book. Voucher Specimens were brought to laboratory and prepared according to the conventional herbarium technique (Jain and Rao, 1976). The morphotaxonomical description of plants and their identification was done by consulting different floras (Flora of M. P.: 1993, 1997, 2001; Cook, 1967; Chopra *et al.,* 1956; Jain *et al.,* 1991; Walton, 1980). After confirmation and noting of ethnobotanical information about the plant species, the subsequent visits were planned in proper flowering period and to confirm the folk medicinal uses

Enumeration:

In the following enumeration, the up-to-date botanical names of the plant species have been arranged alphabetically for easy along with ethnobotanical uses in detail (**K** for Korku; **G** for Gond and **N** for Nihal).

***Abutilon indicum* (L.) Sweet, Hort. (MALVACEAE). T.N.-Tutti/Kanghi/Kharaiti(K and N); Kanghi / Vellipival ta Marra (G).**

- The fruits are cooked with little water and made into a paste and then 1 teaspoon of water is added. The mixture is administered orally once daily in early morning for 21 days to cure diabetes.

***Anogeissus latifolia* (Roxb. ex DC.) Wall. (COMBRETACEAE). T.N.-Dharoa/ Dhaunda (K); Dhamora/Adma ta Marra (G); Dhaura (N).**

- 1-2 teaspoon powdered bark is given twice a day for 30 days to control in diabetes.

***Asparagus racemosus* Willd. (LILIACEAE). T.N.-Sarsarmusali (K)**

- Tablets are made from paste of entire plant and taken twice a day with cow milk to cure diabetes.

***Bauhinia variegata* L., Sp. (CAESALPINIACEAE). T.N.- Kanchnog (K); Champe ta Marra (G); Champa (N).**

- Flower paste is mixed with jaggery. Four teaspoons are taken on empty stomach once a day for diabetes.
- Fruits cooked as vegetables and eaten specially to cure diabetes.

***Benincasa hispida* (Thunb.) Cong. (CUCURBITACEAE). T.N.- Bhura Kaddu (K).**

- 4 teaspoon pulp of fruit is given on empty stomach once a day in diabetes.

***Boerhavia diffusa* L., Sp. (NYCTAGINACEAE). T.N.- Phathari (K and N); Bijkhopara**

ta Chidur (G);

- Plant is cooked as a leafy vegetable and eaten specially to cure diabetes.

Butea monosperma **(Lam.) Taub. (FABACEAE). T.N.- Khakhari/Pharas (K); Palas ta Marra (G); Khakhara (N).**

- 11 flowers are soaked in 200 ml water in a copper pot overnight and mashed next day in the morning and filtered. Filtrate is given as a single dose each day, on empty stomach, for 30 days to cure diabetes.
- 20 gm root powder mixed with equal quantity of root powder of *Bombax cieba* is given twice a day to cure diabetes.

Cassia absus **L. (CAESALPINIACEAE). T.N.- Bankulthi (K); Beda (N).**

- 20 gm root powder mixed with equal quantity of root powder of *Bombax cieba* is given twice a day to cure diabetes.

Cassia fistula **L. (CAESALPINIACEAE). T.N.- Bhanaka-Bhungru (K); Bahawa to Marra (G); Bahala(N).**

- 1 teaspoon stem bark powder is given with water twice a day to control diabetes.

Catharanthus roseus **(L.) G. (APOCYNACEAE). T.N.- Sadasuhagni ta Chidur (G).**

- 20 ml flower extract is given once a day on empty stomach to treat diabetes.

Coccinia grandis **(L.) Voigt, Hort. (CUCURBITACEAE). T.N.-Dhorkakri (K); Indravan ti Veli (G); Kundru (N) .**

- 1 cup leaf juice is given once a day on empty stomach for 3 months to control diabetes.

Curculigo orchioides **Gaertn. (HYPOXIDACEAE). T.N.- Kalimusli (K); Kariyalmusli (G).**

- 50 gm root of plant with 100 gm seeds of *Syzygium jambos* are ground with 50 ml juice of *Momordica charantia.*30 tables are made from this paste and shade dried. 1 table is given with lukewarm water twice a day to control diabetes.

Ficus religiosa **L.,Sp. (MORACEAE). T.N.- Pipri (K); Alli ta Marra (G); Pipal (N).**

- 1 teaspoon bark is taken twice a day for 1 month to control diabetes.

Grewia tiliifolia **Vahl, Symb. (TILIACEAE). T.N.- Mothi Dhaman (K).**

- Decoction of leaves (about 10 ml) is used twice a day for 15 day in diabetes.

***Gymnema sylvestre* (Retz.) R.Br.ex Schult. (ASCLEPIADACEAE).T.N.- Medsinghi/ Gudmar (K and N).**

- 2-3 leaves are chewed twice a day regularly in diabetes.

***Helicteres isora* L.(STERCULIACEAE). T.N. Korajbothi/Aattwafalli/Marurphalli(K); Muradsheng ta Marra(G);Aithafalli (N)**

- 20 ml fresh root juice is given twice a day after meals in diabetes.

***Lagenaria siceraria* (Mol.) Standl. (CUCURBITACAE).T.N.- Tumdi/Tumba (K).**

- Powder of 5 seeds is given to patient twice a day in diabetes.

***Momordica charantia* L., Sp.Pl.(CUCURBITACEAE).T.N. Kartola (K).**

- 20 ml leaf juice is consumed with water twice a day to cure diabetes.

***Pterocarpus marsupium* Roxb. (FABACEAE). T.N.- Bija/Bijasal (K,G and N).**

- 20 ml decoction prepared by powdered heartwood is consumed with water twice a day to cure diabetes.

Result and Discussion:

A family wise analysis of all the 19 plants shows that Cucurbitaceae emerges as the largest family contributing 4 species, followed by Caesalpinaceae (3 species) and other important families are with one species each. The present study also reveals that mostly Climbers/twiners (6) and Trees (6) are used by tribals of East Nimar, to treat diabetes. Herbaceous plants rank second (4 species) followed by the shrubs (3 species).

Conservational Aspect:

Conservation of nature is an ancient tradition in India where the biodiversity is mostly preserved on religious grounds. The tribals have their own ways of protecting medicinally, economically and culturally important plants. They impose taboos, totems and the social and religious restrictions on cutting or harming plant species. In case of mistake or violation, punishment is given. Not only individual species but entire forest tracts are conserved. Such pockets are known as 'sacred groves' which show optimum growth of vegetation. This phenomenon can be considered a good example of conservation as well as judicious use of natural resources.

Some Suggetions

- The tribals should be provided with suitable technology complete in all respects to increase their efficiency in resource conservation and management practices.
- More studies to include all the remaining plants used by these tribals should be conducted to explore, identify and record their uses.
- It is imperative to create a databank of these plants and their uses for strengthening and evolving low cost, highly effective and ecofriendly healthcare system without any side/adverse effect.
- Pharmacological studies have to be conducted to understand the mechanism of action of the active compound of the plant and also to study the effects of ingredients of a mixture.

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ECOLOGICAL STUDIES ON BARKI, YELVAN-JUGAI AND AMBA GHAT REGION OF WESTERN GHAT OF MAHARASHTRA

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Abstract:

The Western Ghats comprises mostly undulating low hills mountain ranges covering 160,000 square kilometers an area with length 1600 kilometer north to south. It is parallel to the western coast of the Indian peninsular covers states of India including Kerala, Tamil Nadu, Karnataka, Goa, Maharashtra and Gujarat. It is recorded as world heritage site by UNESCO and hot spot of biodiversity in 2012 (Myers *et al*., 2000). In Maharashtra, the western ghat ranges are commonly known as Sahyadri and Sahya Parvatam in Kerala. The hill stations situated in this region including Matheran, Lonavala-Khandala, Mahabaleshwar, Panchgani, Amboli Ghat, Kudremukh and Kodagu. Barki, Yelvan-Juagi and Amba Ghat are ecological diverse places frequently visited by students for study and research. The study on ecological parameters were carried out and elaborately discussed with different aspects. Our study indicated that the ecosystem was well balanced with there all constraints including biotic and abiotic. The producer plays key role in balancing the terrestrial and aquatic ecosystem studied there in. The study of succession recorded complex phenomenon and attain climax stage. This study will helpful to understand the ecological status and ecosystem updation of Barki, Yelvan-Jugai and Amba ghat region of Western ghat of Maharashtra. The assessment of the status of species in these regions widely indicated the status of biodiversity. This information provides scope for monitoring biodiversity trend and helps in establishing priorities for species conservation.

Keywords: Barki, Yelvan-Jugai, Amba ghat, Western ghat, Maharashtra, Ecological Studies etc.

Introduction:

The Western Ghats are known for their high biodiversity and endemism.It is identified as one of the world's eight hot hotspots of biological diversity. The western ghat covered with evergreen forest, receives about 350 inches of rain fall every year. It showed 25% of India's biodiversity and new flora and fauna species are discovered every year here. Around 178 species of amphibians, 157 species of reptiles, 220 species of fishes and approximately 650 flora species are found in the Western Ghats.

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It is recorded as world heritage site by UNESCO and hot spot of biodiversity in 2012 (Myers *et al*., 2000). In Maharashtra, the western ghat ranges are commonly known as Sahyadri and SahyaParvatam in Kerala. The hill stations situated in this regions including Matheran, Lonavala-Khandala, Mahabaleshwar, Panchgani, Amboli Ghat, Kudremukh and Kodagu. Barki, Yelvan-Juagi and Amba Ghat are ecological diverse places frequently visited by students for study and research.

The study on ecological parameters were carried out and elaborately discussed with different aspects. Ecological studies dealing with interaction among organism and their surrounding environment. Barki, Yelvan-juagi and Amba ghat is one of the most important botanical place where species variation are greatly observed. Blooming of flower was changed in every 15 days.

Our study indicated that the ecosystem was well balanced with there all constraints including biotic and abiotic. The producer plays key role in balancing the terrestrial and aquatic ecosystem studied there in. The study of succession recorded complex phenomenon and attain climax stage. This study will helpful to understand the ecological status and ecosystem update of Barki, Yelvan-Jugai and Amba ghat region of Western ghat of Maharashtra. It's high time that we should realize the need to find effective way that draws a parallel between development and environmental protection.

Methods:

The botanical study tour was arranged for ecological and environment study of this region. The present report includes status of ecological constraints and wide discussion on their interaction for stability of ecosystem. The field observations were carried out and plants identification with the help of taxanomical tools. The water samples from aquatic

ecosystems were collected and studied in laboratory conditions. The measurements of various environmental parameters were carried out with ecological instruments. The species diversity including dominant, rare and endemic species of plants were recorded on the basis of common observations. The aquatic ecosystem and Hydrarch succession among them were also studied and discussed.

Field Observations and Discussion:

Species Diversity:

The Western Ghats is one of the biologically richest areas harboring more than 3500 species of flowering plants consisting of about 27% flowering plants of India (Pascal, 1963). The Western Ghats are the most important range for many plants, majorities are dominant and some are rare endemic. This region has a potentiality gene pool of many plant species (Ramakrishna *et al.,* 2001). The Western Ghats harbour a healthy population of plant and animal speciesof India with a fairly high degree of endemism. It is also a region that includes several unique ecosystems and harbours a large number of threatened and endemic species. The following plant species recorded as dominant, endemic, endangered and rare species from studied area.

Biodiversity in studied area:

a) Dominant vegetation	*Eriocaulon stillta, Eriocaulon tuburosa, Utricularia sps., Disophylla stelata, Cynotis axillaries*
b) Rare species	*Drocera indica, Waghatia spicata, Ipigenia indica, Cyanotis tuberosa*
c) Endemic species	*Habnaria longicalcarata, Striga sulphuria, Utricularia cerinifera, Ceropegia hirsute, Exacum paniculata*
d) Other Association	*Anelima sps, Rotala sps, Drocera indica, Habnaria longicalcarata, Striga lutea*

Aquatic Ecosystem:

The aquatic ecosystems of the Ghats include mainly lotic and lentic systems. This lentic ecosystem classified as ponds and lakes while the specialized waterfalls, cascades and streams constitute together aquatic lentic systems with rivers like Hiranykeshi, Kadavi and Dudganga with specialised aquatic plants, insect, amphibia and fish. The river tributaries received from temporary monsoon flowed to slowly in moving pools and lakes contiguous to the Ghats.

Water, an elixir of life and is provided by these ecosystems to other components. These ecosystems present in these regions include specialized plant and animal species that are adapted to live in specific region. The aquatic ecosystems are characterized by quality of water including salinity, rate of flow, clarity and oxygen content. The flora and fauna of region in streams and rivers depends on the clarity, the rate of flow and oxygen content. The changing the flow patterns of rivers led to serious problems like a loss of productivity in the aquatic ecosystem (Bharucha, 2008).

The studied pond ecosystem showed producers, consumers and decomposers with different abiotic constraints. We arranged field visit to in and around the Barki fall region for the study of ecosystem. We select small pond and small stream for same. We studied and observed detailed organism as producers, consumers and decomposers. Our observations were recorded as follows.

ABIOTIC COMPONENTS:

Date	:-	28 Sept., 2019.
Visited place	:-	Barki and Yelvan-jugai
Selected area	:-	Near Yelvan, Malakapur.
Altitude	:-	115 meter from sea level.
Direction	:-	West ward from kolhapur
Soil	:-	Red laterite.
Light	:-	Cloudy and misty in rainy season.
Water	:-	Heavy Rainfall around 450cm.
Soil temp	:-	10-12^{O}c.
Soil ph	:-	7 to 7.5
Atmospheric temp	:-	15-18^{O}c. approx.
Relative humidity	:-	65%

Biotic Components:

The biotic components include producers, consumers and decomposers.

Producer:

The producer mainly includes macrophytes and phytoplanktons. The microphytes are occurred on edges of ponds; the macrophytes are mosses like Pogostemon and number of ephemerals includes *Ceratophllum, Utricularia, Erioculon stellata, Disophyllaindia, Anelima, Cynotis axillaries, C. tuberose, Habenaria longiculcarata, Rotallasps., Ipigenia*

indica, grass sps. and crustose and foligae lichen. The phytoplanktons are algae like *Spirogyra, Anabaena* and *Diatoms* were also recorded in this aquatic ecosystem.

Consumers:

The primary consumers like zooplankton comprises *Ciliates, Flagellates, Soruallli*, and Crustaceans like Copepods warms were present. The secondary consumers are the carnivores, which feed on herbivores like insects, grasshopper, variety of spider, butterfly. Tertiary consumers are frog, toads, small fish, lizard. Several bacteria and fungi mostly form actinomycetes are decomposers in this area.

Succession:

The replacement of one plant species by other is generally termed as succession. It is very common and regeneration of plants any ecosystem takes places. The **Hydrarch** type of succession is discussed. The hydrach type of succession starts in pond and becomes progressively shallow towards pond. The various serial stages are found during study of aquatic plant succession. The stages are Plankton stage, Submerged stage, Floating stage, Reel Swamp flag, Marsh meadow stage, Woodland stage and Climax stage.

1) **Plankton stage:** These are the pioneers. It includes minute autotrophic organisms like flagellates, diatoms, unicellular and filamentous green algae like Spirogyra.
2) **Submerged stage:** The soft mud mixed with organic matter form pioneers that favor the growth or submerged plant like Utricularia, Rotala etc.
3) **Floating stage:** The area is invaded by the species of floating plant like Disopyla, Spirodela and Anelima.
4) **Reel Swamp flag:** It includes plant like Scripussps, Astracantha, and after their death they produce abundant organic matter, so new substation developed in the same place that changes in to a marshy soil where another vegetation start to grow.
5) **Marsh meadow stage:** This stage shows growth of *Cyperoustuberosus, Scripussps, Grass sps* and *Eriocaulonstillata* and such many other annuals.
6) **Woodland stage:-**It includes some perennial plants species like Dipacadi, *Ipigenia, Habanera* which after completion of life they live in the form of underground modified stem like corm, bulb and rhizome for next season. Some annuals and periniial herbs also stand well and perish them. It is followed by shurby plants like *Acanthus species*, *Carisacarndasa Ixora* species etc.
7) **Climax-stage forest:-**It includes many trees, which have shade in the form of varied shaped canopy. They grow in a medium to greater height . All provides new colonies

for insects, birds and other animals. It occupies and well furnished whole ecosystem in that area to tolerate by mesophytic plants like *Mangifera indica, Randiasps. Memycelo numbellatum, Terminalia chebula, T. bellerica, Anageiosus latifolia* etc.

The assessment of aquatic ecosystem and there succession clearly focused on aquatic biodiversity and stability of ecosystem presents therein. The aquatic ecosystem characterized by their abiotic features such as quality of water, rate of flow, clarity and oxygen content. The interaction between biotic and abiotic constraints indicated the stability of ecosystem through energy flux and food chain regulation. The aquatic ecosystem in YelwanJugai and Amba ghat is showing stable flow patterns within certain limit and indicated highest productivity in the aquatic ecosystem (Bharucha, 2008). The succession studied in this region showed climaxed stage. The studied are is frequently visited by people therefore over-exploitation, water pollution, flow modification, destruction of habitat takes place. The human activities might be superimposed ecosystems presents in studied area (Dudgeon *et al.,* 2006).

Conclusion:

The assessment of the status of species in these regions widely indicated the status of biodiversity. This informationprovides scope for monitoring biodiversity trendand helps in establishing priorities for species conservation.

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ECOLOGY AND DISTRIBUTION PATTERN OF LICHENS IN TROPICAL FORESTS OF KOPPA TALUK, KARNATAKA

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Abstract:

In the preset chapter we have focused on lichen ecology in the tropical forests of Koppa taluk, Western Ghats of Karnataka. A total of 36 species of lichens were identified belongs to 15 genera from Nine lichen families. The Shannon-Winner diversity value is to be 3.34 and Simpson's richness value is 0.031. Deciduous forests have shown rich diversity of lichens (27 spp.) than semi-evergreen forests. More epiphytic lichens were growing on the trunk of the trees (25 spp.). Twenty four different host trees were recorded from the study area. Trees < 25 cm GBH (girth at breast height) sustained the growth or more lichens (33). *Randia dumetorum*, which has moderate bark texture, supported for five species of lichens. The assemblage of distinct species at different sites indicates restrictive species distribution and signifies the need for protecting large areas for lichen conservation.

Keywords: lichen, biodiversity, photobiont, tropical forest

Introduction:

Lichens are complex organisms involving in a symbiotic relationship between a photobiont and a mycobiont (Pinokiyo *et al.,* 2006). Epiphytic lichen communities are diverse, their richness may be greater or equal to that of vascular plants (Pipp *et al.,* 2001). In a heterogeneous forest land, the diversity of lichen is variable, as the supporting host trees provides space for different types of lichens (Sequiera and Kumar, 2008). These epiphytic lichens commonly cover the bark of trees (corticolous) as well as leaf surfaces (follicolous). Thus in tropical forests lichens show characteristic distribution patterns related to their growth forms (Lakatos *et al.,* 2006).The knowledge of the degree of host specificity of lichens serves a useful purpose in the estimation of their diversity and

conservation (Sequiera and Kumar, 2008). Dead and decaying trees are the favorite habitat of many epiphytic lichens; lichen diversity is often higher on living trees, hence the trees are vitalas a habitat (Hauck, 2005). Macrolichen species in low land rainforests are restricted to the canopy (Cornelissen and Steege, 1989), whereas information on vertical microlichen distribution in tropical forests is virtually lacking.The epiphytic forest dwelling lichens have a long generation time as well as poor dispersal and colonization abilities (Juriado *et al.,* 2009). These are sensitive to climatic stress because of their location in the canopy and because they are dispersed by wind (Esseen and Renhorn, 1998). Hence these are actively used in monitoring of air quality (Saipunkaew *et al.,* 2005; Pande *et al.,* 2006). Epiphytic lichens may also be affected by altered forest microclimate (Esseen and Renhorn, 1998) and serve as indicators of forest health (Pipp *et al.,* 2001). The trees benefit from the input of the lichen. For example, Oaks colonized by lichens received an increased deposition of nitrogen, phosphorous, and water from local rainfall and fog dripping (Werth and Sork, 2008). Epiphytic lichen assemblage and their host specifications have been intensively studied throughout the world (Esseen and Renhorn 1998; Pipp *et al.,* 2001; Hauck 2005; Saipunkaew *et al.,* 2005; Werth and Sork 2008; Juriando *et al.,* 2009). However only scanty information's is available for Indian lichens (Upreti and Chatterjee 1999; Pande *et al.,* 2006; Sequiera and Kumar, 2008). Hence the main objective of this paper is to elucidate the host specificity of epiphytic lichens and to evaluate the important host species exploited by them.

Materials and Methods:

Study area:

Koppa taluk, a part of Western Ghats that comprise an eclectic collection of lichens, comes under Malnad region (hilly area with dense forest) of Chickmaglur district, Karnataka, located at 75º15' to 72º20' E and 13º30' to13º35' N with 700 m to 844 m MSL altitude. The study area enjoys generally cool climate throughout the year and remains pleasant during the summer season. The temperature of this region varies between 18º C and 31º C, where as the annual rainfall is nearly 1,600 to 3,400mm. For the present study, different types of forests such as semi-evergreen and deciduous forests were selected at random.

Surveying and sampling:

Frequent field visits were undertaken from November 2007 to June 2008. A random sampling method was used to document the lichen diversity by laying two belt transects (10 m x 50m) in each type of selected forest. In each transect all substrates were thoroughly

searched for the occurrence of lichens. Only representative lichen specimens were collected and packed in brown paper bags, brought in polythene bags to the laboratory. The altitude was recorded with a hand-held GPS (Garmin e-trex, USA). RH (digital thermo-hygrometer, 288CTH Euro lab), temperature and microhabitat data were recorded in each transect.

Identification and preservation:

Collected lichen specimens were dried for 1-2 week to remove all moisture content from the sample and identified on the basis of their morphology, type of fruiting bodies, anatomy and chemistry following recent literature (Walker and James, 1980; Awasthi, 2000). All lichen specimens were preserved in the herbarium of the Department of Applied Botany, Kuvempu University, Shankaraghatta, Shimoga, Karnataka and voucher specimens were submitted to the herbarium of National Botanical Research Institute, Lucknow (LWG), India. Shannon and Simpson's Diversity values, SIV and other diversity indices were calculated by using respective formulas (Magurran, 1988; Cottam and Curtis, 1956).

Results and Discussion:

Diversity and distribution pattern:

A total of 36 lichen species belonging to 15 genera and 9 families are found in the tropical semi-evergreen and deciduous forests of Koppa taluk (**Table1**). As many as 67 colonies are sampled over 3000 m^2 area. Physciaceae is the largest family in the study area with 11 species, where as both Chrysotrixaceae and Lecanoraceae represented by single species (**Fig. 1**). The foliose lichens were dominant in the study area (59%) followed by crustose (33%) and fruticose (8%). These numbers are subjected to change depending on the vegetation type (**Fig. 3**). *Heterodermia incana* is the most frequent species, also has highest SIV value (16.67) followed by *Graphina* sp. with SIV of 12.5 (**Table 1**). Highest density is shown by *Graphina* sp. (2.33), *H. incana* and *Leptogium* sp. are comes later with the density of 2.0 each. *Graphina* sp. is also the most abundant (7) species of lichen. The recorded lichen species in different vegetations in the study area showed Shannon – Winner diversity value (H') of 3.34 and Simpson's richness value (D) is found to be 0.031.

There are considerable differences in species composition and abundance between the two forest types. The semi-evergreen forests harbored 27 species while 7 species were exclusive to the deciduous forests and2 species of lichens are common in both forest types (**Fig. 2**). However 42.86% in semi-evergreen and 29.63% in deciduous forest are

microlichens and remaining are macrolichens. Fruticose lichen is not found in semi-evergreen forests.

Lichen-host specificity:

Among the three habitats viz. main trunk, branches and fallen twigs, as many as 25 species are found on main trunk, whereas 4 and 9 species are found on branches and fallen twigs respectively (**Fig. 4**). A total of 24 different host trees are recorded from the study area. The GBH of the trees documented in the transect area ranges between 4 to 150 cm. They are divided into 5 girth classes. Below 25 cm girth plants wires the growth of added number of lichens (33), compare to highest girth class (**Fig. 5**). Main trunk supports maximum number of lichens (**Table 1**).Trees with rough bark such as *Schleichera oleosa, Myristica doctyloides*, and *Terminalia tomentosa* supported more number of fruticose and foliose lichens. Whereas smooth bark plants supported more number of crustose lichens. *Randia dumetorum,* which has moderate bark texture supported with five different lichen species. Here bark moisture and externally derived moisture may be influence the growth of epiphytic lichens.

A forest is a habitat with complex ecological gradients is an important habitat for a rich assemble of lichens (Sequiera and Kumar, 2008). Number of species or any other ranks of taxonomic organization in a site (species richness) and their compositional change across different habitat types (species turnover) within a landscape are important parameters of biodiversity that have wide applications such as environmental monitoring and conservation evaluation (Negi 2000). Results revealed that the epiphytic lichen assemblages do vary depending upon the types of habitats under various external pressures (such as the disturbance by humans and livestock grazing) in terms of both of these community level biodiversity attributes. Species richness and composition of lichens on tropical semi-evergreen and deciduous forest varies. Lichen abundance also varied with in tree crowns because of exposure (Esseen and Renhorn, 1998). Competition for light is an important factor than the moisture for growth of lichen (Upreti and Chatterjee, 1999). These facts are emphasized by the result deciduous forest comprises more number of lichens than the semi-evergreen forests. The open canopy forest and widely branched deciduous trees are more favorable for epiphytic lichen growth. Although the evergreen and deciduous forests are found in a complex mosaic often at the same altitude, the lichen floras (corticolous) of the forests are different (Wolseley and Aguirre-Hudson, 1997).

More number of lichens is found on tree bark than any other substratum, reflecting the importance of woody component of the forest as a major lichen habitat (Pinokiyo *et al.,*

2008). Bark texture and chemistry are probable factors determining host preference. Accurate measurements of bark texture and experiments of toxicity of bark to epiphytic species are needed to clarify the causes of host preference (Cornelissen and Steege, 1989). Young trees support the lichen communities dominated by crustose forms, followed by a few foliose and fruticose forms (Upreti and Chatterjee, 1999), mature-trees (young tree have less GBH, where as mature trees has high GBH) sustain climax communities dominated by foliose and fruticose lichens rather than the crustose (**Table 1**).

The effect of trunk size on lichen species richness on the tree bole has proved to be negative in boreo-nemoral forests (Juriado *et al.,* 2009). Pande (2006), density data indicated that with the increase in diameter of the tree, the number of thalli decreased (*Parmelia cristifera* present on the tree having GBH over 100 cm is an exception).The present study result in evidence for there is a relationship between size of the bole and the lichens (by providing information that number of lichens decreases with the rise in GBH. Our results agree with the study of in the Pinokiyo *et al.* (2008) in which epiphytic lichen abundance and diversity was linked to their structural diversity. Because epiphytic lichen communities may be slow to establish (Pipp*et al.,* 2001), they could not grow in disturbed sites hence the profusion of the lichens in an area indicates that the place is serene. Consisting with the tested hypothesis epiphytic lichens have large potential as indicators of edge effects because of the frequent occurrence of more sensitive pendulous lichens like *Usnea, Ramalina* in forest edge microclimate.

Conclusions:

Epiphytic lichens may be strongly affected by the specific environmental conditions. Distinct species assembles at different sites show restrictive species distribution, and it signifies a need for protection of large areas for lichen conservation. Changes in environmental conditions are rapidly, reflected in the lichen flora, and quantitative sampling of individuals (Wolseley and Aguirre-Hudson 1997). Hence it is necessary to protect habitat before thinking of conserving and improving lichen diversity. Lichen abundance also varies depending on tree crowns because of exposure. Hence open canopy forest showed higher diversity than that of closed canopy forest.

Table 1: Frequency, Density, Abundance and species importance value of lichen species with their growth form and host plant in the different vegetation types

Species Name	G	Fre	Den	A	SIV	Host species	VT
Bulbothrixi sidiza (Nyl.) Hale (Parmeliaceae)	F	0.33	0.33	1	4.17	*Syzygium cumini*	SE
Chrysothrix sp. (Chrysothrixacae)	C	0.33	0.33	1	4.17	*Sapium insigne,Croton caudatus*	DD
Dirinariacon fluens (Fr.) D.D.Awasthi (Physciaceae)	F	0.33	0.33	1	4.17	*Syzygiumcumini*	DD
D. applanata (Fee) D.D.Awasthi (Physciaceae)	F	0.33	0.33	1	4.17	*Hopeaponga*	SE
Graphina celata Stirton (Graphidaceae)	C	0.33	0.33	1	4.17	*Gnetumula*	DD
Graphina sp. (Graphidaceae)	C	0.33	2.33	7	12.5	*Terminalia tomentosa, Erithrina superba, Calyptoterics florigunda, Randia dumetorum*	DD/ SE
Graphis aphanes Mont andv.d.Bosch (Graphidaceae)	C	0.33	1.00	3	6.94	*Ficus tsjahela, Symplocos racemosa, Terminalia tomentosa*	DD
*G. celatastirton (*Graphidaceae)	C	0.33	0.33	1	4.17	*Randia dumetorum*	DD
*G. longiramea (*Graphidaceae)	C	0.33	0.33	1	4.17	*Gnetum ula*	*DD*
Heterodermia diademata (Taylor) D.D. Awasthi (Physciaceae)	F	0.67	0.67	1	8.33	*Calyptoterics florigunda, Terminalia paniculata*	DD
H. dissecta (Kurok.) D.D. Awasthi (Physciaceae)	F	0.67	0.67	1	8.33	*Calycopteris floribunda*, *Terminalia paniculata*	DD
H. incana (Stirton) D.D.Awasthi *(*Physciaceae)	F	1.00	2.00	2	16.67	*Croton caudatus, Ligustrum gamblei*, *Randia dumetorum*	DD / SE
H. obscurata (Nyl.)Trevis. (Physciaceae)	F	0.33	0.67	2	5.56	*Hopeaponga,Sapium insigne*	DD
*H. pseudospeciosa (*Kurok.) W. Culb. (Physciaceae)	F	0.33	0.33	1	4.17	*Randia dumetorum*	DD
*H. speciosa (*Wulf.) Trevis. (Physciaceae)	F	0.67	1.33	2	11.11	*Sapium insigne*, *Terminalia paniculata*	DD
*H. albidiflava (*Kurok.) D.D.Awasthi (Physciaceae)	F	0.33	0.33	1	4.17	*Croton caudatus*	DD

Lecanora indica Zahibr. (Lecanoraceae)	F	0.33	0.33	1	4.17	*Randia dumetorum*	DD
Leptogium burnetiae Dodge (Collemataceae)	F	0.33	1.00	3	6.94	*Clausena dentata*, *Mappia foetida*, *Randia dumetorum*	SE
L. chloromelum(Sw.) Nyl. (Collemataceae)	F	0.33	0.67	2	5.56	*Syzygium cumini*	DD
*Leptogium*sp. (Collemataceae)	C	0.67	2.00	3	13.89	*Clausena dentata, Paramignya monophylla*	SE
Ocellularia diacida Hale (Thelotremataceae)	C	0.33	0.33	1	4.17	*Schleichera oleosa*	DD
Parmotrema cristiferum (Taylor) Hale (Parmeliaceae)	F	0.33	0.33	1	4.17	*Symplocos racemosa*	SE
P. hababianum (Gyeln.)Hale(Parmeliaceae)	F	0.33	0.33	1	4.17	*Sapium insigne*	DD
P. reticulatum (Taylor) Choisy (Parmeliaceae)	F	0.33	0.33	1	4.17	*Paramignya monophylla*	DD
P. stuppeum (Taylor) Hale (Parmeliaceae)	F	0.33	0.33	1	4.17	*Clausena dentata*	DD
P. tinctorum (Despr.exNyl.) Hale (Parmeliaceae)	F	0.33	0.67	2	5.56	*Paramignya monophylla, Terminalia paniculata*	DD
Parmotrema sp. (Parmeliaceae)	F	0.67	0.67	1	8.33	*Eliocarpus serratus*	DD
*Porinaamericana*Fee (Trichotheliaceae)	C	0.33	1.33	4	8.33	*Hopea ponga, Randia dumetorum*	SE / DD
*P. innata (*Nyl.) Mull. Arg.(Trichotheliaceae)	C	0.33	0.33	1	4.17	*Hopea ponga*	SE
Pyxine coccifera (Fee) Nyl. (Physciaceae)	F	0.33	0.33	1	4.17	*Terminalia paniculata*	DD
*P. minuta*Vain. (Physciaceae)	F	0.33	0.33	1	4.17	*Mappia foetida*	DD
*Ramalina divericata (*Ramalinaceae)	Fr	0.33	0.33	1	4.17	*Ziziphus xylopyrus*	DD
*Thelotrema canarense*Patw. andKulk.(Thelotremataceae)	C	0.33	0.67	2	5.56	*Hopea ponga*, *Aporosa lindliana*	SE
Thelotrema sp.(Thelotremataceae)	C	0.33	0.33	1	4.17	*Paramignya monophylla*	DD
Usnea ghattensis G. Awasthi (Parmeliaceae)	Fr	0.33	1.00	3	6.94	*Dimocarpus longan, Gordonia obtuse, Myristica doctyloides*	DD

G: Growth form, F: Foliose, Fr:Fruticose, C:Crustose,Fre: Frequency, Den: Density, Ab: Abundance, SIV: Species Importance Value,

VT: Vegetation type, SE: Semi-Evergreen, DD: Deciduous, H: Habit, M: Main trunk, F: Fallen twig, B: Branch

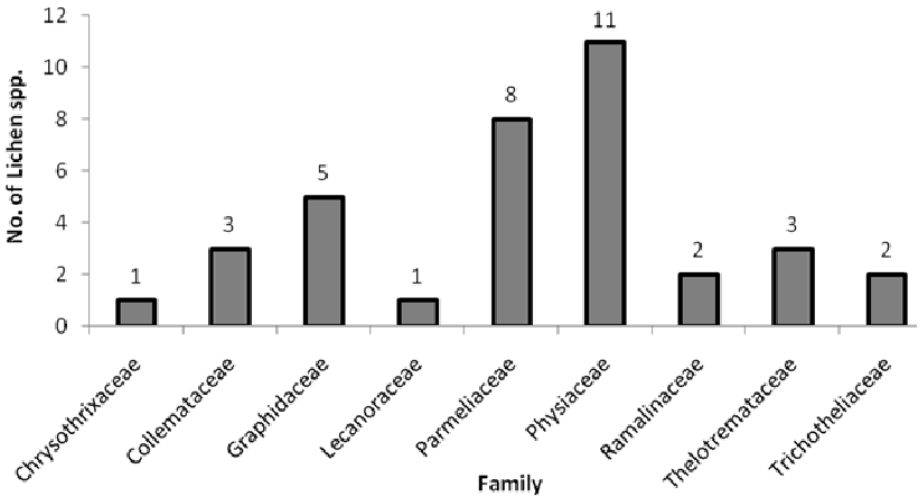

Figure 1: Different family composition in the study area

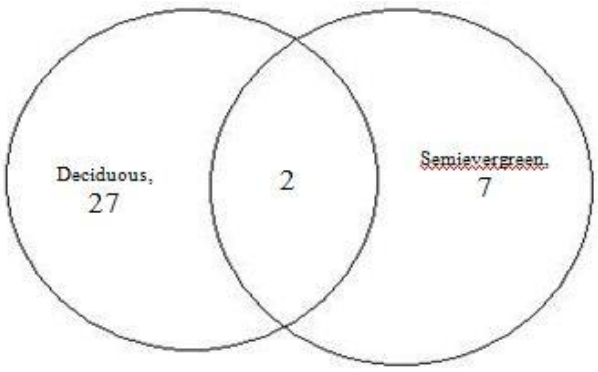

Figure 2: Species composition of lichens in different forests

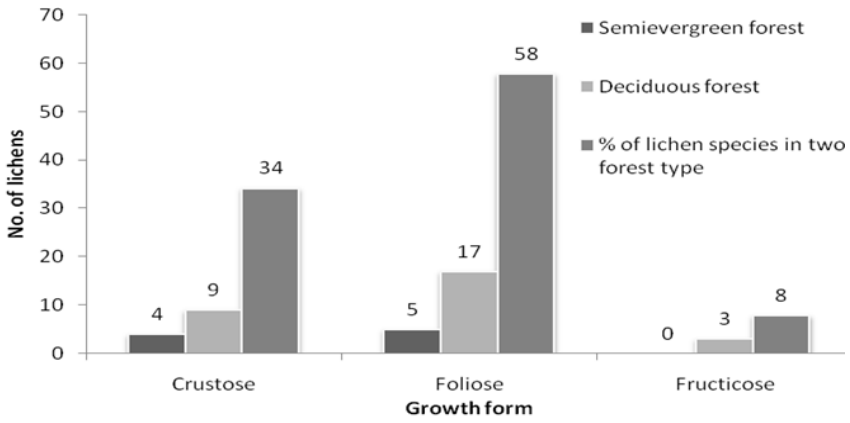

Figure 3: Number of lichens in different forest types and their growth form

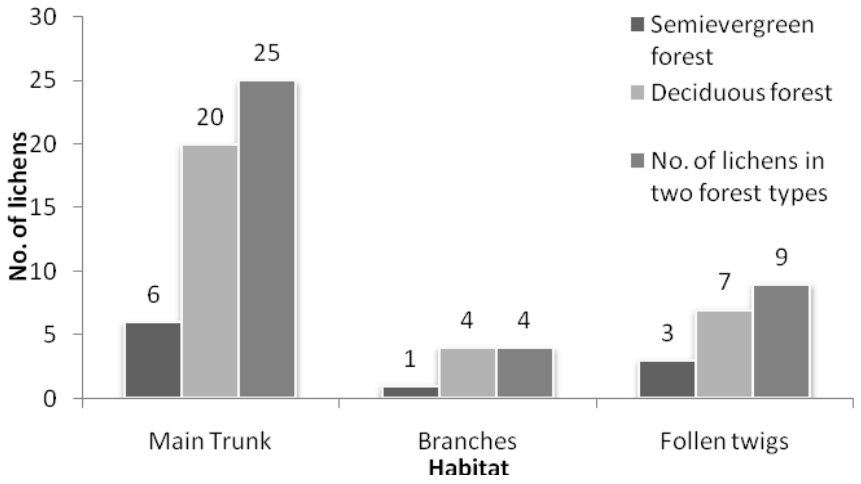

Figure 4: Relationship between substratum and No. of lichens

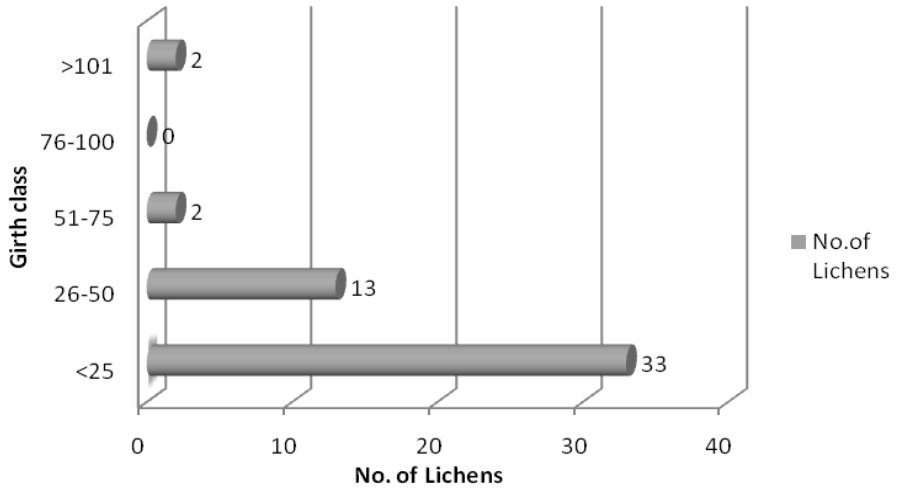

Figure 5: Diversity of lichens according to the GBH of host trees

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ROLE OF PROBIOTICS AND PREBIOTICS IN HUMAN HEALTH

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Abstract:

Nutrition for good health is the current focus of the consumers all around the globe. A varietyof food and food products are being tried and a number of scientific studies are going on for identifying the food for their specific health benefits. Functional foods are such specialized category of which supplies nutrients and along with it components that contribute to the positive health benefits and cure of illnesses. Probiotics and Prebiotics are increasingly being used in functional foods and dietary supplements and there is a strong relationship with human gut and health. Microorganisms are the natural inhabitants of human gastrointestinal tract also known as gut microbiota. The fermentation of Nondigestible substrates like dietary fibres and endogenous mucus is carried out by gut microbiota. Growth of specialist microbes is also supported by fermentation resulting in the production of short chain fatty acids (SCFAs) and gases. The major SCFAs are Butyrate, Propionate and Acetate.The health benefits of probiotics date back to centuries when fermented milk was drank for health purposes. Consumption of specific strains of probiotics is associated with a range ofhealth benefits. A wide range of food products are crowding the market with the existing dairy-based products such as buttermilk, milk powder, ice-cream, cheese, fermented milks and yogurts. Prebiotics are special plant fibres that help healthy bacteria grow in our gut. This makes the digestive system work better. Thus, Probiotics and Prebiotics are found to have a remarkable influence on human health.

Keywords: Probiotics, Prebiotics, microbiota

Introduction:

Thousands of years ago a Greek Philosopher and father of medicine Hippocrates first conceived the notion that food could serve as medicine and once wrote “Let food be thy medicine, and let medicine be thy food. “Now in recent times the concept of food having

medicinal value has upsurged as functional food which refers to "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains."

In this article we have made an attempt to provide a brief overview of Probiotics and Prebiotics which are increasingly being used in functional foods and dietary supplements and their relationship with human gut and health.The human gut which is also known as the second brain plays an important role in human health maintenance and contains a wide array of microorganisms which regulate disease condition and maintenance. Each individual is bestowed with a unique gut microflora which changes depending upon the disease condition. Microorganisms are the natural inhabitants of human gastrointestinal tract also known as gut microbiota; it has been reported that there are 1010–1012 live microorganisms per gram in the human colon. Microbiota refers to the entire population of microorganisms that colonizes a particular location; and includes not just bacteria, but also other microbes such as fungi, archaea, viruses, and protozoans (Lederberg and McCray,2001). These natural inhabitants in our stomach, large and small intestine plays an important role in human health. The majority of these microorganisms, which are mostly anaerobes, live in the large intestine (Sekirov *et al.,* 2010). Joshua Lederberg introduced the concept of the human microbiome first to the scientific community, who defined it as 'the ecologicalcommunity of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease' (Lederberg and McCray, 2001).

Among diverse factors such as, exposure to antibiotics, lifestyle, genetics etc., diet is the most important factor in shaping the gut microbiota composition and functions (Collins and Reid, 2016). The human microbiome, defined as the collection of microorganisms that reside within our body, has coevolved over the history of mankind, and has been overlooked as determinants of health and disease. Gut microbes are key to many aspects of human health including immune, (Louis *et al.,* 2016) metabolic (Zhang *et al.,* 2015) and neuro behavioral traits. Different levels of evidence support the role of gut microbiota in human health, from animal modelsand human studies (Goodrich,*et al.,* 2014; Sonnenburg and Sonnenburg, 2014; Beaumont*et al.,* 2016; De Palma G,*et al.,* 2017; Wiley *et al.,* 2017; Rothschild *et al.,* 2018).

What does the gut microbiota do?

The fermentation of Nondigestible substrates like dietary fibres and endogenous mucus is carried out by gut microbiota. Growth of specialist microbes is also supported by fermentation resulting in the production of short chain fatty acids (SCFAs) and gases

(Falony *et al.,* 2016).The major SCFAs are Butyrate, Propionate and Acetate are produced. The major contribution is that of butyrate which provides energy for human colonocytes, apoptosis of colon cancer cells is also induced by it. Butyrate also activates the intestinal gluconeogenesis having beneficial effects on glucose and energy homeostasis (Wong *et al.,* 2006). Butyrate is essential for epithelial cells to consume large amounts of oxygen through ß oxidation, generating a state of hypoxia that maintains oxygen balance in the gut, preventing gut microbiota dysbiosis (De Vadder *et al.,* 2014). Propionate is transferred to the liver, where it regulates gluconeogenesis and satiety signalling through interaction with the gut fatty acid receptors (Wong *et al.,* 2006). Acetate—the most abundant SCFA and an essential metabolitefor the growth of other bacteria—reaches the peripheral tissues where it is used in cholesterol metabolism and lipogenesis, and may play a role in central appetite regulation (Byndloss *et al. ,* 2017).

Brief history of probiotics:

The health benefits of probiotics date back to centuries when fermented milk was drank for health purposes. Discovery of Bifidobacterium dated back to 1899 by Henry Tessler from Paster Institute in Paris from the intestine of breast-fed infants, who were reported to have fewer diarrheal episodes. In 1907 Eli Metchnikoff, a Russian scientist first proposed the idea of using probiotics for health benefits. A strain of *Escherichia coli* was isolated in 1917 and was used to treat patients affected by shigellosis outbreak. Since then, several others documented uses of probiotics are available in the literature, but well-designed clinical studies and data are lacking (Frost *et al.,* 2014). The word "probiotic" comes from Greek, and it means "for life". Most probably, it was Ferdinand Vergin who invented the term "probiotic" in 1954 (Islam, 2016). Sometime after that, in 1965, Lilly and Stillwell described probiotics as microorganisms stimulating the growth of other microorganism (Virgin, 1954). The definition of probiotics has been modified and changed many times. To emphasise their microbial origin, Fuller (1989) stated that probiotics must be viable microorganisms and must exert a beneficial effect on their host (Lilly and Stillwell, 1965). On the other hand, Guarner and Schaafsma (1998) indicated the necessary use of an appropriate dose of probiotic organisms required to achieve the expected effect (Fuller, R.1989).The current definition, formulated in 2002 by FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) working group experts, states that probiotics are "live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Guarner*et al.,* 1998). The definition was maintained by the

International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2013 (Food and Agriculture Organization (FAO), 2002).

Traditionally, there are many different species of probiotics widely used. The Saccharomyces cerevisiae (boulardii) is the most widely used yeast strain. Other bacterial probiotics mainly comprise of Lactobacillus species and Bifidobacterium species. These include *L. rhamnosus, L. plantarum, L. sporogens, L. reuteri, L. casei, L. bulgaricus, L. delbrueckii, L. salivarius, L. johnsonii, and L. acidophilus*, etc. On top of these, *B. bifidum, B. bifidus, B. lactis, B. longum, B. breve (Yakult), and B. infantis* are also commonly used. Other probiotics commercially available include Streptococcus thermophilus, Streptococcus acidophilus, Lactococcus lactis, Enterococcus SF68, and Escherichia coli Nissle 1917 (serotype O6:K5:H1) (Hill *et al.,* 2014).

Health benefits of probiotics:

In 1974 Mann and Spoerig discovered that lowering of blood cholesterol occurs in people who drank yogurt fermented with wild strains of Lactobacillus sp. this open new dimension to probiotic study. In one study cells of Lactobacillus acidophilus when added to infant formula have shown to decrease levels of serum cholesterol, the same results were also found in adult human experiments (Harrison *et al.,* 1975; Gilliland *et al*., 1985; Gilliland and Walker, 1989; Buck and Gilliland, 1994; Fijan, 2014). In 1994, the World Health Organization deemed probiotics to be the next-most important immune defence system when commonly prescribed antibiotics are rendered useless by antibiotic resistance (Gill and Guarner, 2004). Thus the term microbial interference therapy was given to the use of probiotics in antibiotic resistance.

Consumption of specific strains of probiotics is associated with a range of health benefits (Kailasapathy and Chin, 2000), although strong scientific evidence exists only for a small number of conditions. The health benefits supported by adequate clinical data or promising animal data include prevention and treatment of diarrhoeal disease (acute infantile diarrhoea, antibiotic associated diarrhoea, and nosocomial infections), prevention of systemic infections, management of inflammatory bowel disease, immunomodulation, prevention and treatment of allergies, anticancer effects, treatment of cholesterolaemia, and alleviation of lactose intolerance. The health benefits are strain specific. Studies have been done and the facts are established but still further studies are required to establish the claims. At present, several well-characterized strains of Lactobacilli and Bifidobacterium are available for human use to reduce the risk of gastrointestinal (GI) infections or treat such infections (Teitelbaum and Walker, 2002), other species which are

currently being used include filamentous fungi (e.g., *Aspergillus oryzae*), yeasts (e.g., *Saccharomyces cerevisiae and Saccharomyces boulardii*) and Bacillus sp.

Figure 1: Health benefits of probiotics

Probiotics and food products:

A wide range of food products are crowding the market with the existing dairy based products such as buttermilk, milk powder, ice-cream, cheese, fermented milks and yogurts (Stanton, 2001; Salminen *et al.,* 2005). The other probiotics based products include soy based products, nutrition bars, Cereals, and a variety of juices (Ewe *et al.,* 2010). A thorough evaluation criteria need to be followed before introducing the probiotic strain into the product, its viability, compatibility and safety during processing, Packaging and storage condition need to be considered. for example, a product like Cheese seems to have a number of advantages as compared to yoghurt because here pH plays a very important role in survival and growth of the probiotic and as delivery system for viable introduction in the gastrointestinal tract (Medina and Jordano, 1994; Sheehan, 2007). The issues of probiotic stability and viability issues offering new options for their incorporation in new media and subsequent satisfaction of the increasing consumer demand can now be solved with the current advancement in technological innovations using microencapsulation

technologies which also protects the bacteria from damage caused by external environment. Manufacturers can now provide the consumers with a dry form of probiotic bacterium with the introduction of a straw delivery system. Viable spores of a spore forming probiotic are also nowadays available which offers advantages during processing.

Prebiotics:

Prebiotics are special plant fibres that help healthy bacteria grow in our gut. This makes the digestive system work better. The gut microbiota affects intestinal functions, such as metabolism and integrity of the intestine. A prebiotic is defined as a "nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid, 1995) thus it promotes the growth of lactic acid bacteria in the colon and exert a inhibitory effect on *Salmonella* sp. or *Escherichia coli* thus limiting their proliferation. According to Gibson, the prebiotic should fulfil the following criterion it should be resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; fermentation by intestinal microflora and selective stimulation of the growth, and/or activity of intestinal bacteria associated with health and wellbeing (Stowell, 2007).

The most common prebiotics include Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and trans-galacto-oligosaccharides (TOS). Gut microflora ferments the prebiotics and produces lactic acid, butyric acid, short chain fatty acids and propionic acid, these products have multiple effects on human body thereby improving the quality of human life against a variety of diseases like vascular diseases, Cancers, Obesity and mental disorders.

Conclusion:

Probiotics and Prebiotics have a remarkable influence on human health, scientists all over the world are trying to determine the fundamental mechanisms of the functional foods so that these supplements can enhance the quality of human health. The focus to control and heal some of the disorders using these dietary substances is gaining momentum so that the gut microbiota can be fed properly with these supplements to make it healthier, which in turn can impact the overall health.

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Dr. Sonal D. Kamble was awarded doctorate for her thesis entitled, "Sedimentary diatoms and other multi-proxy paleolimnological studies of the Paradgaon Lake of Nagpur District and the Ghodajhari Lake of the Chandrapur District, Maharashtra for trophic status and paleoclimatic interpretations" from Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur in 2017. She is the recipient of Rajiv Gandhi National Fellowship, India. She has nearly eight years of experience in research and three years of teaching experience to PG student. She presented her research findings on various national and International platforms. She has three publications to her credit and three more under revision in International Journal.

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