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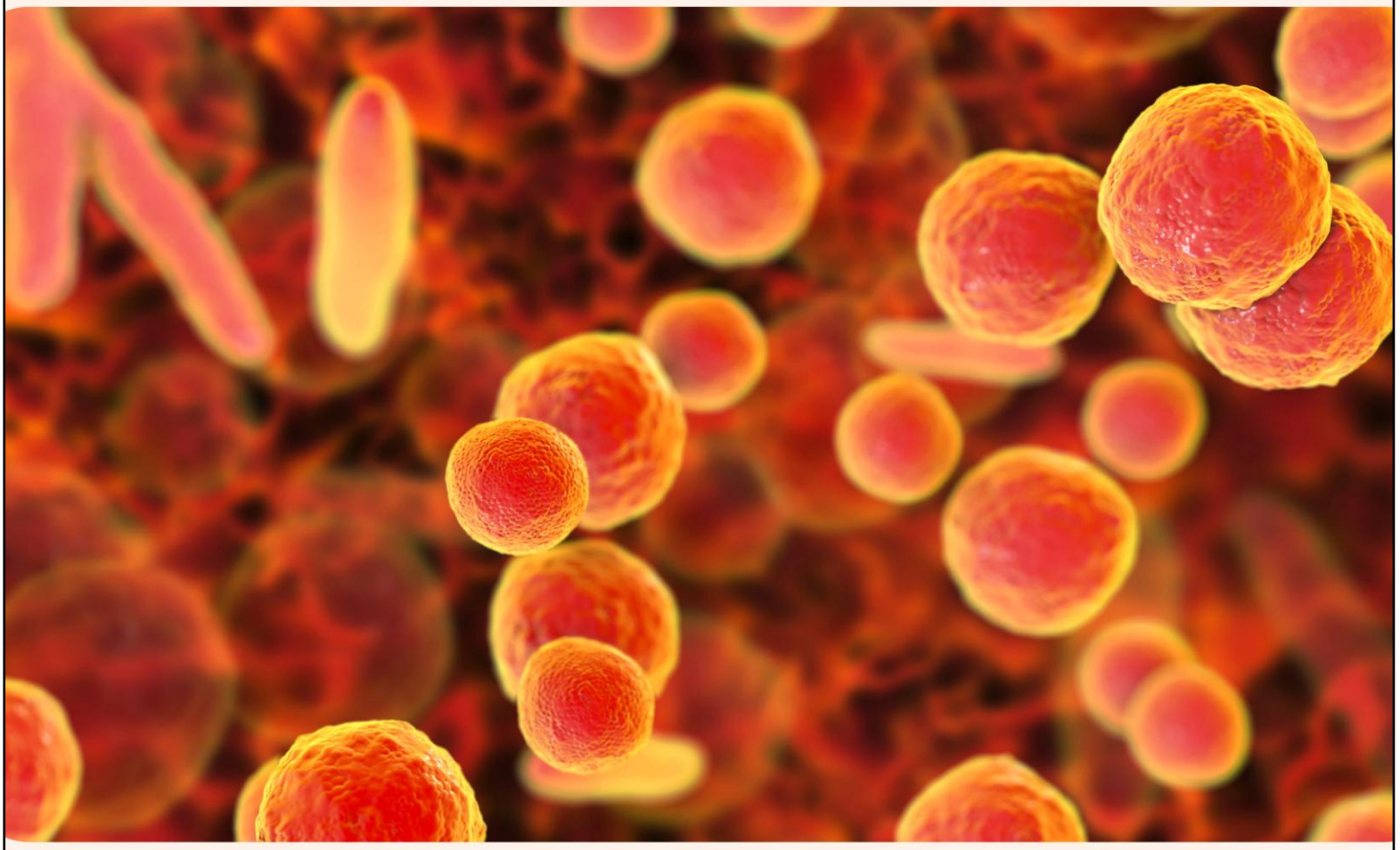
Advances in Microbiology

Volume III



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PREFACE

We are delighted to publish our book entitled ***"Advances in Microbiology Volume III"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of microbiology and life science 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 microbiology and life science. 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.

- Editors

Advances in Microbiology Volume III

CONTENTS

IN VITRO ANTIFUNGAL ACTIVITY OF *PARTHENIUM HISTEROPHORUS* AGAINST *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* CAUSING PANAMA WILT OF BANANA

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Abstract:

Fusarium oxysporum f.sp. *cubense* (Panama Wilt of Banana) is an important disease that causes wilt disease in Banana crop in India and all over world. Management through chemical fungicides causes resistance in pathogen and causes damage to non targeted organisms. Congress grass, *Parthenium hysterophorus* L., of the family Asteraceae (tribe: Heliantheae), is notorious weed distributed all over. Therefore in present investigation in vitro biological control of *Fusarium oxysporum* f. sp. *cubense* was conducted by using plant extract from stem of *Parthenium histerophorus*. Potential of *Parthenium histerophorus* was tested against the *Fusarium oxysporum* f. sp*cubense*by food poisoning technique.

Keywords: *Fusarium oxysporum* f.sp. *cubense*, Panama wilt, *Parthenium histerophorus*

Introduction:

Biological control of plant pathogens is preferred over the hazardous chemical based products. The plants serve as food, medicines, raw product for industry and antifungal sources. The plant extracts serve as ecofriendly and chief antifungal source. Banana (Musa spp.) is an important source of human nutrition, providing food and income to millions of people in the world. Banana is cultivated in Asia, Australia, Africa, North and South America (Rangaswami, 2002). The banana is attacked by many fungal pathogens. Among them Fusarium wilt of banana is most destructive disease (Stover, 1962; Sebasigari, 1988). Panama wilt is caused by *Fusarium oxysporum* f.sp. *cubense* (Smith, 1910; Snyder and Hansen, 1940). In the present investigation solvent and aqueous stem extract of *Parthenium histerophorus* were tested for their antifungal activity against *Fusarium oxysporium* f. sp. *cubense*.

Material and Methods:

Crude stem extract preparation for this study, 20.0 g dry powdered material was extracted with 200 ml of powder each plant was extracted in 95% alcohol by (1:5 w/v) and condensed to serve as stock extract. The toxicity of stock extract was determined against *Fusarium oxysporum* f. sp. cubense sensitive and resistant isolates to benomyl by food poisoning technique (Mishra and Tiwari, 1992) at four different concentrations. Plates containing CDA supplemented with plant extracts at four concentrations with three replicates were inoculated with a 6 day old culture of *Fusarium oxysporum* f. sp. *cubense.* 8 mm agar disc of fungal culture was prepared with the help of sterile cork borer and kept upside down on agar plates, incubated at 28 ± 2°C. Plates without plant extract served as control. Radial growth of *Fusarium oxysporum* f. sp. *cubense* was measured at different intervals. This procedure was repeated for the water extract of same plants instead of alcoholic extracts. The proportion of water and alcohol was 95:5 respectively.

Table 1: Effect of *Parthenium histerophorus* stem extract on radial growth (mm) benomyl sensitive isolate of *Fusarium oxysporium* f. sp. *cubense*

Concentration %	Plant extract	Days			
		2	4	6	8
10	Alcoholic	09.66	12.66	15.33	19.33
	Aqueous	24.33	55.00	61.66	70.33
25	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	19.66	47.33	54.00	64.33
50	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	17.66	38.66	45.66	53.33
75	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	14.66	31.66	37.66	44.66
100	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	9.66	21.00	32.33	37.33
	Control	21.00	42.33	62.00	80.00

Significance by two way analysis of variance (ANOVA)

	Alcoholic	Aqueous
Between days F	1.40	56.90
Between concentrations F	16.39	15.43
C.D. at P=0.05	12.50	6.90
C.D. at P=0.01	17.31	9.55

Table 2: Effect of *Parthenium histerophorus* stem extract on radial growth (mm) benomyl resistant isolate of *Fusarium oxysporium* f. sp. *cubense*

Concentration %	Plant extract	Days			
		2	4	6	8
10	Alcoholic	09.33	10.66	11.66	13.66
	Aqueous	20.33	41.33	55.33	64.00
25	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	17.00	39.33	51.33	58.33
50	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	12.66	31.33	42.33	52.33
75	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	11.33	21.66	29.00	38.00
100	Alcoholic	00.00	00.00	00.00	00.00
	Aqueous	00.00	16.66	26.33	32.66
	Control	25.00	45.33	64.33	80.00

Significance by two way analysis of variance (ANOVA)

	Alcoholic	Aqueous
Between days F	1.18	86.09
Between concentrations F	20.02	32.67
C.D. at P=0.05	11.17	5.55
C.D. at P=0.01	16.31	7.69

Results and Discussion:

Alcoholic and aqueous stem extract of *Parthenium histerophorus* was evaluated against benomyl sensitive and resistant isolates of *Fusarium oxysporium* f. sp. *cubense* in vitro. Alcoholic stem extract had completely inhibited the mycelial growth of both sensitive and resistant isolates of *Fusarium oxysporium* f. sp. *cubense* at 25 % concentration. However, the tested aqueous stem extract was significantly less effective.

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PRECISION FARMING COMPONENTS AND IMPORTANCE OF PRECISION FARMING: A REVIEW

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Abstract:

Farming is a practice to grow the crop, forest, fishery, livestock, or any enterprise for sustaining livelihoods and generating income, and maintaining health. Precision farming as a concept refers to a crop management strategy that employs, site-specific information to well manage production inputs and outputs. Precision farming /agriculture is known as site specific crop management or satellite farming. It helps achieve cost minimization, yield optimization and benefit maximization through GPS, GIS, Decision support system, remote sensing data acquisition, variable rate technology (VRT), sensor-controlled atomization and many others. For performing any management practice, The components work in concord. While GIS can create a geo-referenced wholesome map of a field, Remote sensors and GPS can provide location-specific parameter values; and VRT are provide chemical application in a controlled manner.

The terms precision farming and precision Agriculture are used simultaneously both the term broadly point to information communication technology-based agriculture management system to characterize, integrate, manage and monitor, spatial and temporal variability for soil, crop with in field for profitable sustainability and Environmental protection inter to critically observing and responding in the field variability.

Keywords: Precision farming, GPS, GIS, VRT, Sustainability, Environmental

Introduction:

Precision farming is a relatively modern, new, and mostly technology-driven approach. It is a philosophy or management approach to the cultivation of crops by considering spatially and temporal variation in Rainfall, soils and adopting management techniques to reduce efficient use

of inputs, cost and reduce environmental pollution. Precision farming is a farming management system based on the use of modern technologies at every stage of work. Usually, a field has heterogeneous zones, and technologies allow identifying of such zones and managing this variability. As a result, farmers use seeds, fertilizers, and pesticides more efficiently; this also helps to increase the harvest.

Precision farming can be defined as a farming system, which enables profit to be maximized and where inputs (seed, fertilizer, tillage operations, and chemicals) are verified according to the yield potential of individual parts of a field. Precision farming facilitates the best use of inputs, resulting in increased gross margins with minimized impact on the environment. Variable Rate Technology (VRT) and site-specific agriculture is also known as Precision farming (Sahoo *et al.*, 2002). It is an information and communication-based agricultural production system. We have to gather information for managing agricultural production such as information of crop, environment, and situation then according we have to manage the production system. Both the terms precision farming and precision agriculture are simultaneously applied and almost the same. This is to characterize, manage, monitor, spatial and temporal variability on such crops with in field for profitability, sustainability, and environment protection. In another sense, it is an information and communication technology-based agriculture system to characterize, interpret manage, monitor, spatial and temporal variability of soil, crop in a field. In the sense of (Palaniappan, 2002). Precision farming is also referred to as smart farming, GPS (Global Positioning System), and site-specific based farming. Precision farming is the best solution to identify the causes of variability within the field and to carefully tailor soil and management of crop to fit in cultivated land (Gautam and Sharma, 2002). Thus, precision farming which is based on information and knowledge is a new combined technique for the scientific management of modern agriculture (Reddy, 2016). It is a complete system that takes full advantage of available agricultural resources, reduces pollution to protect the environment and promotes sustainable agriculture.

Importance of precision farming:

It is defined as the application of technologies and principles to manage spatial and temporal variability associated with all aspects of agricultural production (pierce and Nowak, 1999). PF is improving productivity and so reducing the greenhouse gas emissions per kg grain, meat, or milk produced and by being better prepared for climate change by using more efficiently or improving soil structure maximizing efficient use of inputs by locating soil health deficiencies and varying seed applications accordingly. PF controlled Traffic Farming can bring soil structure benefits and by reducing waste. This was optimizing nitrogen fertilizer use, thereby

helping to reduce the amt. of nitrous oxide released from the soil. The importance of precision farming is:-Dissemination of modern farm practices to improve quality, quantity and reduced cost of production, and developing favourable attitudes. It was reduce chemical application in crop production to efficient use of water resources. Precision farming changing the socio-economic status of farmers. Dairy farmers can also save around 20 % on labour using robotic milking systems and livestock farmers can further benefit from EID (Electronic Identification) systems**.** And Crop producers save costs by optimal use of inputs like fertilizers and pesticides, whilst also boosting profit through increased yields.

Components of precision Farming:

The major components include the Global Positioning System (GPS), variable rate application (VRA), Geographic information systems (GIS), Remote sensing, Yield monitors, Computer simulation models, and Gird sampling.

Global Positioning System (GPS):

GPS is a navigation system based on a network of satellites that helps user's record positional information (longitude, latitude and elevation) with an accuracy of b/w 100 and 0.01 m (Lang 1992). GPS allows farmers to locate the exact position of field information, such as soil type, weed invasion, water holes, pest occurrence, boundaries and obstructions. Global Positioning System satellites broadcast signals that allow GPS receivers to calculate their actual position. This information is based on real-time, means that continuous position facts is provided while in motion. Precise of location information at any time that allows cropand soil measurements to be mapped on field. The system with light or sound guiding panel (DGPS), antenna, and receiver. The system, allows farmers to reliably identify field locations so that inputs (seeds, pesticides, fertilizers, herbicides,and irrigation water) can be applied to an individual field, based on performance criteria and previous input applications(Batte 2000). Global Positioning System receivers, either carried to the field or mounted on implements, allow users to return to specific locations to sample or treat areas. Uncorrected GPS signals have an accuracy of about 300 feet.

Variable-rate application (VRA):

Variable rate application refers to the application of parameters like seeding, lime application, fertilizer use, etc. based on the area, location, and soil conditions, etc. Depending upon whether the VRA technology is being used with or without a GPS, there are two methods:

1. Map-based VRA:

In this method of VRA makes use of a GPS receiver. It adjusts the application rate by making use of a prescription electronic map. A prescription map is an Electronic data file, this Map provided all the necessary and specific information regarding the input rates in all field zone. As the users move through the field using the field position from the global positioning system receiver. Map-based VRA also uses maps based on previous measurements which are then implemented using various strategies which are based on information like topography, soil type, soil color, crop yield and remotely sensed images, etc., and this information is crop-and location-specific (Grissoet al.2011).

2. Sensor-based VRA:

In this type of VRA does not require prescription maps or GPS. The sensors installed on applications detect the soil properties of land as well as characteristics of the.Thus, a continuous flow of information is collected based on the sensor report which is then transferred to the control system which calculates the input rates and sends the that information to the controller, which was delivers in the input of the location. Sensor-based VRA has the advantage of providing real-time data using the real-time sensors, unlike the map-based VRA which makes use of previously collected data to vary the application rate inputs.

Geographic information systems (GIS):

GIS is a kind of computerized map. Provide information on field topography, soil types, surface drainage, subsurface drainage, soil testing, irrigation, chemical application rates, and crop yield GIS is a powerful set of tools for collecting, storing, and retrieving the data at will, transforming and displaying the spatial data for a particular purpose (Burrogh and McDonnell, 1998).GIS is a powerful set of tools for collecting, storing, and retrieving the data at will, transforming and displaying the spatial data for a particular purpose (Burrogh and McDonnell, 1998).

Geographic information systems (GIS) is a powerful tools for collecting data, storing, and retrieving the data at will, displaying and transforming the spatial data for a particular purpose (Burrogh and McDonnell,1998). The ability of GIS to analyze and visualize theenvironments of agricultureand workflows has proved to be more beneficial data to those which are involved in the farming industry. Geographic information systems (GIS) can be used to produce images, not just maps, but drawings, animations, and other cartographic products. Geographic information systems playing important role in production of agriculture throughout the world by helping farmers to increase their production, reduction of their costs and manage their land.GIS applications such as Soil amendment analyses, crop yield estimates, and erosion identification

and remediation.GIS is a layer-based and thematic system that provides the flexibility to overlay and review the indices for various changes to the site. GIS provides ways to overlay different 'layers of data: the ecological conditions, the actual physiognomy and human pressure indices (Sood *et al.,* 2016).

Remote Sensing:

Remote sensing has a great potential for precision farming as it provides the solution of monitoring the spectral and spatial changes over time at high resolution. The new modern-day technologies in agriculture have to be induced into the ongoing mechanism of farm practices which needs to be monitored regularly. RS provides a better option for precision agriculture like providing frequent turn-around time (24-48 hrs), low-cost data, High spectral resolution, high spatial resolution, and high temporal resolution (10-15 days).

Yield monitors:

Yield monitors are measuring the yield of cropwhich is a devices installed on harvesting equipment. The yield data from the monitor is recorded and stored at regular intervals along with positional data received from the Global Positioning System unit. Geographic information systems (GIS) software takes the yield data and produces yield maps.

Computer Simulation Models:

A computer crop simulation model of the growth and development of crops was validated using data sets obtained from field experiments. The Computer crop simulation model was used to provide information concerning management options such as the timing and quantity of nitrogen fertilizer applications and to quantify the weather-related risks of crop production. Computer simulation models were linked to a GIS to provide information at a regional level.

Gird sampling:

Grid sampling involves in dividing the field into several small and equal divisions. To do this tractor is fitted with a dish antenna to receive signals from the satellite and samples of soil are mechanically taken from every sub-division. Samples are tested in a modern laboratory of soil testing including chemical and physical characteristics of soil and recorded. Using the test results colourgraine are created for the entire field. The colour grains are stored in the computer for various functions. This helps in balancing the soil fertility of the field. Fertilizers are then automatically applied at variable rates only to where they are needed as indicated by colour grains. Grid sampling brings uniformity of soil fertility, high economic yield, which could not be achieved by implementation other methods.

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SOIL AND NUTRIENT MANAGEMENT PACKAGE FOR ACCELERATING QUANTITY AND QUALITY MULBERRY (*MORUS ALBA*) LEAF PRODUCTION FOR STRENGTHENING SERICULTURE SECTOR IN INDIA

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Introduction:

Mulberry (*Morus alba*) is a foliage crop cultivated extensively in India under different conditions for rearing silkworm (*Bombyx mori*). Along with atmosphere, soil constitutes the natural medium which supports the growth of plants on earth surface. Soil not only serves as reservoir of food and water needed by the plants, but also provides a mechanical anchorage to them. Soil may be defined as thin layer of earth crust made up of disintegrated and decomposed rocks, containing complex mineral compounds, organic matter, water, air and living organisms like bacteria, fungi, protozoa, insects, worms etc. Different experiments are carried out to develop a soil-cum-nutrient management package for mulberry cultivation. Effect of different treatment combinations comprising of NPK, farmyard manure (FYM), lime and boron (B) on important soil properties, yield and quality of mulberry (*Morus alba*). Integrated soil application of NPK @150:50:50 kg ha^{-1}, FYM @10 t ha^{-1}, lime as per requirement (3t ha^{-1} in present case) and foliar application of boric acid solution (0.1%) was the most effective soil-cum-nutrient-management package for the production of higher leaf yield (Dandin *et al.*, 2003). As per the leaf quality is concerned, increased moisture content, total NPK and B content of the leaf are also attributed to the package. Recent estimate indicates that the total are under mulberry cultivation is 26,398 ha in the NER of the country (CSB, 2016). Mulberry flourishes well in soils which are deep, flat, fertile, well drained, loamy to clayey, porous with good moisture holding capacity. Recent research estimated that Dependence on and curiosity about soil, exploring the diversity and dynamics of this resource continue yield fresh discoveries and insights. New avenues of soil research are compelled by a need to understand soil in the context of climate change, green house gas and carbon sequestration. Interest in maintaining the planet's biodiversity and in

exploring past cultures has also stimulated renewed has also stimulated renewed interest in achieving a more refined understanding of soil.

Formation of soil

Soil formation is very slow but ceaseless biochemical process. Soil is formed by the mechanical disintegration and chemical decomposition of parent rocks. There are the three principal kinds of parent rock. (i) Igneous rock (ii) sedimentary rock (iii) Metamorphic rock. Rock weathering may be physical or chemical. The principal agent of rock weathering is heat, water, wind, plants and animals, while the chemical decomposition of rocks is brought about by solution, hydrolysis, carbonation, oxidation and reduction etc.

Soil suitable for mulberry garden

Selection of soil is of prime importance in mulberry cultivation. At the time of selection, it is important that the quality of soil of mulberry field has a profound influence. The fertility of soil ultimately affects the growth of the silkworms and also quantity and quality of the cocoons produced. In this respect, mulberry is quite distinct from other crops like paddy, wheat etc. The soil of mulberry plantation must be capable of maintaining the mulberry plants for prolonged maximum productivity of quality leaves. The ideal mulberry soils are the ones which contain adequate amount of all essential elements in the forms which are readily accessible to plants and are in good physical condition to support and contain the right amount of water and air for desirable root growth. Since mulberry is a deep rooted, perennial, hardy and monoculture crop, the soil should be capable of supplying sufficient air, water and nutrients even in the deeper layers. Though mulberry is tolerant to a wide range of soil conditions, it grows well on loamy soil of high fertility. In general, the soil for mulberry should be deep, well-drained, and clayey loam to loam in texture, porous, fertile and with good water retention capacity. Slightly acidic soils with a pH value around 6.8 which are free from injurious salts **(Sarkar, 2000).** Saline and alkaline soils and also highly acid soils should be avoided and if not possible, should be suitably reclaimed.

Soil reaction (pH)

One of the excellent physiological characteristics of the soil solution is its reaction (pH). Since micro-organisms and higher plants respond so markedly to their chemical environment, the importance of soil pH and the factors associated with it, has long been recognized. The term pH is from the French word Pouvoir hydrogene or hydrogen power. The soil pH is the negative logarithm of the hydrogen 6 ion concentration in solution. The soil reaction is an indication of the acidity or basicity of the soil and is measured in pH units. The scale goes from 0 to 14 with pH 7 as the neutral point. At pH 7, hydrogen ion (H^-) concentration equals the hydroxyl ion (OH)

concentration. From pH 7 to 0, the soil is increasingly more acidic and from 7 to 14 the soil is increasingly more basic (alkaline). Slightly acidic soils with a pH value around 6.8, freefrom injurious salts are ideal for good growth of mulberry plants. The acidic as well as alkaline soils are not suitable for the growth of mulberry. But both types of problematic soils: acidic and alkaline, are encountered in mulberry gardens.

Table 1: Correlation between pH and degree of acidity

pH	Reaction
6.6 to 7.5	Nearly neutral
6.1 to 6.5	Mildly acidic
5.6 to 6.0	Moderately acidic
5.1 to 5.5	Strongly acidic
4.6 to 5.0	Very strongly acidic
4.5 and below	Extremely acidic

Nutrient management package for accelerating quantity and quality mulberry (*Morus alba*)

Mulberry (*Morus alba*) a perennial plant cultivated as a seasonal crop for its foliage to feed silkworms (Bombyx mori) demands high doses of organic manures and inorganic fertilizers. As it is grown for its foliage, it cherishes under desirable levels of pH (6.5-7.5), Electrical Conductivity (EC-<1.00dS/m), Organic Carbon (OC: 0.65-1.0%), Available Nitrogen (N: 250-500kg/ha), Phosphorous (P: 15-25kg/ha), Potassium (K: 120-240kg/ha) and Sulphur (S: 10-15ppm) in the soils (Sarkar, 2000). Therefore, for its sustainable growth and leaf production, it has been prescribed to maintain desirable levels of soil fertility by supplying optimum amount of NPK @350:140:140kg/ha/yr and 20MT FYM/ha/yr for mulberry gardens (Anonymous, 2011). Even though the use of recommended doses of manures and fertilizers plays a pivotal role in mulberry leaf yield and quality, but not found satisfactory at the farmers level resulting in low mulberry yield and soil fertility. Further, due to hectic crop schedules and frequent harvesting of leaf shoot biomass, depletion of soil fertility status of mulberry gardens has become a regular phenomenon. It is also reported that blanket recommendation of fertilizers leads to over or underuse of fertilizers leading to ultimate deterioration of soil health. Frequently cultural operations, inorganic fertilizer applications, imparting of diseases and pest control measures and

industrial emissions and effluents are not only altering and depleting the soil nutrient status, but also polluting the groundwater resources. Therefore, frequent supplementation of essential macro and micronutrients along with the sufficient manuring for conditioning the soils and balancing the soil nutrient status for enhanced quality mulberry leaf production is essential. Rearers emphasized the need for the balanced fertilization and their impact on the quality mulberry and cocoon production. Cluster Promotion Programme (CPP) was implemented under the 12th five-year plan during 2012-2019 in India for boosting the bivoltine sericulture with a target of >5000 MT/yr raw silk production (Anonymous, 2011). Under this program, the state and Central Government jointly organized 178 clusters all over India out of which 14 clusters were implemented in Andhra Pradesh (Anonymous, 2011). Though we could successfully achieve the targeted bivoltine raw silk production with all the above mentioned efforts, we are still lagging behind in succeeding the 3A & above grade silk production and not gaining the anticipated market rates from the sericultural farming community. The reasons behind are many, but low-level adoption of technical knowhow and lacking the judicious imparting of the recommended package of practices in mulberry cultivation has a crucial role. Though, the farming community has been extended the suitable package of practices for the production of the quality mulberry leaf, soil nutrient status depleted due to the continuous and long term harvesting of the mulberry crops (ranging from 10-15 years) and supplementing with the insufficient doses of manures and fertilizers. Further, draining of soil nutrients occurs due to heavy and untimely rains and drought spell situations limiting the nutrients uptake and availability. However, lacking the technical knowhow of the adoption of soil testing and imparting analysis based amelioration of garden soils are the main reasons of soil nutrients depletion. Therefore, it is necessary to assess the current soil nutrient status of prominent bivoltine sericultural areas and to extendthesoil analysis based amelioration recommendation in the form of 'Soil Health Cards' for improving their mulberry soils and to enhance the mulberry quality and cocoon production.The essential nutrient elements required by mulberry plant are exclusively of inorganic nature. Intensive cropping systems have led to an increase in the demand of the crucialnutrients in the soil.Thus, in addition of essential organic and inorganic nutrients to attain the soil status is necessaryfor thesustainable leaf productionin mulberry. The desired nutrients required for accelerating quantity and quality mulberry (*Morus alba*) are as soil pH (6.8) (Sarkar, 2000). Most of the cluster soils (99 percent) showed an ideal range of soluble salts (<1.0 dS/m), Organic carbon (0.65-1.0 percent) (Chen *et al.,* 2009). In case of essential macronutrients, available Nitrogen (N) (250-500kg/ha), Phosphorous (P) 15-25kg/ha and Potassium (K) 120-240kg/ha respectively (Kar *et al.,* 2008)**.** In case of micronutrient sulphur (S), 61% of the mulberry gardensoil were registered to be rich in

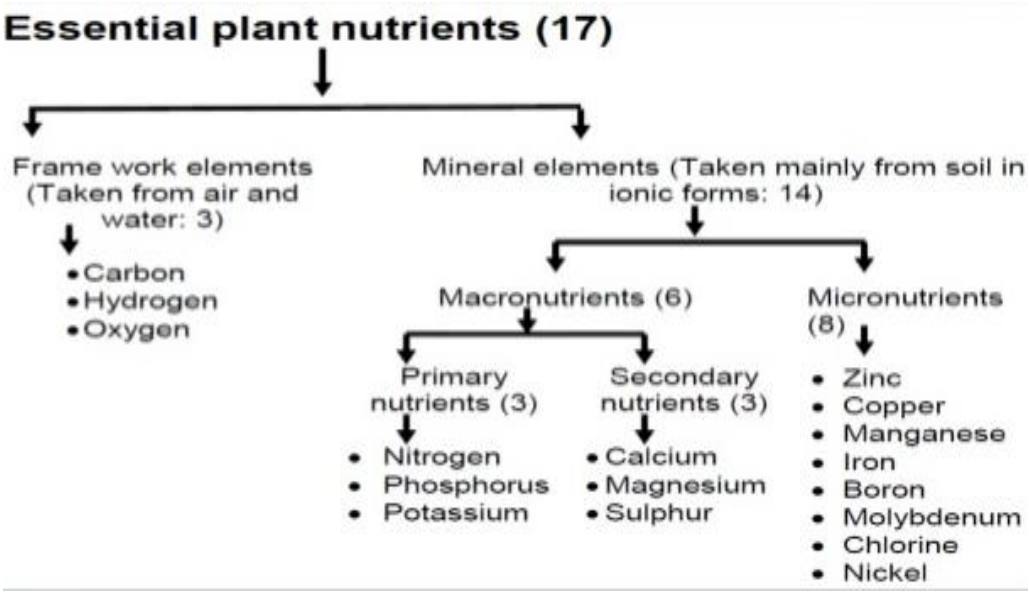
sulphur (>15ppm/ha), 25% showed the admissible range (10-15ppm/ha) whereas only 14% soils were recorded withlow levels of S (<10ppm/ha) indicating that most of the mulberry garden soils are rich in sulphur (Kar *et al.,* 2008).

Seventeen chemical elements are directly involved and important to plant's growth and survival, are classified into two groups. These chemical elements are divided into two main categories

Non-mineral group

Mineral group

1. **Non-Mineral Nutrients:** The Non-Mineral Nutrients may enter plants through either the soil or atmosphere. These nutrients are found in air and water. These are hydrogen (H), oxygen (O) & carbon (C).
2. **Mineral Nutrients:** The 13 mineral nutrients, which come from the soil, are dissolved in water and absorbed through a plant's roots. These are not enough nutrients in the soil for a plant to grow healthy. That's the reason why many farmers and gardeners use fertilizers to the soil for acceleration. The mineral nutrients are divided into two groups
    - **Macronutrients**
    - **Micronutrients**



Macronutrients

Macronutrients can be broken into two more groups: Primary and Secondary nutrients. The primary nutrients are Nitrogen (N), Phosphorus (P), and Potassium (K). These major nutrients usually are lacking from the soil first because plants use large amounts of them for their growth and survival.

Recommended dosage and schedule for NPK application

Manure	Nitrogen	Phosphorus	Potassium
Cattle dung(fresh)	0.3-0.4	0.1-0.2	0.1-0.3
Horse dung (fresh)	0.4-0.5	0.3-0.4	0.3-0.4
Sheep dung (fresh)	0.5-0.7	0.4-0.6	0.3-1.0
Poultry manure	1.0-1.8	1.4-1.8	0.8-0.9
Raw sewage(fresh)	2.0-3.0	-	-
Cattle urine	-	-	0.5-1.0
Horse urine	1.2-1.5	Traces	1.3-1.5
Human urine	0.6-1.0	0.1-0.2	0.2-0.3
Sheep urine	1.5-1.7	Traces	-
Ash, coal	0.73	0.45	0.53
Ash, household	0.5-1.9	1.6-4.2	2.3-12.0
Rural compost (dry)	0.5-1.0	0.4-0.8	0.8-1.2
Urban compost (dry)	0.7-2.0	0.9-3.0	1.0-2.0
Rice husk	0.3-0.5	0.2-0.5	0.3-0.5
Ground nut husk	1.6-1.8	0.3-0.5	1.1-1.7

(Subbaswamy *et al.*, 2011)

The secondary nutrients are Calcium (Ca), Magnesium (Mg), and Sulfur (S). There are usually enough in the soil so that fertilization is not needed. Usually large amounts of Calcium and Magnesium are added when lime is applied to acidic soils. Sulfur is usually found in sufficient amounts from the slow decomposition of soil organic matter.

Micronutrients

Micronutrients are those elements essential for plant growth which are needed in only very small (micro) quantities. These elements are sometimes called Minor elements or Trace elements, but use of the term micronutrient is encouraged by the American Society of Agronomy and the Soil Science Society of America. The micronutrients are Boron (B), Copper (Cu), Iron (Fe), Chloride (Cl), Manganese (Mn), Molybdenum (Mo) and Zinc (Zn). Recycling organic matter such as grass clippings and tree leaves is an excellent way of providing micronutrients (as well as macronutrients) to growing plants.

Macronutrients

1. **Nitrogen (N)**

- Nitrogen is a part of all living cells, proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy.
- Nitrogen is a part of chlorophyll, the green pigment of the plant that is responsible for photosynthesis.
- Helps plants with rapid growth, increasing seed and fruit production and improving the quality of leaf and forage crops.
- Nitrogen often comes from fertilizer application and from the air (legumes get their N from the atmosphere).

Reclamation

- Maintaining soil pH between 6.5 to 7.5
- Application of requisite quantity of nitrogen fertilizers (300-400 kg N/ha/yr. in 4 to 5 split doses after 3-4 weeks of every harvest (Subbaswamy *et al.*, 2011)
- Application of FYM and green manures @ 20 -25 tones /ha/yr.
- Integrated nutrient management and foliar application of nitrogen
- Regular application of nitrogen fixing bio-fertilizers along with FYM.

2. **Phosphorus (P)**

- Like nitrogen, phosphorus (P) is an essential part of the process of photosynthesis.
- Involved in the formation of all oils, sugars, starches, etc.
- Helps with the transformation of solar energy into chemical energy.
- Effects rapid growth.
- Encourages blooming and root growth.
- Phosphorus often comes from fertilizer, bone meal, and superphosphate.

Reclamation

Apply Phosphorous pentaoxide @ 180 kg /ha/yr (Subbaswamy *et al.*, 2011) in equal split doses at alternate crops. It should be applied near to root zone at the depth of 20-40 cm from soil surface.

3. **Potassium (K)**

- Potassium is absorbed by plants in larger amounts than any other mineral element except nitrogen and in some cases, calcium.
- Helps in the building of protein, photosynthesis, fruit quality and reduction of diseases.

- Potassium is supplied to plants by soil minerals, organic materials, and fertilizer.

Reclamation

- Maintenance of soil pH and soil moisture.
- Application of potassium oxide @ 180-240 kg/ha/yr. based on soil testing in two equal split doses after 3-4 weeks of 1st and 3rd harvest (Subbaiah *et al.,* 2016).
- Application of green manure, FYM and tank silt @20-25 tones/ha/yr (Subbaiah *et al.,* 2016).
- Integrated nutrient management and foliar application of potassium.

4. Calcium (Ca)

- Calcium, an essential part of plant cell wall structure, provides for normal transport and retention of other elements as well as strength in the plant. It is also thought to counteract the effect of alkali salts and organic acids within a plant.
- Sources of calcium are dolomitic lime, gypsum, and superphosphate.

Reclamation

- In acid soil apply lime one MT/ha/yr at the interval of every 4-5 years.
- Spray 0.5-1.0 per cent calcium nitrate or calcium chloride over the leaves of deficient plants **(**Subbaiah *et al.*, 2016).

5. Magnesium (Mg)

- Magnesium is part of the chlorophyll in all green plants and essential for photosynthesis. It also helps activate many plant enzymes needed for growth.
- Soil minerals, organic material, fertilizers, and dolomitic limestone are sources of magnesium for plants.

Reclamation

- In acid soil apply lime one MT/ha/yr. at the interval of every 4-5 years (Subbaiah *et al.,* 2016).
- Spray 0.5-1.0 per cent calcium nitrate or calcium chloride over the leaves of deficient plants (Subbaiah *et al.*, 2016).

6. Sulfur (S)

- Essential plant food for production of protein.
- Promotes activity and development of enzymes and vitamins.
- Helps in chlorophyll formation.
- Improves root growth and seed production.
- Helps with vigorous plant growth and resistance to cold.

- Sulfur may be supplied to the soil from rainwater. It is also added in some fertilizers as an impurity, especially the lower grade fertilizers. The use of gypsum also increases soil sulfur levels.

Reclamation

0.1- 0.2 per cent aqueous solution of Potassium sulphate should be sprayed on deficient leaves (Subbaiah *et al.*, 2016).

Micronutrients

1. **Boron (B)**

- Helps in the use of nutrients and regulates other nutrients.
- Aids production of sugar and carbohydrates.
- Essential for seed and fruit development.
- Sources of boron are organic matter and borax.

Reclamation

Spray aqueous solution of one kg Boric acid (Borax)/ha/crop over the leaves of deficient plants (Subbaiah *et al.,* 2016).

2. **Copper (Cu)**

- Important for reproductive growth.
- Aids in root metabolism and helps in the utilization of proteins.

Reclamation

Aqueous solution of one kg copper sulphate /ha/crop should be sprayed over deficient plants (Subbaiah *et al.,* 2016).

3. **Chloride (Cl)**

- Aids plant metabolism.
- Chloride is found in the soil.

Reclamation

Cl is normally not applied to soil. Rain water supplies about 10-100kg of Cl per hectare annually besides its addition through fertilizers like MOP (47.3%). Rate of recommendation is 3-5kg per hectare (Subbaiah *et al.,* 2016).

4. **Iron (Fe)**

- Essential for formation of chlorophyll.
- Sources of iron are the soil, iron sulfate, iron chelate.

Reclamation

- Aqueous solution of one kg Ferrous sulphate / ha/crop should be sprayed over the leaves of deficient plants.
- Fe chelates like EDDHA @100 Kg/ ha should be mixed in soil in iron deficient fields (Subbaiah *et al.*, 2016).

5. Manganese (Mn)

- Functions with enzyme systems involved in breakdown of carbohydrates, and nitrogen metabolism.
- Soil is a source of manganese.

Reclamation

Spray aqueous solution of one kg Manganese sulphate /ha /crop over the deficient plants (Subbaiah *et al.*, 2016).

6. Molybdenum (Mo)

- Helps in the use of nitrogen
- Soil is a source of molybdenum.

Reclamation

Since molybdenum deficiency usually occurs in acidic soils, the most common cure is to lime the soil to a pH of 6.0–6.5, after which Mo deficiency often disappears.

If liming did not fix the deficiency or if the soil pH was already around 6.5, Mo deficiency can be easily corrected with a sodium molybdate or ammonium molybdate foliar spray.

7. Zinc (Zn)

- Essential for the transformation of carbohydrates.
- Regulates consumption of sugars.
- Part of the enzyme systems which regulate plant growth.
- Sources of zinc are soil, zinc oxide, zinc sulfate, zinc chelate.

Reclamation

Aqueous solution of 2 kg Zinc sulphate /ha/crop should be sprayed over the leaves of deficient mulberry plants (Subbaiah *et al.*, 2016).

8. Nickel

- More damage by root knot nematodes.
- Accumulation of ureids in leaves (leaf tip necrosis).
- Critical constituent of the plant enzyme urease for conversion of urea to ammonia

Reclamation

- It can be found as a contaminant in fertilizer and the irrigation water and it is often found in sewage sludge and animal waste.
- Nickel can also be applied as a single element application as nickel sulfate or in a chelated form.

Causes of mineral deficiences

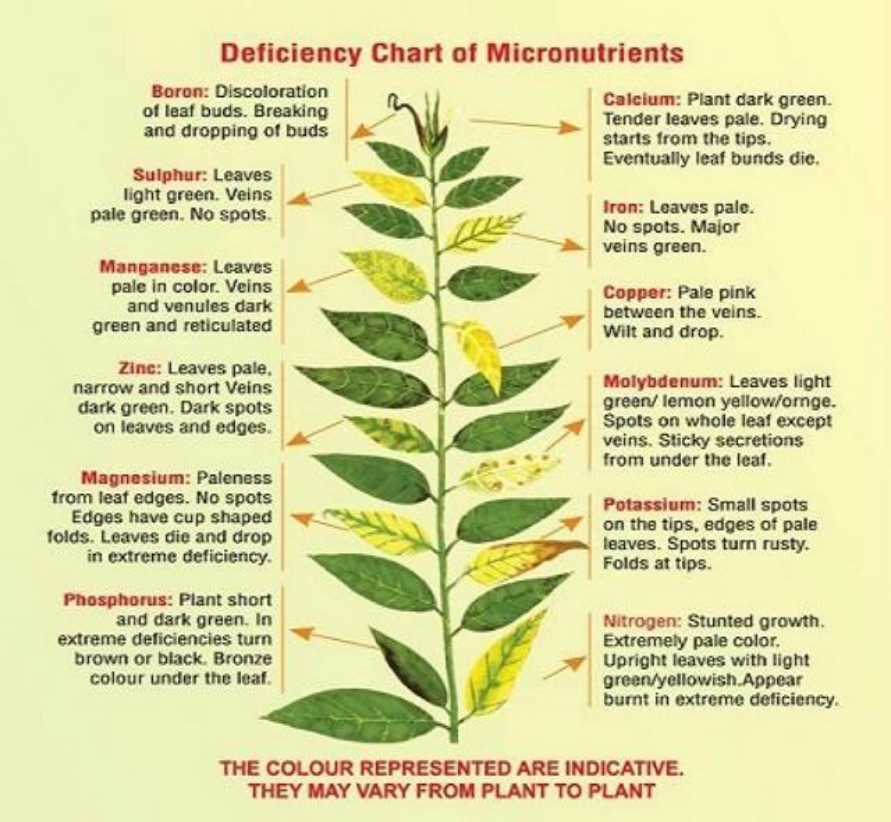
- Inadequate supply of one or more plant nutrients to the soil or imbalance nutrient status.
- Element withdrawals due to continuous cultivation without proper replenishment.
- Imbalance in fertilizer application, especially nitrogen.
- Increasing yield levels leads to higher nutrient requirement.
- Depletion of micronutrients due to intensive cultivation.
- Leaching of micro nutrients in the soil.

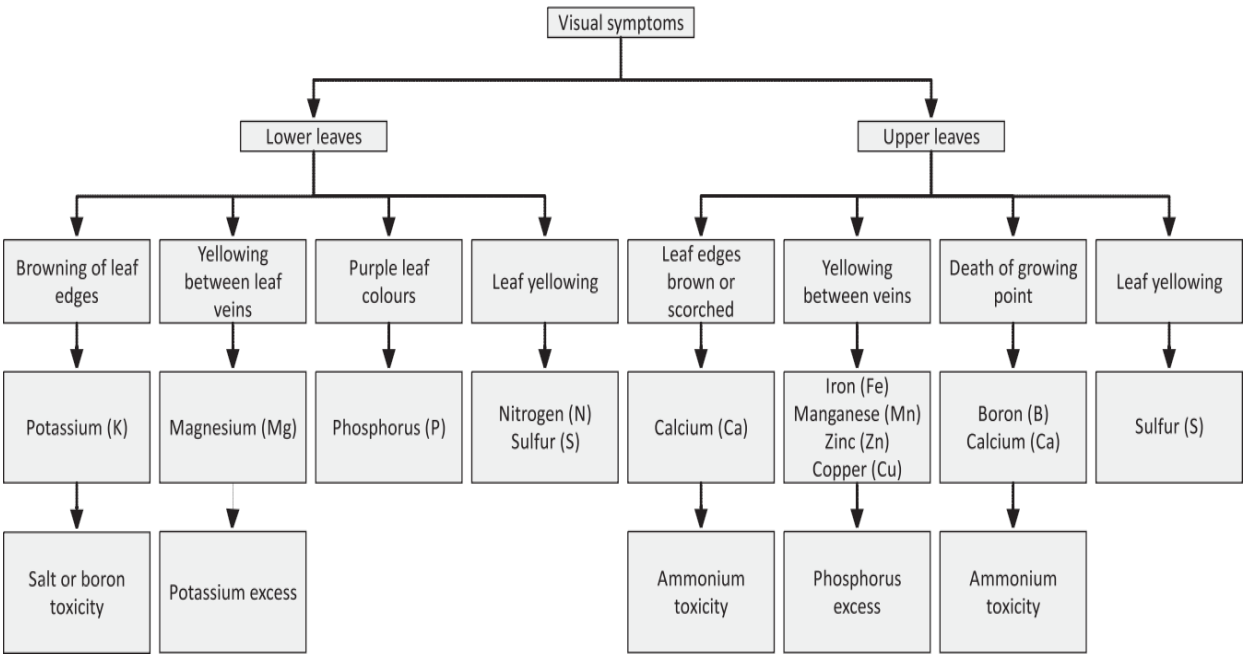
Impact of mineral deficiences

- Primarily effects qualitative and quantitative loss of mulberry leaves.
- Decrease in the intake of K, Ca, Mg, and P causes decrease in body weight of silkworm.
- P deficiency affects the uptake of other minerals elements affecting the growth and economic characters of silkworm.
- Deficiency of Mg, Mn, and Fe reduce the cocoon yield, shell weight and larval duration.
- Zn deficiency is known to decrease the pupal weight, silk filament length and cocoon shell ratio.
- Besides some non- essential (in respect to plants) such as nickel and cobalt has a regulatory role on silk gland development.

Nutrient deficiences

Nutrient disorders in mulberry are induced by inadequate quantity of one or several nutrients. The soil pH can induce nutrient deficiencies in mulberry plants. Poor uptake of nutrients such as boron, copper, and iron can occur if the pH of the soil is above 6.5. Improper supply of chemical fertilizer can lead to nutrient deficiency in the soil, while constant flood irrigation of the land can lead to macro and micro-nutrient deficiencies. Mineral antagonism involves excessive supply of certain elements to plants, which can obstruct the uptake of other nutrients. Application of balanced nutrients based on periodical soil analysis has to be strictly followed to control nutrient deficiency in mulberry. Press mud can be used both as soil reclament and soil conditioner as it prevents soil erosion, crusting and cracking, and adjusts soil pH. Application of Seriboost is an effective way of regulating nutrient deficiency problem in mulberry.





Representing the nutrient deficiencies symptoms

Fertilizer application schedule for mulberry under irrigated conditions

Application time	Row system	Pit system
1st application	60kg N + 60kg P+ 60kg K (As complex fertilizer)Within a month after pruning – early July. 1 harvest- mid August by bottom pruning	60kg N + 60kg P+ 60kg K As complex fertilizer
2nd application	60kg N (As straight fertilizer) Within a month of last harvest- mid September II harvest –early November by pruning	40kg N As straight fertilizer
3rd application	60kg N + 60kg P + 60kg K (As complex fertilizer) Within a month of last harvest- early Dec. III harvest –mid Jan. by pruning	40kg N as straight fertilizer
4th application	60kg N (As straight fertilizer) Within a month of last harvest –mid Feb. IV harvest – late march by pruning	60kg N+ 60KG P +60kg K As complex fertilizer
5th application	60kg N (As straight fertilizer) V harvest- early June by pruning	40kg N as straight fertilizer
6th application	----------	40kg N as straight fertilizer
Total	300kg N +120 kg P+120kg K	280kg N+120kgP +120kg K

Leaf production for strengthening sericulture sector in India

Sericulture in India is an important agro-based cottage industry providing employment to millions in the villages and earning foreign exchange to the tune of 2000 crore of Rupees per year. Indian sericulture is largely dependent on mulberry silk production by the silkworm *Bombyx mori* (Jackson, 2005). The silkworm *Bombyx mori* is essentially monophagous and survives solely on the mulberry leaves *(Morus* spp) (Jackson, 2005). Mulberry is a highly heterozygous and vegetatively propagated species that is prone to prolonged juvenile period. The cultivation of mulberry for raising silkworm cocoon should not aimed only at increased production of leaves per unit area but also leaves of suitable quality for the maximum utilization of the leaf crop produced. The food quality relevant to all the aspects of insect performance including growth, development and reproductive potentialities depends mainly on nutritional

composition. *Bombyx mori* feeds primarily on mulberry leaves and prefers leaves of different maturity viz. tender, medium and mature based on position in different stages of its larval period. Since the quality of silk production is directly proportional to the quality of leaves used as the exclusive feed for silkworms, leaf quality is of utmost importance in sericulture.

The growth and development of silkworm larvae and economic characters of cocoons are influenced largely by the nutritional quality of mulberry leaves. Various characters are responsible for success of cocoon crop which involves mulberry leaf (38.2%) followed by climate (37%), rearing techniques (9.30%) and silkworm race (4.02%), Silkworm eggs (3.10%) and other factors (9.60%) etc (Jackson, 2005). Nearly 70% of the silk protein produced by the silkworm is directly derived from the protein of the mulberry leaves. Hence, choice of mulberry leaves suitable for healthy growth of silkworm is one of the important factors in sericulture. Mulberry leaves suitable as food for silkworms must contain several chemical constituents such as water (80%), protein (27%), carbohydrate (11%), minerals and vitamins and must have favourable physical features such as suitable tenderness, thickness, tightness etc. in order to render them acceptable by silkworms (Bennet, 2013).

It is clear from the above that mulberry leaf is more viable than any other factor and rearing of silkworm requires specific stages of leaf. Though in tropical condition mulberry can be grown throughout the year but quality of mulberry leaves are still not upto the merit particularly at farmer's level. Quality of leaf is one of the major problems behind the problems of silkworm rearing in tropics and the poor quality of leaf is one of the important factors attributed to the poor productivity of silk per unit area.

The quality of mulberry leaf *varies* significantly with the factors such as soil fertility, agronomical practices, planting system, environmental condition. The quality of leaves is reported to depend upon age of leaf on the shoot, succulency and nutrient content. The nutrient contents of mulberry leaves are known to vary according to the season, variety, age type of harvesting. In addition, feeding with mixed varieties leaves and feeding frequencies are also known to influence the health of the silkworms. The top tender leaves nutritionally richer compared to medium, matured and over matured leaves having less density of pubescence with blunt tip. The incidence and spread of diseases can be minimized and the cocoon characters may improve if silkworm larvae were fed with 65 days old mature leaf.

Conclusion:

India has the unique distinction of being the only country producing all the five known commercial silks, namely, Mulberry, tropical tasar, oak tasar, eri and Muga, of which Muga with

its golden yellow glitter is unique and prerogative of India (Kurose, 2016). Mulberry sericulture is mainly practiced in states such as Karnataka, Andhra Pradesh, Assam and Bodoland (Kokrajhar, Chirang, Baksa and Udalguri districts of Assam), West Bengal, Jharkhand and Tamil Nadu who are the major silk producing states in the country. North East has the unique distinction of being the only region producing four varieties of silk viz., Mulberry, Oak Tasar, Muga and Eri. Overall NE region contributes 18% of India's total silk production (Bose *et al.,* 2018). India is the second largest producer of silk in the world. Among the four varieties of silk produced in 2020-21, Mulberry accounted for 70.72% (23,860 MT), Tasar 8.02% (2,705 MT), Eri 20.55% (6,935 MT) and Muga 0.71% (239 MT) of the total raw silk production of 33,739 MT (Sudhakar *et al.,* 2019). The silk production has been reduced in the country during 2020-21 due to the disruptions caused by the Covid-19 pandemic. The total raw silk production in the country during 2020-21 was 33,739 MT, which was 5.8% lesser than the production achieved during the previous year 2019-20 (Yu *et al.,* 2019) and registered around 86.5% of achievement against the annual silk production target for the year 2020-21. The bivoltine raw silk production declined by 3.4% to 6,772 MT during 2020-21 and by 7,009 MT during 2019-20 (Singh, 2019). Similarly, vanya silk, which includes Tasar, Eri and Muga silk, have reduced by 13.8%, 3.7% and 0.8%, respectively during 2020-21 over 2019-20 (Singh, 2019). The area under mulberry has reduced by 0.8% in 2020-21 compared to previous year (2.38 lakh ha). The export earnings during 2020-21 were Rs. 1418.97 crores (Singh, 2019). The estimated employment generation under sericulture in the country was 8.7 million persons during 2020-21 compared to 9.4 million persons in 2019-20, indicating a reduction of 7.4% (Singh, 2019).

The demand for superior quality bivoltine silk is increasing in India for domestic consumption as well as value added silk products for the export market. The Ministry of Textiles Government of India and Departments of Sericulture in various states provide technical and financial assistance for enhancing the bivoltine silk production.Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen, and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability and known as the "**Queen of Textiles**" the world over (Govindaraj *et al.,* 2018). On the other hand, it stands for livelihood opportunity for millions owing to high employment oriented, low capital intensive and remunerative nature of its production. The very nature of this industry with its rural based on-farm and off-farm activities and enormous employment generation potential has attracted the attention of the planners and policy makers to recognize the industry among one of the most appropriate avenues for socio-economic development of a largely agrarian economy like India.Silk has been

intermingled with the life and culture of the Indians. India has a rich and complex history in silk production and its silk trade which dates back to 15th century. Sericulture industry provides employment to approximately 8.7 million persons in rural and semi-urban areas in India (Govindaraj *et al.*, 2018). Of these, a sizeable number of workers belong to the economically weaker sections of society, including women. India's traditional and culture bound domestic market and an amazing diversity of silk garments that reflect geographic specificity has helped the country to achieve a leading position in silk industry.

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WATER QUALITY INVESTIGATION OF UNEXPLORED FRESHWATER HABITAT -KRISHNA LAKE OF POLLACHI TALUK, COIMBATORE

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Abstract:

Water is an essential element and all organisms rely on water for their survival. Freshwater bodies are important ecosystem located in and around living habitations as they are generally natural ecosystems created by human in landscape suitable for water stagnation. The research study was carried out to determine the quality of water collected from unexplored Freshwater ecosystem of South block of Pollachi, Coimbatore, Tamil nadu, India. Water sample collected were analysed for the Physico-chemical parameters using the standard methods. In Physico-chemical analysis, parameters such as pH, turbidity, total dissolved solids (TDS), etc., were analysed and compared with standards of water quality. The results were indicated and discussed.

Keywords: Physico-chemical analysis, BIS, Freshwater, pH and turbidity.

Introduction:

Water is the basis of all organisms to sustain on the earth. Among different sources of water, freshwater bodies like lakes and ponds are found throughout the world Meena Sundari (2012). Fresh water habitats are quite a small portion of the earth surface as compared to marine and other terrestrial habitats, but their significance to human is greater than their areas. Fresh water is the most suitable and cheapest source for domestic and other needs and they act as convenient waste disposal systems. An undeniable bond exists between freshwater bodies and human beings. These are the prime sources of water used for drinking and domestic purposes, irrigation, industrial usage, and also aid in ground water recharge. Since all the freshwater ecosystems are used for drinking and domestic purposes, their quality is to be maintained and should be monitored periodically. Several studies were carried out to check the quality of freshwater bodies. The increased demand of water as a consequence of population growth, agriculture, industries and construction has forced environmentalists to resolve the chemical,

physical and biological characteristics of natural water resources Regina and Nabi (2003). Anthropogenic activities consequently cause water pollution which has brought a variable water crisis. Physico-chemical analysis is the primary consideration to determine the quality of water for its best utilization like drinking, irrigation, etc. The objective of the present study was to assess the Physico-chemical parameters of the unexplored freshwater ecosystem at Ambarampalayam, Pollachi, Coimbatore district, Tamil nadu by estimating the various parameters like pH, Temperature, Total Dissolved solids, Total Alkalinity, Total Hardness, Phosphate, Nitrate, Turbidity, Fluoride, Chloride, Dissolved Oxygen, Iron and Conductivity, etc.

Material and Methods:

Study area

Pollachi taluk is one of the headquarters in Coimbatore district, Tamil nadu, India, which is located about 40 km to the south of Coimbatore, consider to be the second largest town in the district. Pollachi is located at latitude 10.654ºN and longitude 77.0048ºE with an average elevation of 293m (Fig.1). The economic status of the town majorly depends on agriculture. One freshwater habitat was selected from South block of Pollachi Taluk, Coimbatore. Krishna pond is the Freshwater habitat present in the south block of Pollachi, belongs to Zamin Uthukuli Town Panchayat. The Tamil nadu water supply and drainage (TWAD), Department of rural development and Panchayat have the responsibility of providing safe drinking water supply for these areas in the state.

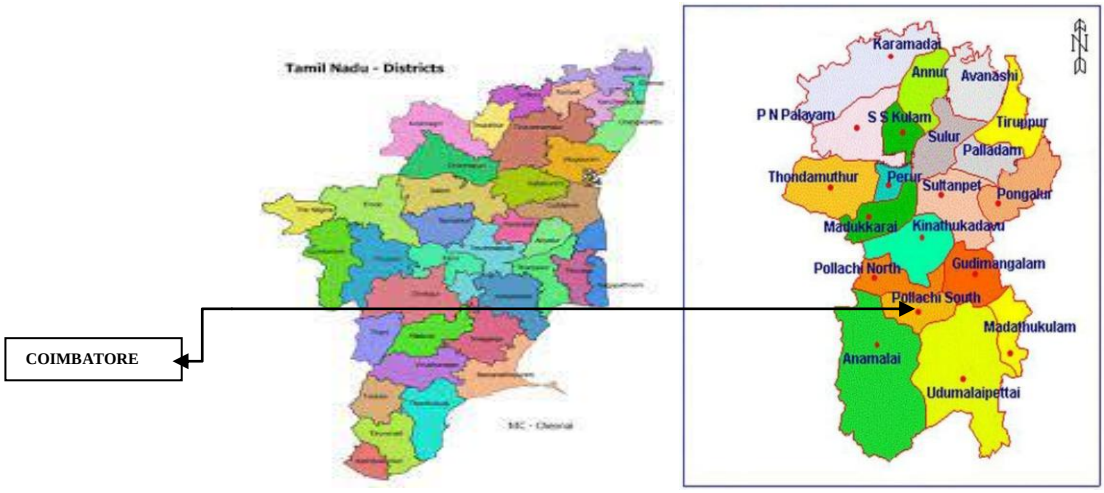

Figure 1: Map of the Sampling location

Sampling procedure

Table 1: Test methods of physico-chemical parameters of water

Sr. No	Characteristic BIS 10500:2012	Unit	Test Method / Instrument	Permissible Limit
1	Temperature	°C	-	-
2	Appearance	-	-	-
3	Colour	-	IS 3025 (Part 4)	-
4	Odour	-	IS 3025 (Part 5)	Agreeable
5	Turbidity	NTU	APHA 23rd Edition 2017-2130 B	5
6	Total Dissolved Solids (TDS)	mg/L	APHA 23rd Edition 2017-2540 C	2000
7	Electrical Conductivity	Micro mho/cm	APHA 23rd Edition 2017-2510 B	-
8	pH	-	IS 3025 Part 11- 1983	6.5-8.5
9	pH Alkalinity as CaCo3	mg/L	IS 3025 Part 23- 1983	-
10	Total Alkalinity as CaCo3	mg/L	IS 3025 Part 23- 1986	600
11	Total Hardness as CaCo3	mg/L	IS 3025 Part 21- 2009	600
12	Calcium as CaCo3	mg/L	APHA 23rd Edition 2017-3500 Ca B	200
13	Magnesium as Mg	mg/L	APHA 23rd Edition 2017-3500 Mg B	100
14	Sodium as Na	mg/L	APHA 23rd Edition 2017-3500 Na B	-
15	Potassium as K	mg/L	APHA 23rd Edition 2017-3500 K B	-
16	Iron as Fe	mg/L	APHA 23rd Edition 2017-3500 Fe B	1.0
17	Manganese	mg/L	APHA 23rd Edition 2017-3500 Mn B	0.3
18	Free Ammonia as NH3	mg/L	APHA 23rd Edition 2017-4500 NH3 C	0.5
19	Nitrite as NO2	mg/L	APHA 23rd Edition 2017-4500 NO2 B	-
20	Nitrate as NO3	mg/L	APHA 23rd Edition 2017-4500 NO3 B	45
21	Chloride	mg/L	IS 3025 Part 32- 1988	1000
22	Fluoride	mg/L	APHA 23rd Edition 2017-4500 F D	1.5
23	Sulphate	mg/L	IS 3025 Part 24- 1986	400
24	Phosphate	mg/L	APHA 23rd Edition 2017-4500 P D	-
25	Tidys Test 4hrs as O	mg/L	APHA 23rd Edition 2017-4500 O- B	0
26	DO	mg/L	APHA 23rd Edition 2017-4500 O- C	-
27	BOD	mg/L	APHA 23rd Edition 2017-5210-D	-
28	COD	mg/L	APHA 23rd Edition 2017-5220-B	-
29	Chromium	mg/L	IS.3025(Part 52)	0.05
29	Copper	mg/L	IS.3025(Part 42)	1.5
30	Zinc	mg/L	AAS	15.0
31	Nickel	mg/L	AAS	0.02

The water sample was collected during morning hour (i.e. between 7.00am and 9.00am) in polyethylene bottle of 2L capacity. The water sample was collected in two litre polyethylene can which was previously cleaned, rinsed and washed with deionized water and then rinsed with sample several times and closed with airtight lids, the water sample was analyzed for about 31 water parameters. The sample container was named properly and handed over to the State level Water Testing Laboratory of Tamil nadu Water Supply and Drainage (TWAD) Board, 24 hours from the time of collection. The water analysis were conducted at, Tamil nadu, Water Supply and Drainage Board (Coimbatore) and GRG Food Quality Testing Laboratory (Coimbatore) using standard methods (BIS 10500 : 2012) and quality assurance procedures (Bureau of Indian Standards (BIS) IS 3025-50:2001 and Bureau of Indian Standards (BIS) IS:10500:2012).

During each sampling events water temperature was measured in the field immediately with the help of glass thermometer, Turbidity, TDS, electrical conductivity, pH were measured following the standard methods Marimuthu and Rajendran (2017). The phenolphthalein alkalinity (PA), total alkalinity (TA), total hardness, fluoride, chloride, chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved oxygen (DO) and Tidy's test were also measured. The amount of Na, K, free ammonia, nitrite, nitrate, sulphate, phosphate and other heavy metals were analyzed using methods like spectrophotometer, flame photometry and AAS Kamalanandhini *et al.* (2018). The nature of the tests and some of the parameters tested were given in the Table 1.

Results and Discussion:

The physico-chemical parameters such as pH, electric conductivity, alkalinity, dissolved oxygen, total dissolve solid, calcium, magnesium, chloride, biological oxygen demand, nitrate, total hardness, etc of water were analysed for the water samples collected from the freshwater habitats of Pollachi Taluk and the results were given in the Table 2.

Appearance, Colour and Odour are the organoleptic and physical quality of water which can be observed, these parameters were found be neutral, where water was found to be clear, colourless, odourless in the lake water analyzed. Temperature is the most important physical factors in aquatic environment, the water temperature was found to be in the range of 25.4°C. The similar kind of observation with similar trends on different water body was reported by Priyatharsini and Dhanalakshmi (2016). The Total dissolved solids (TDS) in water are due to presence of all organic and inorganic substances. The most desirable limit of TDS is 500 mg/L and maximum allowable limit is 1500 mg/L. In the present study, the TDS value was found to be

in the range of 823 mg/L in the sample collected. The same range of TDS showed in the result, which is found to be in accordance with the present study Udhayakumar *et al.* (2016).

Table 2: Physico-chemical parameters of water

Sr. No.	Characteristic	Unit	Permissible Limit	Sample 1
1	Temperature	°C	-	25.4
2	Appearance	-	-	Clear
3	Colour	-	-	Colourless
4	Odour	-	Agreeable	None
5	Turbidity	NTU	5	1
6	Total Dissolved Solids (TDS)	mg/L	2000	823
7	Electrical Conductivity	Micro mho/cm	-	1210
8	pH	-	6.5-8.5	7.6
9	pH Alkalinity as $CaCO_3$	mg/L	-	0
10	Total Alkalinity as $CaCO_3$	mg/L	600	308
11	Total Hardness as $CaCO_3$	mg/L	600	272
12	Calcium as $CaCO_3$	mg/L	200	54
13	Magnesium as Mg	mg/L	100	33
14	Sodium as Na	mg/L	-	121
15	Potassium as K	mg/L	-	12
16	Iron as Fe	mg/L	1.0	0
17	Manganese	mg/L	0.3	0
18	Free Ammonia as NH_3	mg/L	0.5	13
19	Nitrite as NO_2	mg/L	-	1
20	Nitrate as NO_3	mg/L	45	0
21	Chloride	mg/L	1000	168
22	Fluoride	mg/L	1.5	0
23	Sulphate	mg/L	400	3
24	Phosphate	mg/L	-	0.27
25	Tidy’s Test 4hrs as O	mg/L	0	0.24
26	OD	mg/L	-	4
27	BOD	mg/L	-	13
28	COD	mg/L	-	124
29	Chromium	mg/L	0.05	0
29	Copper	mg/L	1.5	0.001
30	Zinc	mg/L	15.0	0.012
31	Nickel	mg/L	0.02	0

pH is a method of expressing the concentration of hydrogen ion, it determines whether the water is acidic or alkaline in nature. The permissible pH limit for freshwater is 6.5-8.5. The water sample analysed in the present study was found to have pH values within the permissible limit of 7.6, and it was slightly alkaline in nature as discussed in the study of Devendra Podhade *et al.* (2020). The Electrical conductivity is a valuable tool in assessing water quality, which is indicative of the total concentration of dissolved ions. The recommended value of EC as per BIS is < 2000 micro mho/cm. The value of 1210 micro mho/cm is found in the water sample which was analysed in the present study. The same range of result was observed in the research of Kamalanandhini (2019). Turbidity of water is in the range of 1NTU. It is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms. Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample Shobana *et al.* (2014). Total Alkalinity in water is due to the salts of weak acids and bicarbonates of highly alkaline water. Large amount of alkalinity imparts a bitter taste, harmful for irrigation as it damages soil and hence reduces crop yields. The most desirable limit is 200 mg/L and maximum allowable limit is 600 mg/L. In the present study, the water sample's total alkalinity level was found to have 308 mg/L. The same range of alkalinity was found in Ukkadam lake studied by Jeyaraj *et al.* (2016). Total Hardness of water is due to the concentration of salts. In particular, it is due to the concentration of multivalent metallic ions of calcium and magnesium. An increase in hardness causes scale deposition and scum formation Devendra Podhade *et al.* (2020) and Chennakrishnan *et al.* (2018). The desirable limit of Total Hardness is 200 mg/L - 600 mg/L. In the present study, the sample has 272 mg/L which is found to be in permissible limit. The presence of ammonia at higher than permissible levels is an important indicator of faecal pollution. Taste and odour problems as well as decreased disinfection efficiency are to be found if water containing more than 0.2 mg of ammonia per litre. In present study it is absorbed that, water sample was found to have the ammonia content, which is higher than the BIS's level such as 13mg/L. But in WHO Guidelines for drinking-water quality it has been mentioned as surface waters may contain up to 12 mg/L World Health Organization (1996). Calcium and magnesium play an important role in water quality analysis, in water, calcium serves as one of the vital micronutrients in organisms. Magnesium is found to be associated with calcium in all types of waters, but its values remains generally lesser than the calcium Sreenivasulu and Damodharam (2016). In present study, the values of calcium was found to be 54 mg/L, while magnesium ranged about 33 mg/L, which is found to be in

permissible limit. Concentration of Sodium and potassium in the water samples were within safe limits, whereas in the study Manoj *et al* (2012) observed the value of Surface Water, for which the concentration of Na^+ was 415 mg/L, while that of Ca_2^+ is 375, which is found to be higher than the permissible limit. The distribution of heavy metals (Cr, Cd, Zn, Mn, Fe and Cu) in water samples has been evaluated, where all the results obtained revealed that the concentration of the heavy metals were found to be absent in the sample which is found to be in contradictory with the results observed by Dagne Bekele Bahiru (2020).

There is no fluoride concentration observed in the water sample observed in the present study, but, in the study Pradeep Kumar *et al.* (2019) observed four different sample sites of Palar River, having the fluoride content ranging between 0.18 and 0.22 mg/l. Higher concentration of chloride in water is often found in combination with higher sodium concentration. BIS have prescribed 250- 1000 mg/l as the maximum permissible value. If the chlorine value exceeds 1000 mg/l, it is confirmed water is salty, which may affect the taste, palatability and corrosive effect of water Kistan *et al.* (2015). Sodium chloride, soluble salt acts as a source for chlorides. The chloride content of the lake water showed a high level of 168 mg/l. The increased application of fertilizers, use of detergents and domestic sewage greatly contribute to the heavy loading of phosphorous in the water. The BIS suggested the limit of phosphate is 0.1mg/l. Phosphate levels were found to be within the permissible limit in the lake water sample. Phosphate levels were found to be high in all the sampling sites in all the seasons Vincy *et al.* (2020) which is found to be contradictory with the presnt study. The sulphate concentration of the water samples varied about 3 mg/l. The sulphate concentration is within the permissible limit of 200 - 400mg/l. The low sulphate concentration indicates that there is no agricultural runoff and animal wastes being dumped into these habitats Dineshkumar and Natarajan (2020). Nitrates (NO_3^-) are chemical compounds made from the elements nitrogen (N_2) and oxygen (O_2). When plant and animal dies, the bacteria in it break down protein into ammonia (NH_3) which is then broken down by other bacteria to form nitrite (NO_2^-). The resulted nitrite is then consumed by a third type of bacteria to form nitrates (NO_3^-). Nitrate is used mainly in inorganic fertilizers. Nitrate can reach both surface water and groundwater as a consequence of agricultural activity (including excess application of inorganic nitrogenous fertilizers and manures), from wastewater treatment and from oxidation of nitrogenous waste products in human and animal excreta, including septic tanks. In the present study, the Nitrate content was absent, while nitrite was found to be present in sample 1 in the range of 1 mg/l. Tidy's test is the measure of O_2 in mg/l.

According to the BIS standard the Tidy's test as O_2 in mg/l is zero, but in the present study, the samples are found to have 0.24mg/l of Tidy's. Dissolved oxygen (DO) is the

fundamental fuel of life in water Rajamanickam and Nagan (2016). DO in water plays a vital role for all aquatic organisms and is considered to be the factor that reflects the biological activity taking place in a water body and determines the biological changes that are brought out by the aquatic organisms. The Biochemical Oxygen Demand (BOD) is directly linked with decomposition of dead organic matter present in the lake and hence the higher values of BOD can be directly correlated with pollution status and inverse relation with DO level. Chemical Oxygen Demand (COD) is used to indirectly measure the amount of organic pollutant found in the surface water. It indicates the mass of oxygen consumed to oxidize the organic compounds in the water to carbon dioxide, ammonia, and water. The values of COD and DO is found to be neutral, whereas the value of BOD found to be slightly high.

Conclusion:

Monitoring water quality is very important, for the determination of current status for hydrological condition of water and water pollution and longtime safety for contaminated water. The present study integrated hydrochemical characterization methods to examine the freshwater quality and to understand the pollution source of freshwater ecosystem of Krishna Lake, Pollachi, Coimbatore district, Tamil nadu by estimating the various parameters like pH, Temperature, Total Dissolved solids, Total Alkalinity, Total Hardness, Phosphate, Nitrate, Turbidity, Fluoride, Chloride, Dissolved Oxygen, Iron and Conductivity, etc. From the obtained results one can safely conclude that, different levels of anthropogenic inputs have caused wide variations in physico- chemical parameters. Human activities and other biotic factors were implicated as the reasons for the observed variations.

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STATUS AND MANAGEMENT OF NON-COMMUNICABLE DISEASES: A GLOBAL SCENARIO

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Introduction:

The prevalence of non-communicable diseases (NCDs) has become a major public health challenge in the 21st century. In 2018, the World Health Organisation (WHO) reported that NCDs have caused 41 million deaths each year, which is equivalent to 71% of all deaths globally. Cardiovascular diseases account for most NCD deaths, i.e. 17.9 million people annually. This is followed by cancers (9.0 million), respiratory diseases (3.9 million) and diabetes (1.6 million), respectively. It is estimated that each year, 15 million people die prematurely from an NCD between the ages of 30 and 69 years, of which over 85% occur in low- and middle-income countries. The epidemic of NCDs has devastating health consequences for individuals, families and communities, which also threatens to overwhelm health systems across the world. Moreover, high rates of NCDs in low and middle-income countries perpetuate poverty, strain economic development, and burden fragile health systems, making these countries less resilient when emergencies like infectious disease outbreaks or natural disasters occur. The socioeconomic costs associated with NCDs make the prevention and control of these diseases a major development imperative for the 21st century. NCDs also bring about social stigma that hampers a nations prosperity and happiness indices (Jastreboff *et al*., 2019)

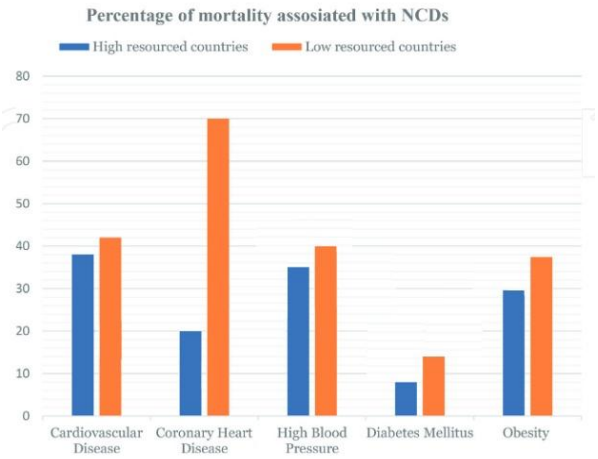

Figure 1: Percentage of mortality associated with ncd in high-resource and low resources countries (source: kassa and grace, 2019)

The outburst of NCDs is driven by factors such as rapid and unplanned urbanization, globalization of unhealthy lifestyles and population ageing. The modifiable risk factors that underlie most NCDs are behavioural issues like tobacco and alcohol abuse, physical inactivity and unhealthy diet .These behaviours lead to the four key metabolic changes, which includes raised blood pressure, overweight/obesity, hyperglycemia and hyperlipidemia. These metabolic changes further manifest as chronic NCDs like hypertension, diabetes mellitus, cardiovascular diseases like stroke and ischemic heart disease (Arroyo and Mincey, 2016).

In terms of attributable deaths, the leading NCD risk factor globally is raised blood pressure (to which 13% of global deaths are attributed), followed by tobacco use (9%), hyperglycemia (6%), physical inactivity (6%), and overweight and obesity (5%). Studies report that more than 80% of heart diseases, stroke, hypertension, type 2 diabetes and over a third of cancers can be prevented by eradicating the common risk factors, mainly tobacco use, unhealthy diets, physical inactivity, and the harmful use of alcohol (Berge *et al*., 2015).

Dietary risk factors of NCDs

The world has seen rapid transition in the food system over the past decade that has occurred with industrialization, urbanization, economic development and market globalization. This is having a significant impact on the health and nutritional status of populations, particularly in developing countries (Fall, 2018). Over these years, the standard of living has improved, food availability has expanded and become more diversified and access to services has increased. On the other hand, there have also been significant negative consequences in terms of inappropriate dietary patterns and corresponding increase in diet-related chronic diseases, particularly among poor people (Fruh *et al*., 2013). Changes in the world food economy are reflected in shifting dietary patterns, for example, increased consumption of energy-dense diets which are high in fat, particularly saturated fat and low in unrefined carbohydrates. There has been an increase in the availability of inexpensive, high calorie foods, often from staple cereal crops, which has reduced hunger for many. This has, however, often been at the expense of diversity and has displaced local, often healthier, diets. Access to diverse, micronutrient rich foods such as fresh fruits, vegetables, legumes, pulses and nuts has not improved equally for everyone and unhealthy foods with salt, sugars, saturated fats, and trans fats have become cheaper and more widely available (Barnes, 2015). Furthermore, global demand for meat, dairy products, sugar sweetened drinks and processed and ultra-processed foods have increased dramatically. These patterns are combined with a decline in energy expenditure that is associated with sedentary lifestyle. Because of these changes in dietary and lifestyle patterns, chronic NCDs including obesity,

diabetes mellitus, cardiovascular disease (CVD), hypertension and stroke and some types of cancer are becoming increasingly significant causes of disability and premature death in both developing and newly developed countries, placing additional burdens on already overtaxed national health budgets.

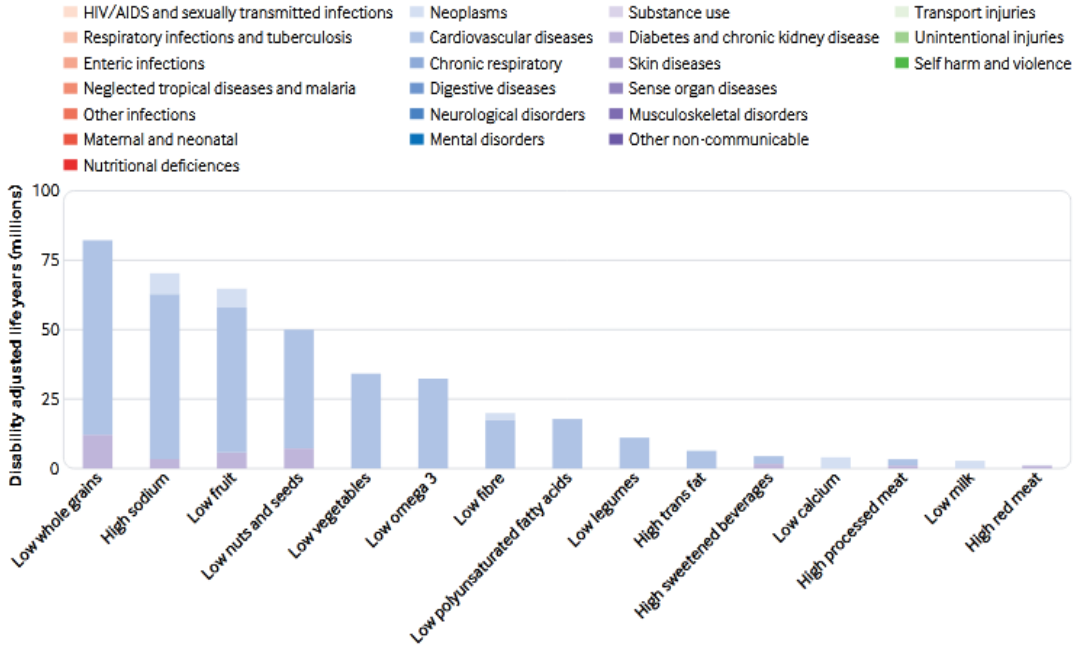

Figure 2: Specific dietary risk factor contribution to disease burden. Total dalys lost by dietary risk factor, global, all ages, both sexes, 2017 (source: branca *et al*., 2019)

Good nutrition, particularly during the fetal development and early childhood period is critical. Two thirds of the world's children donot consume the recommended minimum number of food groups and only one in six children adapts to a minimum acceptable diet. The prevalence of adult obesity has nearly tripled since 1975 (Martin *et al*., 2015). According to the WHO, in 2016, more than 1.9 billion adults were overweight or obese, while there has been a ten folds increase in overweight and obesity among children and adolescents over the same period. (Maternal and child malnutrition and poor dietary pattern are the two major risk factors for disease burden, as measured by disability adjusted life years (DALYs), according to the 2017 Global Burden of Disease data (The Lancet, 2019). Poor diet, as defined by a cluster of dietary risks, is the leading cause of death and is the first or second biggest contributor to NCD disease burden in all six World Health Organization regions. Of these dietary risks, the biggest contributors to the global burden of disease in 2017 were diets that are low in whole grains, high in sodium, or low in fruits, nuts and seeds, or vegetables (Barnes, 2015). Additionally, there is an effect of higher body mass index (BMI) on increased disease outcomes. Many factors influence

consumers' dietary behaviours. This includes personal factors such as culture, knowledge, skills, dietary preferences and time for food preparation. Economic and political factors such as the cost or availability of food also influence dietary choices to a great extent. Information about food, whether through education or marketing, also influences choices (Hamdy, 2016).

Nutrition is coming to the fore as a major modifiable determinant of NCDs, with scientific evidence increasingly supporting the view that alterations in diet have strong effects, both positive and negative, on health throughout life. Dietary adjustments do not only influence the present health status, but also determine the sustainability of health of individuals in the upcoming generations (Chakma, 2014).

Global trends in the strategic approaches for prevention and management of NCD's

The epidemic of NCDs was first identified in the World Health Assembly in the year 1998, which was responsible for 60% of global deaths and 43% of the global burden of diseases. The 'Global Strategy for the Prevention and Control of NCDs (2000)', was established focussing on the four most prominent NCDs which were currently responsible for more than 80% of all premature NCD deaths occurring between the ages of 30-69. These were cardiovascular disease, cancer, chronic pulmonary disease and diabetes. Based on survey evidences, the strategy identified the common preventable risk factors related to lifestyle, namely, tobacco use, unhealthy diet, physical inactivity and the harmful use of alcohol. Action to prevent the vast majority of premature deaths and disease burden was planned on controlling the risk factors in an integrated manner (WHO,200).

The 'WHO framework convention on tobacco control (FCTC)' was the first international treaty adopted under the WHO in 2003 in response to the globalization of the tobacco epidemic. The treaty imposed measures to reduce the demand of tobacco, which included protection from exposure to tobacco smoke, regulation of the contents of tobacco products, regulation of tobacco product disclosures, packaging and labelling of tobacco products and education, communication, training and public awareness (WHO, 2003).

In 2004, the 'Global Strategy on Diet, Physical Activity and Health (DPAS)' was endorsed by the WHO. The strategy identified the five risk factors for NCDs, namely, high blood pressure, high concentrations of cholesterol in the blood, inadequate intake of fruit and vegetables, overweight or obesity, physical inactivity and tobacco use, which were closely associated with diet and physical activity. The strategy was planned to eradicate problems like elevated consumption of energy-dense, nutrient-poor foods that are high in fat, sugar and salt; reduced levels of physical activity at home, at school, at work and for recreation and transport;

and use of tobacco, which were identified as the leading cause of NCDs in all populations across the world (WHO, 2011).

The' Global Strategy Action Plan 2008-2013' was introduced to establish the 'Global strategy for prevention and control on NCDs (2000)' into action (WHO, 2015). Action plans under this strategy were planned to reduce the main shared modifiable risk factors: tobacco use, unhealthy diets, physical inactivity and harmful use of alcohol. To manage the rising epidemic of tobacco use, both price and non-price implementations were introduced such as monitoring tobacco use and protecting people from tobacco smoke in public and workplaces, warning people about the dangers of tobacco, enforcing bans on tobacco advertising, promotion and sponsorship and raising tobacco taxes and prices. The action plan further enforced strong emphasis on promoting healthy diet. It included promoting and supporting exclusive breastfeeding for the first six months of lie and promoting programmes to ensure optimal feeding for all infants and young children. National policies were developed on food and nutrition, with an emphasis on national nutrition priorities including the control of diet-related non communicable diseases. Food-based dietary guidelines were established to promote the reduction of salt levels, eliminate industrially produced trans-fatty acids, decrease saturated fats and limit free sugars (WHO, 2018). The strategy also focussed on providing accurate and balanced information for consumers in order to enable them to make well-informed, healthy choices. It involved preparing a framework and/or mechanisms for promoting the responsible marketing of foods and non-alcoholic beverages to children, in order to reduce the impact of foods high in saturated fats, trans-fatty acids, free sugars, or salt. The strategy included provisions for management of alcohol use by focussing its harmful effects on health in particular on cancers, liver and cardiovascular diseases, and injuries. Promotion on physical activity through national and school-based programmes was introduced (Wang and Wang, 2020).

To update the 2008-2013 Action Plan, the World Health Organisation adopted the 'Global Action Plan 2013-2020' in 2013, with nine voluntary targets for achievement in 2025 (WHO, 2020)

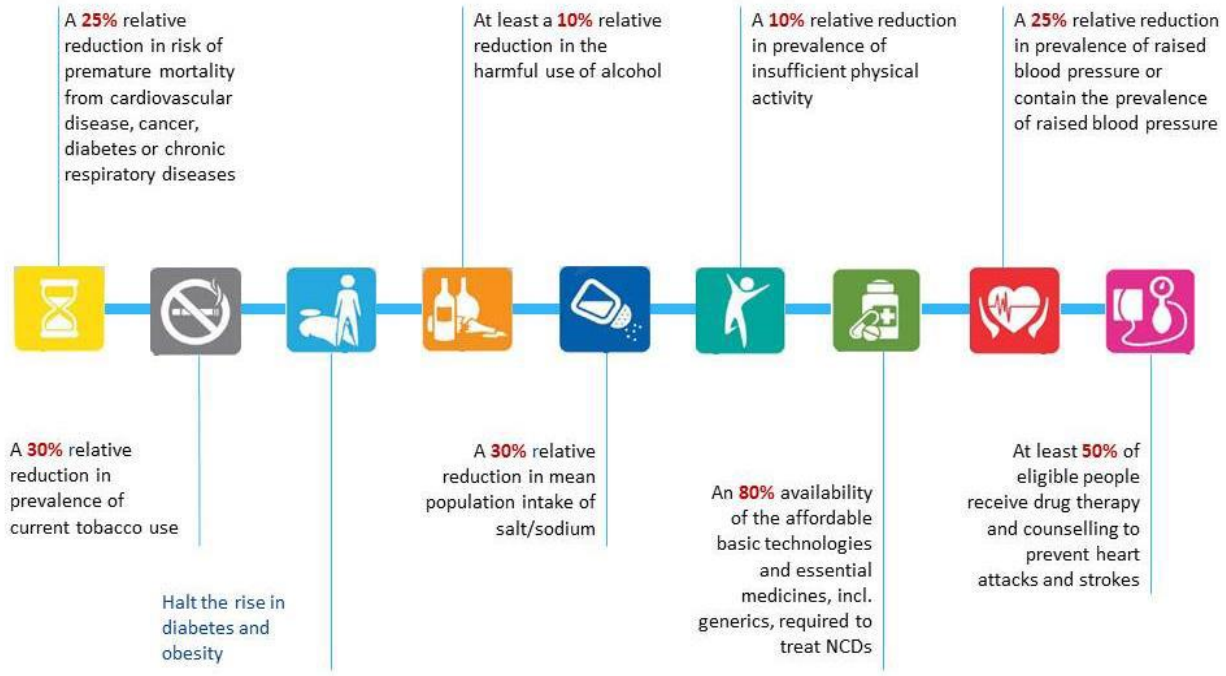

Figure 3: Nine voluntary targets under who global action plan 2013-20 (Source: who, 2017)

The action plan focused on several implementations to control the modifiable risk factors of NCD. Focus was given on improving dietary practices, which was considered to be one of the biggest factors behind several non-communicable diseases across the globe. The action plan emphasised on better nutrition practices, by focusing on glycemic load and its role in diabetes mellitus, cardio-vascular diseases and some forms of cancer. Health and nutrition authorities were channelized to promote on balanced diet, reduced refined food intake, reduced salt intake and increase consumption of a variety of fruits and vegetables. Along with dietary interventions, provisions on restriction on tobacco and alcohol abuse and promotion on physical activity were also included (The Lancet, 2019).

Global dietary interventions to prevent NCDs

The World Health Organisation has recommended certain measures to discourage promotion of unhealthy foods, such as restricting marketing of unhealthy foods to children, taxing sugar and sweetened drinks or banning industrial trans fats and so on (WHO, 2017). To accelerate progress, the United Nations Decade of Action on Nutrition (2016-2025), along with the 2030 Sustainable Development Agenda and Goals and the Paris Agreement, has presented an unprecedented opportunity to transform food systems, eliminate malnutrition in all its forms, prevent diet related NCDs and realise the human rights to food and health. Structural actions to

improve the food environment included implementing a ban on industrially produced trans fats, effective restrictions on marketing of unhealthy foods and beverages to children, ending inappropriate promotion (to parents) of foods for infants and young children, taxing sugar sweetened beverages or unhealthy foods, mandating simplified, interpretive front-of-pack nutrition labelling and introducing nutrition standards for food served or sold in schools, hospitals, and other public institutions (WHO,2020).

Other strategic approaches include adopted worldwide include:

1. Protection and promotion of optimal breastfeeding and complementary feeding. This protects against undernutrition and can reduce the risk of developing overweight and diet related NCDs later in life, while breastfeeding can also protect mothers against some forms of cancer and other NCDs. Measures to promote breastfeeding, including banning advertising of breast milk substitutes and extending paid maternity leave, increased exclusive breastfeeding in Vietnam, for example, from 20% to 62% in five years.
2. Enabling better access to safe drinking water protects against infectious diseases which exacerbate undernutrition while helping prevent obesity by providing an alternative to sugar sweetened beverages.
3. School nutrition programmes can ensure children access diverse diets needed for healthy growth and development, while limiting their exposure to unhealthy foods, and can even work towards broader development goals (Bruins *et al*., 2019).

Conclusion:

The adoption of diet modification towards a healthier, more environmentally sustainable and more equitable at the national or global scale requires a long-term strategic approach. To integrate these three pillars of health, environmental sustainability and equity into diets, interventions must be made across the different entry points of the food system. Interventions targeting food environments, whose composition and influence on choice are associated with poor diets and obesity, have to be included along with agricultural and food supply approaches. Strategies will have to involve fiscal measures, regulatory and trade interventions, industry approaches, context-specific interventions, challenges to defaults and norms of information, and consumer-focused education. These approaches must be sensitive to cultural, social, and economic context, and balance the trilateral goals of health, sustainability and equity. This multi-pronged approach should be one that involves the whole system, including governments, industry and consumers.

The burden of change in the dietary pattern should not be solely placed on the consumer's ability to make healthy choices. Public understanding of nutrition and health of diets, as well as their environmental and justice impacts, would be more effective towards the attainment of the goal. Governments and other food system actors generally favour healthy interventions focused on individual-level efforts. Paradoxically, these interventions can worsen dietary inequities and health consequences. Food choices are driven by systemic and social factors. Therefore, the strategic approaches of dietary intervention must be community oriented.

Lastly, gaps in data need to be filled by improving the quality of dietary data, which will allow for a better understanding of their impacts on a wide-range of outcomes. Individual-level dietary data collection and analysis need to be better standardized, as does the methodology of how these data are collected across survey tools and design.

Dietary data need to be collected across a broad range of countries, with more disaggregation of socioeconomic status that takes equity issues into account. Most of the dietary data comes from high-income country data, particularly in the USA. Focusing on low- and middle-income countries is critical, as these countries are more likely to experience the greatest increase in NCDs, yet there is little research on appropriate interventions or delivery channels.

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THE FISH PATHOGENS: A SPECIAL EMPHASIS ON BACTERIA

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Abstract:

Human population is increasing and if reports are to be believed world's food and feed supply has to grow by 70 % to substantiate the growing population by the end of 2050. Aquaculture sector is the mostrapidly expanding amongst animal food sector. However fish, shellfish and different fish products are frequently threatened by different microbial pathogens and bacteria are the predominant in causing infections. *Aeromonas hydrophila, A. salmonicida, Vibrio sp., Edwardsiella ictaluri, E. tarda, Streptococcus* sp., fewgram-positive *cocci* are to name some of these pre-dominant. Hence, detection of the microbial pathogen is crucial. This ensures the safety of fish, shellfish and different fish products; besides highlighting the presence and prevalence of the pathogen. In a way these assessments provides information about the aquatic habitat where the organisms are residing. There are some which are the natural microflora and there are few which arises as a result of contamination (sewage or introduced by wild animals, livestock, and different feeds). Basically the surface water of aquatic ecosystems is inhabited by large diversity of microbial species. In both the cases the effects on fish is obvious. Besides these, pathogenic profile from the aquatic environment serves as an important ecological indicator. Thus there assessment is important not only for the better health of fish stocks but for providing valuable insight about food chain and importantlyto safeguards human concern.

Introduction:

The fish species are important and inhabits different kinds of habitats – river, lakes, ponds, sea, ocean and others. They also occur in different sizes ranging from tiny cyprinds not even an inch long to whale sharks (*Rhincodon typus*) that may reach approximately 60 feet length or even more. Also fishes are adapted to wide environmental conditions (Dala Corte *et al.*, 2018). In total, there are approximately around 34,000 different fishes worldwide. Accordingly some are tolerated to high salinity, some to low and few could tolerate both. Basically the body characteristics are common to the particular environment they reside. Fishes of clear and sunny

coral reefs are brightly coloured whereas those residing in murky water tend to be mud-coloured. Fishes residing in open blue ocean are silver on their undersides whereas dark on back (Bianco and Nordlie, 2008). Availability of sunlight, nutrient, dissolved oxygen level, pressure and temperature all matters in shaping the distribution of fishes and their adaptibility. Generally, tropical fish are more colourful than fish residing in cold water. Pelagic fishes like Herrings, Tuna etcare those which resideand feed away from the bottom of the sea. They also stay away from shore. As they are adapted for swimming in open water, they have evolved a more elongated and slender body with a narrow caudal peduncle. Demersal fishes or groundfish could be benthic that rests on the sea floor or benthopelagic fish that floats in the water column just above the sea floor. They have wider mouths (at times pointing downwards) and deeper bodies structures like flattened heads, enlarged pectoral fins, modifications of pelvic fins into structures like anti-skid devices. Pelagic fishes are highly nutritious as they contain up to 30% fish oil whereas demersal fish fillets contain little fish oil (Rincon-Sandoval *et al*., 2020)

Microbial population is integral to aquatic ecosystem owing to their involvement in foodweb transfers, nutrient uptake and regeneration, biogeochemical transformations, organic matter production and decomposition and many other roles. They are largely implicated as decomposers, nutrient regenerators and particle producers thus posing as potential food sources for higher consumers. They include the integral water species (*Enterobacteriaceae*, *Flavobacterium*, *Acinetobacter*, *Moraxella*, *Aeromonas*, *Micrococcus*, *Staphylococcus*, *Streptococcus* and *Clostridium*) (Toranzo *et al*., 1989; Sousa, 1996), residents of soil sediment (*Bacillus*, *Arthrobacter*, *Pseudomonas*, *Agrobacterium*, *Alcaligenes*, *Clostridium*, *Flavobacterium*, *Corynebacterium*, *Micrococcus*, *Xanthomonas*, and *Mycobacterium*)and sewage-derived originating from the industrial effluents, domestic and irrigational sources or the resultant feeds of animalslike *Escherichia coli*, *Campylobacter* sp., *Hepatitis* A, *Giardia* sp., *Salmonella*sp., *Shigella*sp., *Legionella* sp., *Cryptosporidium* sp., *Vibrio*sp.,*Acinetobacter* sp., *Plesiomonas shigelloides*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia*, *Lactococcus garviae*, *Streptococcus iniae*, *Kocuria rhizophila* etc. The fish habitat plays a significant role in determining the gut microbiota. Freshwater fishes pre-dominantly represented family Enterobacteriaceae, Vibrionaceae, Moraxellaceae, Gammaproteobacteria, Alcaligenaceae, Clostridiaceae whereas family Aeromonadaceae, Leuconostocaceae showed prevalence in seawater fishes. Be it lentic or lotic ecosystem, the presence and prevalence of bacteria is indispensable. However, bacterial presence in different organs like kidney, spleen, liver, brain etc is concerning as it is indicative of infection (Joshi *et al*., 2016).

In natural conditions, these different infectious agents co-exist with their host. However factors like over-crowding often worsen the situation and trigger the dissemination of infection and subsequently disease outbreaks. In this review, an attempt of highlighting the major bacterial pathogen, basic microbial techniques employed to identify them and probable precautionarymeasures being practiced has been tried.

Common bacterial pathogen in aquatic ecosystem

- ***Aeromonas* infections:** *Aeromonas* spp. are commonly found in water and fishes are continuously exposed to them. During stress like over-crowding or during fish handling and transportation they get more proned to infection. *Aeromonas* infection is characterized by typical skin ulceration with characteristics lesions in skin and gill region. This motile Gram negative bacteria often triggers systemic infection and causes MAS (Motile *Aeromonas septicimea*). Few characteristics species are *Aeromonas hydrophila, A. Sobria, A. caviae, A. salmonicida.* They are the opportunistic pathogen and often in association with other bacteria like *Pseudomonas spp., Shewanella putrefaciens, Acinetobacter spp. or Stenotrophomonas* causes serious threats to aquaculture.
- ***Pseudomonas* infections:** *Pseudomonas spp.* are ubiquitously found in the environment. They are psychrophilic in nature and hence develop well at low temperatures. Besidesthey are the dominant microflora. At higher temperatures of 10ºC, they are quickly replaced by competing, mesophilic microorganisms like *Aeromonas.* Some *Pseudomonas spp (P. putida or P. luteola)* have been reported from internal organs of fish. They are reported to cause strawberry disease in rainbow trout (*Oncorhynchus mykiss*) and tench (*Tinca tinca*). These are the red marked syndrome characterized by multifocal, rough reddened areas. Basically they are inflammatory skin lesions on the flanks of affected fish. Similar systemic infections with typical symptoms of septicaemia were also observed in crucian carp (*Carassius carassius*) and silver carp (*Carassius gibelio).*
- ***Flavobacterium* infections:** *Flavobacterium spp*. are thenatural inhabitants of aquatic environment. They also occur in the gill microflora of healthy fish. Flavobacteriosis is caused by three species: *F. columnare,* which causes columnaris disease, *F. branchiophilum* causing bacterial gill disease (BGD), and *F. psychrophilum,* predominantly associated with cold water disease (CWD) or rainbow trout fry syndrome

(RTFS). Cyprinids are caused by the first two species, while *F. psychrophilum* is mainly isolated from salmonids; though the latter also cause severe infections in cyprinids.

- ***Acinetobacter* infections:** These short plump rods are widely prevalent in nature including aquatic habitat. *Acinetobacter* in association with *Aeromonas* or *Chryseobacterium spp*. readily cause disease. They are very concerning to aquaculture for being carriers of antimicrobial resistant genes. Hence, they are largely implicated in the spread of drug resistance (Figure 1).
- ***Shewanella putrefaciens* infections:** The causative pathogen *Shewanella putrefaciens* is a halophilic bacterium and are mainly involved in food spoilage process. In both cultured and ornamental fishes, they have been reported to promote infection. Clinically, the infected fish shows lethargy, skin darkening, skin lesions and ulceration.
- **Infection caused by Gram positive bacteria**: *Lactococcus garviae* and *Streptococcus iniae* are found in water as well as soil sediments. The symptoms of their infection are overlapping. Exopthalmia is the typical characteristics along with skin darkening, anal oedema, petechiae in theeyes, on the gill covers, and at the base of the fins. Often haemorrhages in the swim bladder, liver, spleen, and kidney are seen along with stomach inflammation. Nervous whirling rapid movements from the bottom to the surface or in the opposite direction, resulting from meningitis or encephalitis are reported too (Austin, 2011) (Figure 2).

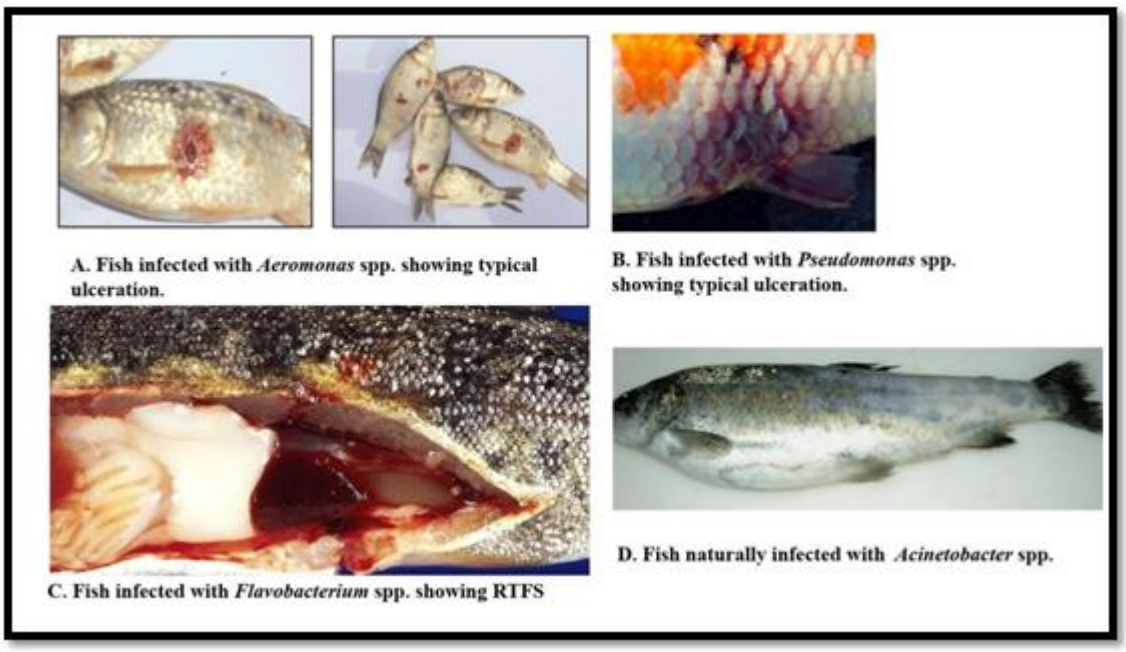

Figure 1: Characteristics of bacterial infection in fishes (Presentation 1)

Figure 2: Characteristics of bacterial infection in fishes (Presentation 2)

Bacterial evaluation from aquatic environment – The basic strategies employed

Water is a very adapted medium for propagation and dissemination of microbial pathogens. In the context of bacteria, it also holds true. The sequentialsteps involved are as follows:

Isolation of bacteria by membrane filtration technique is readily used.Here the sample water is passed through 0.45 µm pore-sized filter (like cellulose nitrate membranes, Whatman Laboratory Division, Maidstone, England) using a water pump (model Sartorius 16824).At appropriate dilution, they are spread on plates of selective medium like mFC agar for faecal coliforms, mEndo for total coliforms, nutrient agar for heterotrophic bacteria, and *Aeromonas* selective medium for *Aeromonas* and *Pseudomonas*. The entire process is followed as per manufacturer's instructions and usually in triplicates. At appropriate temperature like 37°C except for mFC agar which were incubated at 45°C for 24 hours. The colonies were enumerated, characterized, and recorded. The results were expressed as the number of faecal coliforms, total coliforms, *Pseudomonas* and *Aeromonas* in 100 mL of water, and heterotrophic bacteria in 1 mL of water. Blue colonies from mFC agar (presumptive coliforms), metallic-sheen colonies from mEndo agar (presumptive total coliforms), and yellow (presumptive *Aeromonas*) and green colonies (presumptive *Pseudomonas*) from *Aeromonas* selective media are indications of positive colonies.

Purification of colonies: For this basically streak plate technique is utilised. In this process, the sample/inoculum is diluted by streaking it across the plate. While streaking in successive areas of the plate, the inoculum is diluted to the point where after growth single colony would grow. This means there is only one bacterial cell deposited at the later stages of streaking. Pure cultures can be obtained by picking the well-isolated colonies and re-streaking on fresh sterile plates.

Antimicrobial Susceptibility Testing: This test was performed test using the Kirby-Bauer disk diffusion technique. The standard antimicrobial discs used are as follows: Ampicillin (AP) 10 µg, cephalothin (KF) 5 µg, streptomycin (S) 10 µg, erythromycin (E) 15 µg, chloramphenicol (C) 30 µg, neomycin (NE) 30 µg, amoxycillin (A) 10 µg, ciprofloxacin (CIP) 5 µg, trimethoprim TM 25 µg, kanamycin (K) 30 µg, and oxytetracycline (OT) 30 µg. The pure culture are spread on the plate and Antibiotic discs placed using sterile needles followed by incubation at appropriate temperature like 37°C for 24 hours. The antibiotic inhibition zone diameters (IZD) aremeasured and values obtained are used to classify isolates as being resistant (R), intermediate resistant (I), or susceptible (S) to a particular antibiotic using standard reference values according to National Committee for Clinical Laboratory Standard, now Clinical and Laboratory Standards Institute (CLSI).

Analytical Profile Index (API) 20 Test: These are done using API strips as per manufacturer's instructions (BioMérieux, 69280, Marcy I'Etoile, France) and the organisms are identified to species level using API software. The kits include strips that contain up to 20 miniature biochemical test chambers containing dehydrated media having chemically-defined compositions for each test. They usually detect enzymatic activity, mostly related to fermentation of carbohydrate or catabolism of proteins or amino acids by the inoculated organisms.A bacterial suspension is used to rehydrate each of the wells and the strips are incubated at appropriate temperature (37 °C) for 18 to 24 hours required for optimum growth of bacteria. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. All positive and negative test results are compiled to obtain a profile number, which is then compared with profile numbers in a commercial codebook (or online) to determine the identification of the bacterial species.

The tests done are as follows:

ONPG: test for β-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside

ADH: decarboxylation of the amino acid arginine by arginine dihydrolase

LDC: decarboxylation of the amino acid lysine by lysine decarboxylase

ODC: decarboxylation of the amino acid ornithine by ornithine decarboxylase

CIT: utilization of citrate as only carbon source

H_2S: production of hydrogen sulfide

URE: test for the enzyme urease

TDA (Tryptophan deaminase): detection of the enzyme tryptophan deaminase: Reagent- Ferric Chloride.

IND: Indole Test-production of indole from tryptophan by the enzyme tryptophanase . Reagent- Indole is detected by addition of Kovac's reagent.

VP: the Voges-Proskauer test for the detection of acetoin (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway

GEL: test for the production of the enzyme gelatinase which liquefies gelatin

GLU: fermentation of glucose (hexose sugar)

MAN: fermentation of mannose (hexose sugar)

INO: fermentation of inositol (cyclic polyalcohol)

SOR: fermentation of sorbitol (alcohol sugar)

RHA: fermentation of rhamnose (methyl pentose sugar)

SAC: fermentation of sucrose (disaccharide)

MEL: fermentation of melibiose (disaccharide)

AMY: fermentation of amygdalin (glycoside)

ARA: fermentation of arabinose (pentose sugar)

Extraction of Genomic DNA and PCR for the identification of culture species: DNA from the bacterial isolates are extracted using commercially available bacterial DNA extraction kit like peqGOLD (PEQLAB Biotechnologie GmbH 12-3450) according to manufacturer's instructions. As a following step the concentration of the isolated sample is determined spectrophotometrically using NanoDrop ND 1000, Thermo Scientific followed by checking the integrity of the purified template DNA onstandard 1% (w/v) agarose gel.

The identification of the isolatesare done on the basis of screening against specific amplicons like *gyrB* and *ecfX*in case of *Pseudomonas, aerA and hylH* for *Aeromonas*.After amplification through standard PCR cycling conditions, the PCR products are subjected to electrophoresis. The visualization under UV light confirms for the isolate as evident from the size of band appearing on the stained gel. The confirmation is ultimately done by data analysis and comparison with the existing database.

16S ribosomal RNA PCR and sequencing or rDNA sequence analysis has become a major tool in the determination of relationships between bacteria, and readily used for

identification purposes. The 16S rRNA gene codes for the RNA component of the 30S subunit of the bacterial ribosome. It is ubiquitously presentin all bacterial species. The bacterial species harbour one to several copies of the 16S rRNA gene. 16S rRNA gene sequencing is by far one of the most common methods targeting housekeeping genes to study bacterial phylogeny and genus/species classification. DNA-DNA hybridization is astandard technique for identifying bacterial species. However, DNA-DNA hybridization is little complicated, hence 16S rRNA gene sequencing is used as a tool to identify bacteria at the species level and assist with differentiating between closely related bacterial species (Mulamattathil *et al*., 2014).

Bacterial evaluation from fishes – The routine protocol conducted

The identification of pathogens is important for effective disease control in aquaculture sector. Clinical and sub-clinical diagnosis of infected fish and the environment at different stages like harvesting, re-stocking are important and work as an "early warning". A variety of methods for pathogen detection and identification are done from blood and tissue samples like IFAT (Indirect Fluorescent Antibody Technique), IHC (ImmunoHistochemistry), ELISA (detection of pathogen/antibodies), PCR (one cycle/nested/reverse cross blot hybridisation/RT-PCR/quantitative PCR) and in situ hybridisation. The tests are conducted to screen against *Aeromonas salmonicida, Aeromonas hydrophila, Photobacterium spp., Renibacterium salmoninarum, Mycobacterium spp., Vibrio anguillarum, Flavobacterium psychrophilum* etc (Gono *et al.*, 2013).

Conclusion:

An evaluation of bacterial profile is crucial to aquaculture. The fish and fish products are routinely consumed by people across the globe. The fishes are continuously subjected to infectious pathogen specially bacteria. In this aspect, the probable presence of bacterial population in their habitat and at different stages of their growth critically determines the health of animals including human being who are consuming them. Effective health management is typically required in various fish farms. Some problems arise from accumulation of toxins and nutritional imbalance, besides pathogen attack. Usually fish health management emphasizes on minimizing stress in cultivated fishes, confinement of disease outbreak to affected pond and reducing losses from disease outbreak. General preventive measures includes selection of healthy fish seeds, proper density and rational culture,fish farm management, qualitatively uniform ratio and quality food, good water quality and prevention of fish body from injury. Water quality monitoring is primary criteria for fish farm management as the medium is responsible for different vital functions of fish *viz.* movement, feeding, digestion, assimilation, growth,

responses to stimuli, reproduction etc. The microbial techniques mentioned earlier in the article are the standard and routine protocol for routine examination and regular upkeep.

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MICROBIAL QUALITY CONTROL OF RAW MILK AND ISOLATION OF SPOILAGE CAUSING MICROORGANISMS

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Abstract:

Microbial quality of raw milk is crucial for the production of quality dairy products. Since milk is an excellent medium, microbes can easily grow in it. Microbial spoilage of milk often involves the degradation of protein, carbohydrates, and fats by the enzymatic activity of microorganisms. This leads to the deterioration of texture, color, odor or flavor of the milk, rendering it unpalatable or incompatible for human consumption. The purpose of this study was to show the effects of various storage temperatures on the pH, and titratable acidity and microbial load of raw milk. In two identical experiments, raw cow and buffalo milk samples were stored at 10°C, 25°C, 37°C and 55°C. All samples were tested at every 24hr intervals for a period of 7 days. Both cow and buffalo milk samples stored at 25°C and 37°C represented spoilage indicators like increase in acidity and microbial load much more quickly than those stored at 10°C and 55°C. The results also demonstrate that raw milk samples with or without refrigeration can be tested up to 24 hours without showing an appreciable shift in response due to bacterial spoilage.

Keywords: Titratable acidity, Microbial spoilage, Raw milk, Standard plate count.

Introduction:

India is the largest producer of milk in the world (Nangia, 2021). Milk & its products have been an integral part of Indian cuisine since the Vedic period. Raw milk obtained from cattle is a nutritious medium for the growth of various microbial communities (Garnier, 2017), hence, microbial spoilage has great concerns regarding the safety and quality of raw milk. Milk can be exposed to microbial contamination by unhygienic milking practices, and also from other sources like animal feces, contaminated water, animal hides, during transport etc. Contaminated raw milk can harbor pathogens, such as *Brucella*, *Campylobacter*, *E. coli*, *Listeria*, and

*Salmonell*a (Valente, 2019), which can pose serious health issues. Hence it becomes mandatory to conduct microbial quality control of raw milk before it can be used for consumption or further processing.

In India, raw milk is usually pooled from rural household producers at a dairy plant before further processing. The Indian government has devised various regulations and platform tests to assess the quality of the incoming milk before it is accepted (*National Dairy Plan Phase I*, n.d.). Milk is usually sterile during secretion from the udder, but it can be contaminated by unhygienic milking, handling, storage, and other pre-processing activities. The microorganisms that are principally involved in spoilage are psychrotrophic organisms which are usually destroyed by pasteurization temperatures, however, some like *Pseudomonas fluorescens, Pseudomonas fragi* can produce heat stable proteolytic and lipolytic extracellular enzymes which are capable of causing spoilage. Some species and strains of *Bacillus, Clostridium, Corynebacterium, Arthrobacter, Lactobacillus, Microbacterium, Micrococcus*, and *Streptococcus* can survive pasteurization and can grow at refrigeration temperatures (Yuan *et al.*, 2019).

In the present study, raw cow & buffalo milk samples were obtained from the dairy farms and platform tests were performed to ensure the quality. The samples were then incubated at different conditions to study their spoilage mechanism and isolation of spoilage causing microorganisms was carried out using nutrient medium.

Materials and Methods:

1. Collection of milk samples

Cow milk and buffalo milk samples for the study were obtained from Gadre Dairy Farm, Kalyan Taluka, Mumbai. Samples were collected from the dairy farm immediately after milking and transported to the lab in sterile containers. The milk samples were stored in previously autoclaved glass bottles and refrigerated until use.

2. Platform test:

The milk samples, once received at the lab, were subjected to the following platform test.

a. Organoleptic Evaluation:

Samples were checked for any off-smell. The appearance of milk was observed for any visible dirt or foreign matter. The color of milk was recorded and also checked for the presence of abnormal colors. The consistency of milk as normal, watery, thick, ropy, and

slimy was determined. The temperature of milk at the time of receiving was determined using a digital thermometer.

b. Clot on Boiling (C.O.B) Test

5ml milk sample was taken in a test tube and boiled for 10 min. If there is clotting, coagulation or precipitation, the milk has failed the test. Since it contains acid (pH<5.8) or rennet producing microorganisms or the milk has an abnormally high percentage of proteins like colostral milk. Such milk cannot stand the heat treatment in milk processing and must therefore be rejected.

c. The Alcohol Test

This is based on instability of the proteins when the acidic milk is acted upon by alcohol. The test is done by mixing 2 ml milk with 2 ml of 70% alcohol in a test tube. If the tested milk is of good quality, there will be no coagulation, clotting or precipitation.

d. Titratable acidity

Fresh milk has natural acidity of 0.16 - 0.18%. Bacteria that normally develop in raw milk produce lactic acid. In this test 9 ml of the milk was taken into the conical flask, 1 ml Phenolphthalein is added and titrated with 0.1N sodium hydroxide until a faint pink color appears and the amount of alkali required is measured. From this, the percentage of lactic acid can be calculated as per BIS Standard.

e. Methylene Blue Dye Reduction Test

This test is based on the principle that methylene blue (oxidation- reduction dye), which is blue in its oxidized state, is reduced to a colorless compound as a result of the metabolic activities of bacteria in milk. The time taken for the reduction of the dye (methylene blue reduction time) is influenced by the number and types of bacteria present in milk. The greater the numbers of organisms present in milk and greater their activity, the more rapidly the dye is reduced.

3. Microbiological Quality Control

a. Standard Plate count

The initial count of viable microbes present in the milk samples was determined by spread plate technique, where 0.1ml of the 10 fold dilutions of milk was pour plated on sterile Glucose Yeast Extract Agar and incubated at room temperature for 24 hr and then colony count (cfu/ml) was calculated (Munsch-Alatossava, 2007).

b. Laboratory Pasteurization count

This test is performed to determine the load of heat resistant thermophilic bacteria which can survive pasteurization and cause spoilage later. 5ml milk is heated at 63°C for 30 min and then counts are performed similar to SPC.

c. Psychrophilic plate count

The psychrophile count is used to detect microbes which can grow in cold storage during preservation and cause spoilage. Counts are performed similar to SPC and plates were incubated at 5ºC for a week.

d. Thermophilic plate count

The thermophile count detects the heat-loving spore-forming bacteria present in the milk sample. Counts are performed similar to LPC and plates were incubated at 55ºC for 48 hr.

e. Coliform count

This count indicates fecal contamination and the presence of pathogens in the given sample. Counts are performed using Violet Red Bile Agar plate, incubated at 37ºC for 24 hr.

4. Spoilage Analysis

After performing the platform tests, the milk samples were subjected to spoilage study by incubation at various temperatures. The milk samples were properly labeled and three sets of each of the milk samples were stored in Sterile Glass bottles with cotton plugs under aseptic conditions and incubated at 10° C, 27° C, 37° C, and 55° C. After every 24 hr the milk samples were subjected to organoleptic evaluation, titratable acidity, microbial viable count (SPC) and isolation of spoilage causing microbes. The isolates were maintained on sterile GYE slants.

5. Extracellular enzymatic activity of the spoilage causing bacteria

Microbes causing milk spoilage produce extracellular protease and lipase enzymes. All isolated colonies obtained on the above SPC plates were plated on agar plate supplemented with 1% casein and 1% with tributyrin (Himedia) for detecting their proteolytic and lipolytic potential, respectively.

Results and Discussion:

1. Quality control of Milk

Table 1.1 represents the results of the platform tests and microbial quality control of the cow and buffalo milk sample. All values are represented by the mean value calculated of all the samples. As per the BIS standard, all the milk samples were found to be of good quality and can be used for human consumption after pasteurization and can also be used for further processing.

Table 1: Results of the platform tests and microbial count of the raw mil sample

Sr. No.	Name of the test	Cow milk sample	Buffalo milk sample	BIS Standard*
1.	Organoleptic Test:			
	i. Color	White	White	Absence of abnormal reddish, bloody, bluish colors
	ii. Odor	No abnormal odor	No abnormal odor	Absence of off-odors like feed, fishy, barny
	iii. pH	6.7	6.8	6.6 - 6.8
	iv. Temperature	27 ° C	26.5°C	Room Temperature
2.	Clot on boiling. Test	no coagulation	no coagulation	no coagulation on boiling
3.	The Alcohol Test	no coagulation	no coagulation	no coagulation
4.	Titratable acidity	0.11% lactic acid	0.12% lactic acid	0·10 to 0·17 % lactic acid
5.	MBRT	Good quality	Good quality	No decolorization in 30 min.
6.	Standard Plate Count	1.2x10^3 cfu/ml	2.7x10^3 cfu/ml	Not exceeding 2,00,000 - Very good quality
7.	Lab Pasteurized Count	Nil	2 cfu/ml	Presence of appreciable numbers of psychrophilic bacteria = Poor Quality
8.	Thermophilic count	12 cfu/ml	8 cfu/ml	
9.	Psychrophile count	Nil	5 cfu/ml	
10.	Coliform Count	Nil	Nil	Absent in 1:100 dilution of milk

*IS:1479 (Part I) – 1961 (Reaffirmed 1997) & IS:1479 (Part III) - 1977 (Reaffirmed 2003)

2. Spoilage Study

The milk samples which passed the platform test were stored in sterile glass bottles (Fig. 1) and incubated at 10° C, 27° C, 37° C, and 55° C till further assay.

2.2 Titratable Acidity

All values are represented as the mean acidity (% lactic acid) of 3 experimental sets. The Figure 2.1 and 2.2 represents that the titratable acidity increases with incubation period. Incubation at 27°C and 37°C leads to maximum increase in the acidity since microbial growth is more favorable at these incubation temperatures.

Figure 1: Milk samples stored in sterile glass bottles under aseptic conditions

2.3 Standard Plate Count

SPC counts were performed every 24 hr for a period of 7 days and the log of cfu/ml calculated in represented graphically in the Figure 3.1 and 3.2. Microbial load was found to be increasing exponentially with time. Maximum increase was obtained in the samples incubated at 27°C and 37°C (Marouf, 2018).

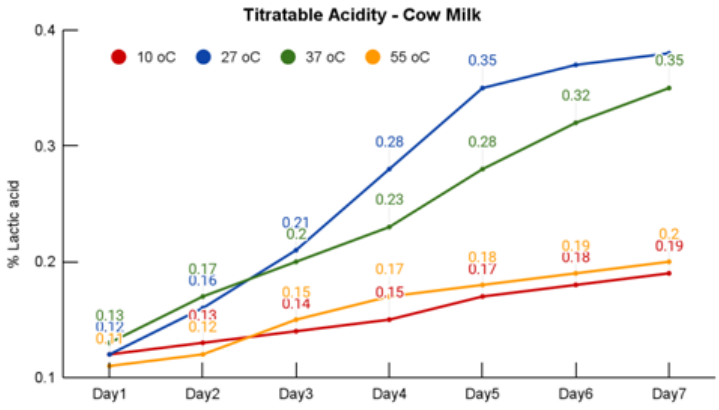

Figure 2.1: Titratable acidity of Cow milk sample

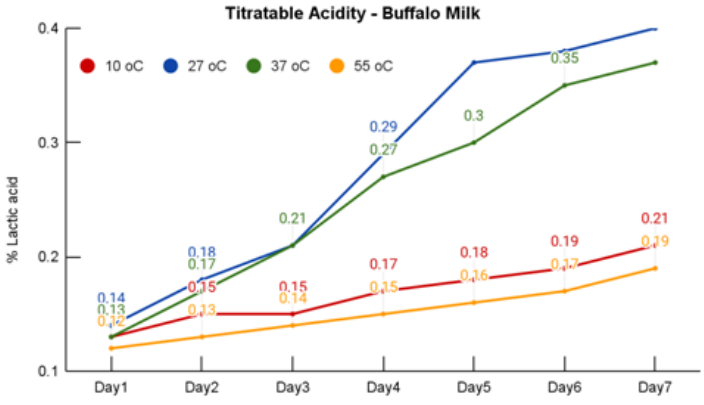

Figure 2.2: Titratable acidity of Buffalo milk sample

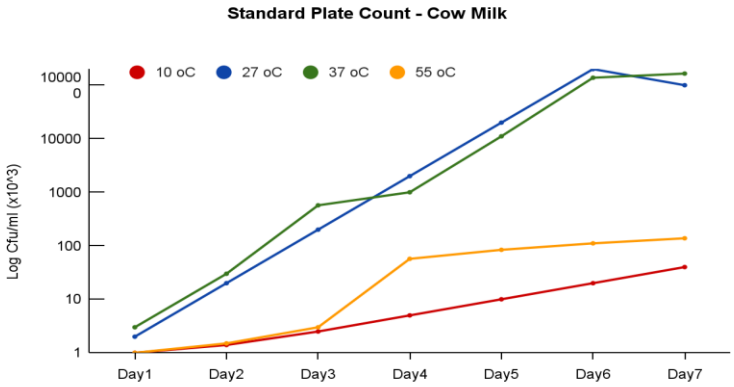

Figure 3.1: SPC of Cow milk sample

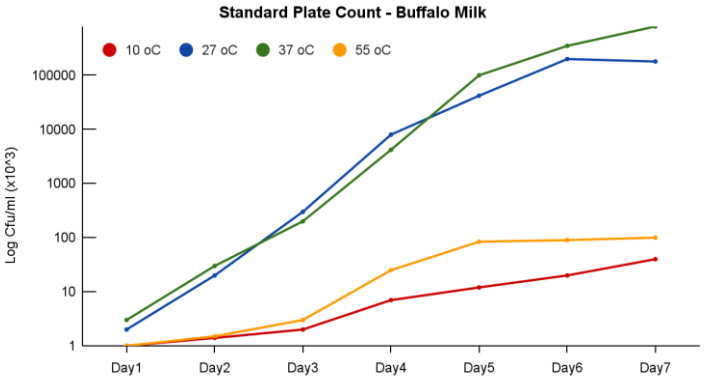

Figure 3.2: SPC of Buffalo milk sample

2.4 Isolation of spoilage bacteria

A total of 40 different isolates were obtained on the SPC plates (Figure 4.1). Isolated colonies obtained were maintained on nutrient agar slants for further tests. The Gram nature of the isolates was determined by Gram staining. Out of 40 isolates, 25 were Gram positive bacillus, 10 Gram negative bacillus and 5 isolates were of Yeast sp (Figure 4.2).

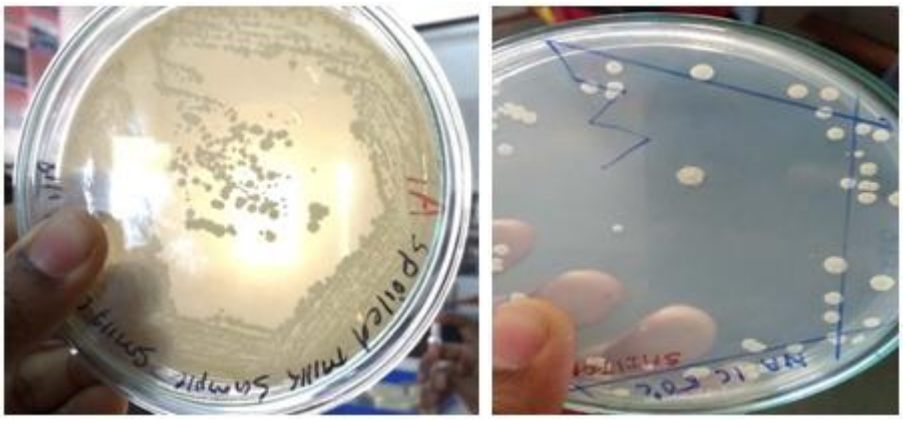

Figure 4.1: Isolation of milk spoilage bacteria

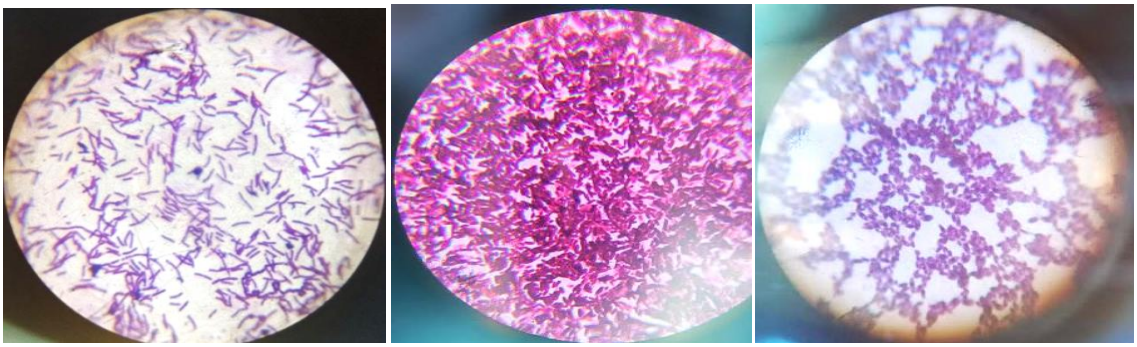

Figure 4.2: Gram staining of milk spoilage bacteria

2.5 Enzymatic Activity of the isolate

Milk samples are spoiled by the action of protease and lipase enzymes produced by the microbes. The activity of the protease enzyme was confirmed by the presence of a clear zone around the colony on the casein agar plate (Figure 5.1). Presence of clear zone around the colony in tributyrin agar indicates lipase activity (Figure 5.2). Out of 40 isolates tested, 32 isolates give positive results for qualitative protease and lipase activity.

Figure 5.1: Protease activity of the isolate

Figure 5.2: Lipase activity of the isolate

Conclusion:

As per the results obtained in the spoilage analysis, the microbial load in the milk samples increases, the pH decreases, and acidity increases with incubation time. Raw milk incubated at 27°C and 37°C exhibits spoilage characteristics much quickly as compared to 10°C and 55°C. The isolated spoilage bacteria will be employed for further identification and characterization studies.

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ADVANCEMENTS IN MICROBIOLOGY TO ESTABLISH SUSTAINABLE AQUACULTURE

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Abstract:

Implementation of sustainability in aquaculture became a prerequisite in this century to meet the demands of the rising population. The aquaculture industry became the popular protein-rich food source in the world as the health benefits and nutrition it guarantees. Thirty percent of the total population is believed to be facing the threat of malnutrition, enhancing the availability of fish food resources can solve this problem in most of the poorer countries. The pressure of contributing to increasing production worldwide has in turn brought the risks of overexploitation and manipulation of aquatic edible organisms. The overcrowded aquaculture cultivation environment and techniques invited hazardous microorganisms which initiated massive mortality and less production. Another area that needed attention was the incorporation of alternative feed ingredients into the fish feed industry. The review covers the recent advances in the alternative feed ingredients which established sustainability. The recent developments in understanding the protein and nutrient requirements have helped to employ formulating better feed with plant by-products, animal by-products and microorganisms especially probiotics. The new techniques of integrating probiotics along with the feed culture and developing fermentation strategy to increase the size, growth, digestibility, antioxidant and immunostimulant properties. The review focuses on the recent developments in alternative feed ingredients and their efficiency in promoting global aquaculture production.

Introduction:

The expansion of the human population has brought significant challenges and stress to the food production sector, which is estimated to feed a population of 9.7 billion by 2050 (Hua *et al.*, 2019). Globally 30 percent of the total population is under the threat of malnutrition due to the deteriorating natural resources and decreasing protein in their diets. This dilemma has broughtthe need to incorporate protein-rich and nutrient-rich food for human consumption. With an increase in the global population, there is also a rising demand for quality-based food, especially in meat, poultry, and aquaculture. Aquaculture can provide affordable protein supplies through the intensive farming of aquatic species, which can compromise the need for animal-

source protein in human diets. The demand for feeding the concurrent growing population has brought the need to produce approximately 150 million metric tons of fishery products and seafood by 2030 (Nations and Food and Agriculture Organization of the United Nations, 2012). The fishery products as a food source are highly recommended for human consumption than the terrestrial organisms because of the specific functional components. These components include the production of health-promoting omega 3 or n-3 polyunsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid, which has proved to aid the prevention of thrombosis and arteriosclerotic diseases (Hosomi *et al.,* 2012). In addition to that, fish are the valuable sources of essential amino acids, minerals (calcium, phosphorous, zinc, iron, selenium and iodine), micronutrients, and vitamins (A, B and D) (National Research Council, 2011; FAO, 2018; Tacon and Metian, 2018).

Challenges faced by the aquaculture industry

Aquaculture is the field of development of edible fish in its complete hygiene, marketed for human consumption. The aquaculture industry is challenged with the hygienic development ofthe fishes and the maintenance of a good diet source to enhance the nutrient and mineral intake of the consumer. According to The state of the world fisheries and aquaculture 2018 reportspresented by the food and agricultural organisations, the annual growth rate of food fish consumption has surpassed meat consumption. This escalation of demand for fishery products has brought high stress, which triggered overfishing and overexploitation of wild fisheries,eventually leading to the impairment of a sustainable ecosystem. The pressure of mass production of forage fishes was prevalent in the aquaculture industry for decades, which invited certain risk factors such as disease management and feed unavailability.

The outbreak of diseases and infectious pathogens is one of the constraints encountered while farming commercial fishers. This issue has emerged due to the unhealthy management ofaquaculture techniques in which fishes are cultivated in stressful and enclosed spaces such as a pond or huge tanks under high-density stocking and overcrowding environments. Apart from that, certain other factors such as environmental, nutritional and metabolic changes also lead tothe proliferation of deadly pathogens. The consistent occurrence of pathogenic diseases become a reason for substantial economic and quantity loss. Addressing this issue, synthetic antibiotics and chemical drugs are used indiscriminately to control the pathogens worldwide, which later inducedmultidrug resistance in pathogenic bacteria (Suva *et al.,* 2017). Prebiotics,probiotics and synbiotics are considered the competent alternatives which have shown promisingresults to hinder the pathogenic attack by developing the host immunity. Recently, metagenomicshas given insights to identify the relationship between the microbiota and ecosystem and theirpathology. Another concern that brings an imbalance to sustainable aquaculture is theconstriction of aquafeed availability. About 75% of farmed fish cultured

are raw materials to form feed, especially carnivorous species. Supplementing quality ingredients that incorporateenough micro and macronutrients in aqua diets cost about 60% of the total cost of production. The innovation of an economically viable alternative aquafeed became a necessity. The scientific community experimented with different options developed from plant by-products, animalby-products, and fermentation methods to improve the quality of feed ingredients and replaceexpensive feed diets (Salin *et al.*, 2018).

Recent advances in aquaculture:

Modern aquafeeds combine raw materials like oil, minerals, vitamins, pigments, by-products, and high protein content substitutes. The essential ingredients for a fish feed are (1) a source of protein, (2) a source of fat, (3) a mineral and vitamin mixture and (4) a source for starch (wheat) that serves as a binder for the production of the pellets. Further, ingredients can be added (5) witha particular function, such as pigmentation of the fish or other feed supplements. The utilisation of offal waste as an ingredient was the remarkable modern aquafeed, which helped solve the problem of disposing 70% of the processing waste of the fish. On the other hand, thorough research is carried out on how seaweed microalgae and macroalgae can be perfect ingredients to complete the nutrient requirement and become a sustainable aquafeed. The sustainability quotient in aquaculture is still a question regarding water quality management, enhancement of breeding by fast maturation, drug use, desired fish production, transgenics, vaccine, nutraceutical and nutrigenomics. Integrating and deploying scientific technologies like nanotechnology by ensuring a next-generation platform for feed formulation, drug and nutrient delivery can guarantee sustainable aquaculture. (Shah and Mraz, 2020) The advancements of technology and innovations have brought more sophisticated and efficient techniques like fermentation, genetic modification, gene mapping to study performance and production traits, functional genomics, metagenomics, etc. which scientifically helped to curtail the gap between sustainability and aquaculture mass production (Browdy *et al.*, 2012). This review evaluates and identifies the potential bacterial strains that can promote immunostimulant character and their mode of action to inhibit pathogenic attacks and analyses the efficient replacement of fish meal by possible alternative ingredients.

The relevance of probiotics for a sustainable aquaculture

The use of probiotics has become a significant factor in the sustenance of the field of aquaculture. Probiotics, a term coined by Parker (Fuller, 1989), are beneficial bacteria that can beadministered as mono and mixed contents of microbial composition along with prebiotics and immunostimulants to help maintain the gut microbial balance. The past decade has witnessed an increased emergence of infectious bacterial and viral diseases, which has cost a high amount ofloss due to the increased mortality rate in the fishes. The indiscriminate usage of

antibiotic and chemotherapeutic drugs to prevent outbreaks has caused a threat of developing environmental pollution, antibiotic resistance, and suppression of the host's immune system. (Van Doan *et al.,* 2020). The ability of probiotic strains towards disease resistance, growth performance, gut morphology, modulation of the gastrointestinal microbiota, feed efficiency by enhancing nutritional qualities and antioxidant enzyme activities has triggered applications of the probiotics and the development for further research to this field. Using probiotics as an alternative approach for antibiotics may develop equilibrium for sustainable aquaculture. It shows established benefits on the host, like ensuring a healthy gut that result in fish growth enhancements. Microbial manipulation in the fish gut has allowed us to understand how the endogenous gut microbiota and fish host have integral roles in mediating immunological enhancement and practical functionality of the mucosal interface within the gastrointestinal tract. The researchers also addressed probiotic

functionality, which validates the usage of probiotics as an alternative method to advocate health management and disease control by ensuring security against pathogenic microbes, inhibition through competition, production of digestive enzymes and vitamins (Tinh *et al.,* 2008). Parvez *et al. (*2006) studied the efficacy of using probiotics for health and disease management by competence shown in modulation and stimulation of the immune system, diminished competition, atopic diseases and allergies anticarcinogenic activities.

The commonly used probiotic component includes gram-positive bacteria and gram-negative bacterias like *Bacillus subtilis, lactobacillus, Micrococcus, Streptomyces, Enterobacter spp, Aeromonas spp., Pseudomonas etc.* Apart from the bacterial component, there are non-bacterial components which are bacteriophages, microalgae and yeast. Studies show bacteriophages such as Myoviridae and Podoviridae enhanced protection against *Pseudomonas plecoglossicida* in Ayu fish (*Plecoglossus altivelis).* Still, one limitation is the chances that lysogenic phages can change non-virulent to virulent strains (Lund *et al.,* 2000) (Rao, and Lalitha, 2015). *Dunaliella salina, Dunaliella tertiolecta, Phaedactylum tricornutum* are some of the microalgae which showed protection against pathogens. Similarly, Microalgae: *Chaetoceros spp., Tetraselamis sp.,* has shown inhibitions against Vibrio spp (Dineshbabu *et al.,* 2019).

The administration of *Saccharomyces cerevisiae* has been reported to show results in promoting immunity in species like Catlacatla, striped bass and Nile Tilapia etc.(Lara-Flores *et al.,* 2003). One of the challenges faced by researchers with probiotics in aquaculture is their route of administration. Using a suitable means for administering probiotics is essential for favourable performance and growth in fishes. Several methods are proposed to assist probiotics to fishes, such as direct administration as dietary supplements (Park *et al.,* 2012). These include using live food, i.e. Artemia and rotifers, or as pellets (Moriarty, 1998). Another simple method practised

for probiotic administration is the direct addition of probiotics to water (Skjermo and Vadstein, 1999). Furthermore, delivery of probiotics via injection to the fish has also been reported (LaPatra *et al.*, 2014). In aquaculture, the most common way of using probiotics are informs like non-viable, viable, freeze-dried and fermentation probiotics (Rao *et al.,* 2010)

The dosage of probiotics plays a vital role in the administration of probiotics in the feed. Determination of the microbial load was the significant step to understand the trend in their adaptation and survival mechanisms which gave a broader understanding of probiotics utilisation. A dietary supplement with 10^8 CFU of Lc. lactis has shown better growth rate, blood respiratory burst activities, lysozyme, serum peroxidase in Japanese flounder (Heo *et al.*, 2013). The application of a combination of both *B. subtilis* and *B. licheniformis* at 109 CFU g-1 has improved the size, growth, leukocyte and phagocytic activity of Rainbow trout. The efficacy of a mixture of strain at 10^7 CFUg/1 has shown the best results in *Perna canaliculus* (Kesarcodi-Watson *et al.*, 2012). The beneficial dosage is at the rate of 105 CFUg/1, probiotics at the rate of 107 CFU ml/1 has implemented healthier stimulatory effects due to the increase in innate immunity (Salinas et al., 2006; Hai, 2015).

Limitations for using probiotics include the time taken to obtain results, improper usage of host and microbe interaction, and organisms' inability not to maintain the target site. Certain bacteria can be harmful to a particular host but can be beneficial to others. Deschrijver, Ollevier and Austin, in their paper, has observed the effects of *Vibrio proteolyticus* and *Vibrio alginolyticus* can be probiotic when administered to Atlantic salmon (*Salmo salar*) and turbot (S*cophthalmus maximus*) in contrast to other species of vibrio, which are harmful to aquatic organisms(Schrijver, De Schrijver, and Ollevier 2000; Austin *et al.,* 1995). Hence the researchers worldwide concentrated on studying the species-specific microorganisms interactions by the screening process and metagenomic knowledge, which promised desired results (Rekiel *et al.*, 2007).

The results obtained in this area have given more insight to the scientists of metagenomics to explore better results with diverse microbial utilisation. The enhancement of probiotic bacterial culture was regulated by studying the factors affecting and influencing microbial growth. The observations suggest that it is unlikely to maintain suitable conditions for establishing stable microbial communities in aquaculture farming. Therefore, there is a requirement of long term exposure of probiotic administration in the rearing water and the ambient environment for the adaptation and dominance of probiotic bacteria. Bioremediation and bioaugmentation use active microbes to improve water quality; the extensive role includes breaking down organic compounds like ammonium nitrate and nitrite by providing a more excellent range of exoenzyme (Gupta *et al.,* 2016). Studies by Dumas *et al.* (1998) suggested that

non-toxic *Cyanobacteria pharmidium bohneri* removed the dissolved inorganic compounds from rainbow trout culture effluent, ensuring better algal growth reduction in blue-green algae and enhancement of dissolved oxygen.

Researchers have provided evidence that probiotics significantly eliminate the aquatic pollution caused by anthropogenic activities, using antibiotics and different chemical compounds. A strategy of non-infecting from diseases is by disruption and destruction of quorum sensing, which was done by enzymatic secretion of *Lactobacillus, Bifidobacterium and Bacillus Cereus* strain by degrading the signal molecules. Some authors have driven further development of probiotics to use in food and generate nutrients and bio controllers for diseases. The supplementation of probiotic bacteria can manipulate the gut microbiota by colonising and multiplying the gut of host-microbiota and overcoming the effect of antibiotics and drugs. Certain water addictive probiotic strains like Bacillus cereus, *Bacillus subtilis*, *Bacillus licheniformis* (Decamp *et al.,* 2008) *Bacillus pumilus* (de Souza *et al.,* 2012), *Pseudomonas fluorescens* AH2 (Gram *et al.,* 1999) were used to enhance growth in rainbow trout, rotifers etc.

The usage of probiotics could directly affect the growth performance of the host fish due to the boost in releasing the digestive enzymes, improving gut morphology and breakdown of indigestible compounds (Van Doan *et al.,* 2018). Pacific white shrimp have shown an increase in final weight and production of digestive enzymes while supplementing them with 10^9 CFU *Lactobacillus plantarum* for 15 days (Zheng *et al.,* 2017). In Crayfish (*Astacus leptodactylus*), adding $10^{7,}$ 10^8, 10^9 CFU of *Lactobacillus plantarum* for 97 days elevated the haemocyte count, semi granular cells, hyaline cells, oxidative and digestive enzymes(Nedaei *et al.,* 2019). The dosage of 10^5 and 10^8in *Bacillus subtilis* has escalated the immune response in mud crab, while inpacific white shrimp, it has increased the growth and digestive enzymes (Einar ringo *et al.,* ,2019). Some probiotic bacteria help produce micronutrients like vitamin B12, which is a cofactor in DNA synthesis and participates in amino acid and fatty acid metabolism. In channel catfish, supplementation of vitamin B12 was unnecessary because intestinal bacteria synthesise 1.4ng of vitamin B12 per day (Wang *et al.,* 2020). Streptococcus cremoris, the dosage of 5 x 106 cells g- 1 in *Penaeus indicus,* has shown resistance towards V*ibrio parahaemolyticus. Bacillus subtitlus* has proved to increase the reproductive performance revealed by Ghosh *et al.,* 2007) (Gupta *et al.,* 2014). They found the use in the gonad somatic index viability, fecundity, and the production of fry.

Selection of a probiotic:

The specificity of Novel probiotic strain is determined by the screening criteria like phenotypeand genotype stability, plasmid stability, carbohydrate utilisation patterns, acid bile tolerance, andproduction of antimicrobial growth, antibiotic resistance and adherence to the intestinal mucosa. A handful of criteria allow us to select and decide whether the

probiotic bacteria can be harmfulto the host or not. This strain should not provoke the formation of antibodies and should have the ability to stimulate the immune system against pathogenic bacteria. Genetically stable probiotic strains that have desirable viability have been checked for pathogenicity, virulence factors, metabolic activity, and antibiotic resistance are the safety criteria of selection contrary to determine functional criteria the tolerance of gastric juice and bile juices immunomodulation antagonistic activity towards gastrointestinal pathogens. The encompassing of the biochemical and physical mechanisms of the gut microbiota present inside the intestine can be studied by understanding the taxonomic and metagenomic framework (Balcazar *et al.,* 2006; Hanning and Diaz-Sanchez 2015). Metagenomic studies reveal the antagonistic and symbiotic relationship between microbes and fish, crustaceans, molluscs, and the information obtained bystudying DNA through sequencing and bioinformatics technology (Gianoulis *et al.,* 2009; Suttle 2007). The introduction of metagenomics in microbiology had brought many benefits, which helped scientists investigate and get more evidence for understanding the diversity and quantityof microbial community by studying the hypervariable region of 16S rDNA of prokaryotes and 18SrDNA of eukaryotes (Hugerth *et al.,* 2014). This technology also helped in evaluating the multiresistant bacteria and their mutations by analysing sequence-based and functional metagenomics. Identifying viral pathogens to understand their mode of action and mechanisms was also done through metagenomics to develop diagnosis and treatment methods for the host fish. All these data stored in the metagenomic libraries can be used to further build profiling fish gut microbes by analysing their genetic background (Yukgehnaish *et al.,* 2020).

Fish microbiota:

Research has shown that the abundance of microbial load in the fish gut showed a descending order trend from proteobacteria, firmicutes, bacteroidetes, fusobacteria and actinobacteria, comprising about 90% of the fish gut among different species (Nayak 2010; Sullam *et al.,* 2012). There will be a difference in the fishes that inhabit different niche systems, the influence of the environment and exposure. These factors have also determined the abundance of other species of microorganisms in the fish gut. The Fish Microbiota has been explored in prior studies which observe the dominance of members of family Enterobacteriaceae like *Acinetobacter, Aeromonas, Flavobacterium, Lactococcus, Pseudomonas, Clostridium, fusobacterium*etc in the gut of freshwater fishes. Whereas *Aeromonas, Alcaligenes, Alteromonas, Carnobacterium, Flavobacterium, Micrococcus, Moraxella, Pseudomonas and Vibrio* are the species that show ascendancy in the marine water fishes. (Gómez and Balcázar 2008; Llewellyn *et al.,* 2014; Talwar *et al.,* 2018). The assemblage pattern of gut colonisation is regulated by the stochastic model assembly (random dispersal), and the deterministic model

(selective host pressure and microbe-microbe interactions) decides the fate and shape of the intestinal community (Ley *et al.,* 2006). The hierarchy of adaptation and survival of the microbialcommunity is dependent on the ability of them to attach them to the mucosal epithelial wall orhow efficiently they can utilise the nutrients, or the amount of genetic material which allows them to adjust to the ecological plasticity offered, which resist them against bacteriophages orpathogens determine their permanence in the gastrointestinal niche (Talwar *et al.,* 2018). Thesegood microorganisms, which are already present inside the gastrointestinal tract, can facilitate an environment to allow or destroy allochthonous (visitors of bacteria counter with the GIenvironment) adherence to the host gut wall. The microbial adherence to the gut wall is seen throughout the development of the gastrointestinal tract, in which evidence suggests that the amount of bacteria is diverse from foregut, hindgut and midgut. This different microbial community composition throughout the mucosa and GI tract serves various purposes andvariations at different trophic levels, different cohabiting species, and other geographic locations.This diversity of microbial species and their ability to form biofilm allow them to withstandhostile environments and adapt to the niche, making them either harmful or beneficial to a hostthat has to be studied efficiently (Sanchez *et al.,* 2016; Kalia *et al.,* 2017).

It is identified that the factors which influence the fish gut microbiomes are dietary intake [source of diet, the composition of the diet, feeding behaviour and probiotic administration], environmental conditions [water quality, habitat, pollution, geographical location], genetic constitution [metabolic profile, response to stress, status of the immune system]. These factorsregulate the normobiosis and symbiosis state of the fish gut microbiome. Many researchersexplore the antipathogenic compounds which are released by the bacteria in recent years as thatarea is merely elucidated fully. The mode of action by which the probiotic bacteria defend from pathogens is by releasing bacteriocin [inhibit pathogens] (Vandenbergh, 1993), siderophores [scavenging iron and deprive competitors] (Witte, 2000)), lysozymes, hydrogen peroxide, alteration of pH value [production of organic compounds] (Sugita *et al.,* 1997)) ammonia, diacetyl and proteases. Another possible way explained theoretically is by the competition of adhesion sites (good bacteria compete for the adhesion receptors to prevent colonisation by pathogens) and competition for available energy for survival (Verschuere *et al.,* 2000).

Prebiotics

Apart from probiotics, studies suggest using immunostimulants, prebiotics, and synbiotics as alternative antibiotics. S.A. Mastan, in his paper, confirms that immunostimulants help improve the immune capacity of fish and shellfish. In the various types of immunostimulants used, beta-glucans were generally the most effective; compounds like levamisole, chitin, and glucancan also produce the desired effects in fish and shrimp. Prebiotics

directly influence fishes' innate immunity, especially the immunosaccharides like fructooligosaccharide, mannanoligosaccharide and beta-glucan. The promising benefits of prebiotics were studied by different authors, which suggested the usage of Arabinoxylooligosaccharides (AXOS), Inulin, Fructooligosaccharides (FOS), Oligofructose, Poly-b-hydroxybutyrate (PHB), Inactive brewer's yeast as prebiotics (Hoseinifar *et al.,* 2016)). The one percentage usage of Arabinoxylooligosaccharides (AXOS) in Siberian sturgeon has shown health-enhancing properties by stimulating the growth of bifidobacteria (Rivière *et al.,* 2014). Inulin and Fructooligosaccharides (FOS) showed an increase in the LAB bacteria after one week of prebiotic administration. Two percentage of inactive brewers yeast showed a subsequent increase in LAB growth after feeding sturgeon forsix weeks. Synbiotics are used as a combination of both prebiotics and probiotics for advanced results. Studies showed evidence that AXOS showed an increase in phagocytic and lysozyme activity in Siberian sturgeon. When AXOS was combined with probiotic Lactobacillus *Lactic sps*, *Bacillus circulans* showed synergistic effects as they boosted innate immune parameters, respiratory burst activities, leucocyte, phagocyte activities and haemolytic complement activities (Geraylou *et al.,* 2012).

Fermentation

Many alternative feed strategies were applied to ensure more sustainability for aquaculture industries. Still, due to the less protein content, antinutritional factor, low palatability, lessdigestibility rate and unbalanced amino acid compositions, different plant by-products andanimal by-products in aquafeeds became limited. Fermentation is the metabolic process thatreleases energy due to the breakdown of complex carbohydrates like lipids and proteins in thefeed to produce metabolites like amino acids, vitamins, antioxidants or alcohol, depending upon the microorganisms used. An increasing number of studies have found the promising benefits offermentation on the feed as an alternative strategy. It guarantees growth production, increased feed efficiency, immunity, and stress tolerance to the aquaculture environment. Fermentation also helps in improving the acceptance of feed by boosting the organoleptic properties. The fermentation techniques applied to cheap and available protein resources have shown evidence ofpromoting plant and animal protein sources (Dawood and Koshio, 2020). The microbialcontamination, indigestible factors and high humidity could be solved using the fermentationmethod. Seafood and animal offal waste comprises 70 % of the waste generated after processingto the markets, fish offal waste itself has generated about 32 million tons of waste. The disposalof such large amounts of waste residue into the environment has brought massive contamination and pollution. Studies on fish powder material from trash fish and fish fillet waste as a feed gave promising results as the former showed 43.84% and

later showed 36.3% of protein content, which was good enough to replace a fish meal (Tugiyono *et al.*, 2020).

The idea of manipulating protein sources from biodegradable offal waste was limited due to the constraints like less digestibility and undesirable contamination by hazardous microorganisms. Employing fermentation technology to biodegradable offal waste has brought a solution for the limitation of utilising them in feed, reviving the proteins, increasing the digestibility, and eliminating contamination by microorganisms. Fermented fish offal waste by *Lactobacillus sp., Rhodopseudomonas sp, Azotobacter sp. and Saccharomyces sps* has been verified to be partially replaced fishmeal up to 30 % and 50% in the diets of Catfish and Carp and Rohu diets (Mondal *et al.,* 2008). Fermented offal fish waste as feed-in freshwater Catfish (*Mystus vittatus*) could partially replace fish meal in the diets and showed better lipid digestion than referral diets (Samaddar *et al.,* 2011).

The fermentation can lead to the production of bioactive peptides useful for the aquaculture industry. The high value-added peptide compounds obtained from fermentation were considerably used to preserve and antioxidant activities. Fermentation helped preserve feed and food for centuries by preventing foodborne pathogens and producing antibacterial compounds (Manivasagan *et al.,* 2014). Extension of feed shelf life by preventing spoilage microorganisms was obtained by fermentation of feed by *Lactobacillus Plantarum*. The peptides recovered from fermented tuna viscera was peptide inhibitors of the angiotensin-converting enzyme, as observed in the paper by Wenno *et al.* (2016). The fermentation of skin turbot with *Aspergillus oryzae* has produced different functional antioxidant peptides that can be used for preservation (Fang *et al.,* 2017). Fermentation can be used to enhance lipid and fatty acid composition. Five different bacterial strains were used to recover fish oil from Sea bass (*Dicentrarchus labrax*) by the fermentation process. This includes the different LAB strains like *Enterococcus gallinarum, Lactobacillus brevis, Streptococcus spp., Lactobacillus plantarum, and Pediococcus acidilactici* results suggested that the fermented oil recovery showed improved initial lipid quality than the acid silage recovered oils (Özyurt *et al.,* 2018).

Types of Fermentation

Submerged fermentation SMF and solid-state fermentation SSF are the two traditionally performed methods of fermentation, which were extensively applied as they assure many qualities like fewer energy requirements, higher production yields and lower wastewater production. In this, the appropriate substrates used in SSF include grains, rice brans, wheat etc.,as the dry medium for the growth of microorganisms. In contrast, SMF had liquid substrate like media, whey, broth, molasses and wet distiller grains, which supports the aqueous medium forthe growth of the microorganisms.

Recently SSF has gained more attention as a better alternative to SMF due to the high-performance capacity, cost-effective technology, higher end-product stability, catabolic repression, optimal growth conditions and less contamination (Halker *et al.,* 2004). The selection of microorganisms, substrates, and raw materials plays a crucial role in determining the rate of fermentation and the choice of microbe and substrate interlinked to obtain the desired products. The challenges included identifying physicochemical factors and physiology where the microorganisms could grow, the pH, temperature, aeration, moisture, thickness, and porosity status of the substrate for optimal growth and activity. Yeast and fungi (aspergillus and Rhizopussps) are the most commonly used substrates in solid-state fermentation. *Saccharomyces cerevisiae, Lactobacillus fermentum* and *Bacillus subtilis* are bacterial sources that have reduced the growth of enterobacteria and shown increased production of lactic acid bacteria in the feed solid-state fermentation (Hu *et al.,* 2008; Costa *et al.,* 2018).

Advancement of Fermentation in aquaculture

Fermentation over a period became the solution to tackle the limiting factors of using plant protein as an alternative to fish meal ingredient. Studies propose fermentation has helped increase fibrinolytic enzyme activity, in vitro trypsin digestibility, increased peptide content, nitrogen solubility, and antinutritional factors in the aquafeeds (Mukherjee *et al.,* 2015). Soybean fermented meal is one the most effective feed ingredients that have shown better results of eliminating the antinutritional factors and increasing the nutritional value of the feed. There is a balanced content of essential and non-essential amino acids present in soybean meal where glutamic acid is present in large amount descended by aspartic acid, arginine, glycine and serine, in the case of essential amino acids, lysine dominates over isoleucine, threonine, tyrosine etc. The microbial fermentation of soybean meal is subjected to both SMF and SSF methods without any conversion of moisture or texture as the meal can be made powder form and aqueous form accordingly. But SSF has shown a better advantage over SMF as there is evidence of up-gradation of nutritional qualities and high productivity rates by utilising waste residues, by-products residues and agro-industrial residues as nutrient sources. The fungal fermentation using Aspergillusoryzae has shown beneficial effects by removing trypsin inhibitors from 2.6 mg/g to zero and also eradicates phytate content making the phosphorus content available in the feed (Feng *et al.,* 2007). Studies observe that fermented soybean meal by *Aspergillus oryzae* compared to unfermented soybean meal, which contains 11g/kg raffinose, 52g/kg and 78g/kg sucrose, is reduced to none as the breakdown of carbohydrates is connected to the alpha-galactosidase produced by aspergillus in the fermentation (Hong *et al.,*2004; Feng *et al.,* 2007).

The bacterial based fermentation using *Bacillus subtilis, Lactobacillus brevis, L. Plantarum* can increase the small size proteins and the contents of arginine, serine, threonine,

aspartic acid, alanine (Teng *et al.,* 2012). Bacterial fermentation of soybean meal has also increased the antioxidant and metal chelating activity due to the increased production of Histidines, serine, valine and lysine, and increased phenolic compounds (Amadou *et al.,* 2011; Tamang *et al.,* 2019). Feeding juvenile parrotfish (*Oplegnathus fasciatus)* with *Aspergillus oryzae* fermented soybean meal has shown the efficiency of increasing non-specific immune response and can enhance the absorption of phosphorus (Kim *et al.,* 2009; Jannathulla *et al.,* 2018). (Ding *et al.* (2015) in their study used a mixture of *Pediococcus acidilactici, Enterococcus aecalis, Candida utilis, Bacillus subtilis, Bacillus licheniformis, Rhodopseudomonas palustri, Saccharomyces cerevisiae* to ferment soybean meal to supplement the growth performance of *Macrobrachium nipponense But* did not show any significant changes even though high soybean fermented meal was included in the diet.

Rapeseed, canola, sunflower, cottonseed and fermented lupin meals are the next considered meal after soybean meal as they have shown promising results in the aquaculture diets; researchers suggest that there has not been an adverse effect on the odour, weight, size, taste of the host fish by the association of the former meals as a feed ingredient. The restriction of using canola meal as a total substitution in the feed was mainly used to the presence of glucosinolates and phytic compounds and dominating fibre contents over protein. This problem was solved by creating canola protein concentrate by aqueous extraction of protein and fermentation using *Saccharomyces cerevisiae*, which approximately contained the same amount of crude protein as in fish meal and high rates of methionine and lysine relative to soybean and corn gluten meal (Enami 2011). Plaipetch and Yakupitiyage in their paper have stated that yeast fermented canola meal using *Saccharomyces cerevisiae* can replace 50 % of fish meal in the Asian brass meal and can also be an alternative to the soybean meal in Nile tilapias feed without any reduction in the survival rate, feed intake and growth of the fish (Plaipetch and Yakupitiyage, 2011, 2013). *Aspergillus niger* fermented combination meal, which included soybean meal, groundnut meal cake, rapeseed meal and sunflower oil and cake has been a suitable protein source for fermented feed formulation *Penaeus monodon* and *Penaeus indicus* (Jannathulla *et al.,* 2018). Cottonseed meal fermented using beneficial microorganisms were observed in the Nile tilapia and parrotfish has shown the reduction of free gossypol and fibre by improving the digestibility, enhanced enzyme activity and protein content (Lim and Lee 2011). The previously mentioned fermented rapeseed and sunflower meals have stated the production of prebiotic oligosaccharides such as arabinoxylans, beta-glucans and xyloglucans during Lacto fermentation (Dossou *et al.,* 2018). The lactobacillus and bifidobacterium can exploit the oligosaccharides produced to enhance the gut's bacterial composition, feed efficiency, and increase digestive enzymes. This, in turn, boosts the microbial gut diversity to have a beneficial immune system. The fermentation with the probiotic effects helped to manipulate the

characteristics of beneficial bacteria such as colonisation, intestinal interaction with gut microbiota, adhesion properties and pathogen inhibition and exclusion properties to the fermented feed (Dawood and Koshio 2020). The fermented mulberry meal has proved to successfully replace fish meal up to 30- 32 % in *Labeo rohita,* and in *Heteropneustes fossilis* 52-53% of fishmeal could be replaced (Ali *et al.,* 2020).

Usage of poultry by-products like poultry feather meal, bone meal and blood meal were fermented with *Bacillus circulans* PB7and results suggested 75% of fishmeal could be replaced accordingly through fermentation (Bandyopadhyay and Das Mohapatra 2009). *Lactobacillus acidophilus* fermented slaughterhouse blood and fish offal waste have shown an ideal 21.11% replacement for the growth of *Labeo rohita (*Samaddar *et al.,* 2015). Another effective method was using fish protein hydrolysate, which is made from whole fish or by-products. FPH accommodates a high amount of bioactive peptides, which can assure immunomodulatory and antibacterial properties. Krill, shrimp and tilapia hydrolysate has shown better resistance against diseases in red sea bream (Khosravi *et al.,* 2015). Fermented feed comprises health benefits through synergistic effects of both prebiotic and probiotic effects in the gastrointestinal tract by maintaining eubiosis and maintaining homeostasis between microbe immune systems.

The recent advancement in fermentation has invented U-loop technology which converts natural gas like methane to highly concentrated protein by providing optimal conditions for methanotrophic bacteria. The end product is proved safe enough to be used as animal and fish feed. The natural strategy of fermented feed to reduce or inhibit microbial contamination and minimise environmental stress has established sustainability in the aquaculture industry.

Conclusion:

Sustainability in fish stock is one important criterion to be taken care of, which showed a decline in the 1960s to 2013 due to overfishing, destructive fishing practices, illegal and unreported fishing etc. which impaired the ecosystem functions. But by 2008 bringing a scientific way of implementing effective aquaculture techniques by regulating the harvest of fishes without disturbing the aquatic diversity has helped in stabilising the maximum sustainable yield by 2015(“14.4.1 Fish Stocks Sustainability | www.fao.org” n.d.)The schedule was built up for 2030, which has a more excellent sustainability vision with better regulation to use natural resources, ensuring sustainable development in the economic, social and environmental context. Certain limitations that occurred to this agenda that had to be confronted for better implementation included developing countries' management and governance capacity to achieve their entire fisheries and aquatic potential. Aquaculture grows faster than any other food-producing industries, which stress is handling the fact projected by FAO, which stated that by

2050, 60 per cent of food might have to increase to feed the population.(Benton *et al.*, 2018)). Apart from the human consumption rates, the number of fish products for feeding the cultured fishes alsoincreases. It is anticipated that by 2030 60 percentage of fish for human consumption comes through aquaculture whereas 40 per cent of fish comes from capturing forage fishes. This means aquaculture has to keep on improving their feed diet ingredients to feed a vast population, which will further increase in the course of time.

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INTERACTION OF GUT MICROBIOME AND HEAVY METALS

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Abstract:

Exposure of heavy metals to humans is becoming a major health concern to the public. Since heavy metal absorption mainly occurs through the gastrointestinal tract, the gut microbiome will be the first group of organisms in the human holobiont to encounter the heavy metals. The gut microbiome plays a vital role in maintaining physiological homeostasis and human health. Dysbiosis of gut microbiota, which is an imbalance in the composition and function of these gut microbes, is associated with a broad range of chronic diseases, including cancer and inflammatory, metabolic, cardiovascular, autoimmune, neurologic, and psychiatric diseases. Heavy metals are known to be toxic to living organisms including microbes; however, their mechanisms of action are not fully understood. Exposure to toxic metals can change the composition, diversity, and function of the gut microbiome. Because of the gut microbiome's strategic localization in the human gastrointestinal tract and its profound impact on the homeostatic regulation of human body function, it is likely that the heavy metal–induced alteration of the gut microbial community mediates at least some of the toxicity of heavy metals in humans. Moreover, the gut microbiome could mitigate heavy metal toxicity by decreasing absorption and increasing elimination. In addition, the gut microbiome may be used as a prime target for monitoring environmental exposures to the toxic metals. In summary, the gut microbiome may be an important explanatory factor of metal toxicity, metal penetrance in humans, and a novel target for monitoring, prevention, and treatment strategies. More research on the impact of interaction between heavy metals and the gut microbiome on human health is warranted.

Introduction:

The human intestinal microbiota is a topic of great interest for its fundamental role in several aspects of host homeostasis, such as nutrition, immune development, metabolism and defense against pathogens (Hill and Artis, 2010). The gut microbiota (GM) is estimated to be

composed of $>10^{14}$ microorganisms: It is represented by a complex system of bacteria, archaea, viruses and fungi who live in symbiosis with the host (Cho and Blaser, 2012). The individual pattern changes over the years and it is subjected to strong variations due to environmental changes, diet, exposure to antigens, infections, drugs, hygiene factors and lifestyle (Breton *et al*., 2013). Several studies have assessed the association between environmental stressors and GM integrity. The interaction between the host and microbiota determines the difference between eubiosis and dysbiosis. This balance can be altered by air pollution, exposure to pesticides and heavy metals, and can promote the development of diseases, such as obesity, type 2 diabetes mellitus, metabolic diseases and cancer (Fenga *et al.,* 2017; Planchart *et al.,* 2018; Rapisarda *et al*., 2018; Rehman *et al.,* 2018). Toxicants in the environment can directly harm the components of the microbiota, but can also be modified by the microbiota to become more or less toxic to the host and/or the microbiota itself (Breton *et al.,* 2013). The effects of heavy metals on the intestinal microbiota are not still largely defined. The aim of the present work is to provide an update of the available evidence in the literature related to the interaction between the GM and heavy metals, focusing on the compounds most widely distributed in the environment or considered biopersistent.

Overview:

Human GM:

The human microbiota is the set of symbiotic microorganisms that coexist in the human body; it is composed of >100 trillion different organisms in a complex system of bacteria and viruses, as well as fungi, archaea and protists (Cho and Blaser, 2012). In the majority of studies, the bacterial constituents of a microbial population were identified by sequencing the 16 sRNA-encoding gene. GM varies among individuals during development, and is dependent on the host and environmental factors (Sommer and Backhed, 2013). This gut microbial community has evolved with its host over a timespan of millions of years and offers benefits to its host through a number of mechanisms, including digestion, detoxification, the production of nutrients, protection against pathogens and the regulation of the immune system (Forbes *et al.,* 2016). The intestinal tract is exposed to a plethora of food-borne and bacterial antigens. The epithelial cell layer prevents a 'too close contact' of these antigens with the immune cells, and thereby also protects the gut from unwanted immune reactions. This is achieved by the sophisticated organization of the intestinal epithelium, which establishes a tightly regulated barrier (Mandel *et al.,* 1993). In adults, the GM is composed of 500 species of bacteria divided into 45 genera and 14 families; however, the individual pattern changes over the years and it is subjected to strong variations due to environmental changes, diet, exposure to antigens,

infections, drugs, hygiene factors and lifestyle. The most frequent microorganisms are commensal bacteria that are beneficial for the host. The analysis of normal bacterial communities has indicated that the enteric microbiota in healthy subjects belongs mainly to 4 phyla: Firmicutes and Bacteroidetes that are the most representative (together they represent 90% of total enteric microbiota), Proteobacteria and Actinobacteria (including Bifidobacterium), which are mainly driven by dietary intake, but are independent of age or body mass index (Arumugam *et al.,* 2011). Fusobacteria, Cyanobacteria and Verrucomicrobia are less represented.

Heavy metals:

Heavy metals are naturally occurring elements that have a high atomic weight and density. Both prokaryotes and eukaryotes have developed strategies to benefit their action and to defend themselves from undue exposure, particularly against those metals showing high reactivity. These metals are commonly spread and many of them are used in human activities, such as manufacturing, mining, agricultural fields, configuring exposure as environmental and occupational risks. Conversely, other metals can be found in water and the food supply chain, posing a safety concern for human health, particularly in developing countries. Arsenic (As), cadmium (Cd), chromium (Cr) and nickel (Ni) are classified as group 1 carcinogens by the International Agency for Research on Cancer. Heavy metals can affect target organs (liver, kidneys, lungs) through different mechanisms and pathways (Richardson *et al.,* 2018). Moreover, these metals are difficult to degrade and their bioaccumulation is dependent not only on the exposure (concentration and frequency), but also on the organism capability to eliminate the metals. The residuals in the gastrointestinal tract can affect the human GM that interacts with heavy metals, and is in part, responsible for the intestinal barrier functioning involved in the control of absorption of toxic metals (Zhang *et al.,* 2016). All studies on humans or different animal models or in vitro, concerning investigations of ≥2 heavy metals, are concordant in concluding that heavy metal exposure, even if for a short period of time, can cause perturbations in the composition, structure and diversity of the GM. The majority of the studies used the amplification and sequencing of the 16S rRNA gene for the microbial community analysis; only 2 studies (Rothman *et al.,* 2019) proceeded with metabolomics approach demonstrating that As, Cd and selenate also influenced several metabolic pathways. When investigated together, different toxicants may interact and their effects, on GM or on health status, could be potentiated.

Human studies:

Laue *et al.* (2020) conducted a study on 179 6-week-old infants the GM was assessed using 16S rRNA sequencing. They found that postnatal exposure to toxic elements was

differentially associated with the infant microbiota. Exposure was negatively associated with microbial diversity, particularly in infants exposed to peripartum antibiotics. As, Cd, manga-nese (Mn), copper (Cu), iron (Fe), lead (Pb), Ni, selenium, tin, and zinc (Zn) were each differentially associated with at least one taxon, in particular with Bacteroides and Lactobacillales, while mercury (Hg) was associated with specific taxa. In addition, on children aged from 3-7 years, other authors investigated potential interactions between toxic metals, the gut microbiome and the development of autism spectrum disorders (ASDs). The levels of Pb, Cd, As, Cu, Zn, Fe, Hg, calcium and magnesium in hair samples were analyzed. Microbial DNA was extracted from fecal samples and 16S rRNA was sequenced. Levels of Pb, As, Cu, Zn, Hg, calcium and magnesium were significantly higher in the ASD group. Different metals were associated with dissimilar perturbations in GM composition and diversity, suggesting the possible pathogenesis of ASD depending on metal absorption and gut microbial community (Zhai *et al.,* 2019).

Animal studies:

Liu *et al.* (2019) conducted a study on rats poisoned first with methylmercury (MeHg) and then treated with sodium selenite (NaSe). Microbial DNA was extracted from fecal samples and 16S rRNA was sequenced. The results suggested that MeHg damaged the composition of microbiota. Following Se treatment, the richness of gut microbial commu-nity was partially reestablished and a similar mechanism can be hypothesized also for human beings. A most recent studyperformed on rats focused on neuro-developmental effects (Qui *et al.,* 2020). Offspring rats were exposed to inorganic As (iAs) and fluoride (F-). DNA was extracted from fecal samples and 16S rRNA gene was sequenced. Noticeable neuro-developmental effects were observed in rats concurrently exposed to iAs and F-so microbiome-based biomarkers of iAs and/or F-may be suggested as early indicators of neuro-developmental deficits. Richardson et al exposed rats to 3 different doses of sodium arsenite, Cd chloride, sodium dichromate, cobalt chloride or Ni chloride by oral gavage for 5 consecutive days. DNA was extracted from fecal samples and 16S rRNA gene was sequenced. Significant variations in GM composition were observed in high doses of Cr and cobalt, but only in As, Cd and Ni exposure in a significant dose-dependent manner. Following As and Ni exposure, bacteria with a huge number of iron-importing gene were excessively represented. The results underline the convenience of the microbiome as an early tool for recognizing specific heavy metal exposures.

Recently, in a study on mice (Ruan *et al.,* 2019), animals were exposed to Cu, Hg or both. DNA was extracted from cecal contents and 16S rRNA was sequenced. All treatment groups showed intestinal histopathological damages and alterations in the diversity of gut microbiota in the cecum of female mice, which can provide new insight into the risk assessment

in intestinal disorders caused by Cu and Hg. Gaulke et al fed mice with diet containing either different Zn concentrations and exposed to As for 6 weeks (Gualke *et al.,* 2018). Zn deficiency and As exposure autonomously changed microbiota composition but, when in combination, the outcomes in microbial community were intensified, in particular marginal Zn deficiency resulted in a higher sensitiveness to As exposure of the GM (Gualke *et al.,* 2018). Li et al treated mice with Cd chloride and As in their drinking water for 2 weeks. Colon and caecum content were collected and 16S rRNA gene was sequenced (Li *et al.,* 2019). Both As and Cd significantly perturbed the metabolome and lowered the GM diversity; however, only Cd significantly decreased the microbial diversity. The number of metabolite interac-tions diminished in a number of genera. Moreover, As and Cd exposure affected pathways involved in metabolic health (Li *et al.,* 2019). Previously, other authors performed a research on cows. Animals were fed supplemental Cu, Zn, and Mn from sulfate minerals, glycinate minerals or Cu and Mnsulfate with glycinate Zn. Treponema Operational Taxonomic Units (OTU) in GM, frequently related to bovine digital dermatitis, were less abundance in cows fed with Cu and Mnsulfate with glycinate Zn. These results suggest a further connection between organic Zn supplementation and better animal health (Faulkner *et al.,* 2017). Rothman et al carried out a study on honey bees administering solutions of 50% sucrose spiked with Cd chloride, pollen patties spiked with selenium or Cd. Cd and selenate exposure perturbed the composition of the GM altering the metabo-lite pathways, particularly those concerning detoxification, proteolysis, and lipolysis, resulting in bioaccumulation of these toxicants (Royhman *et al.,* 2019). In a study conducted on black soldier fly larvae, the authors administered Cu or Cd. Gut DNA samples were analyzed. Cu and Cd exposure noticeably perturbed the GM. The highest levels of exposure significantly reduced the rich- ness of the majority of dominant families; however, in the meantime, other families were enriched, resulting in a total decrease of the GM diversity (Wu *et al.,* 2020). Zhang *et al* examined the GM of Buforaddei from a polluted and relatively unpolluted area (Zhang *et al.,* 2016). The Firmicutes/Bacteroidetes ratio and the number of probiotics in the gut microbiota from the polluted area were decreased when compared to those from the unpolluted are. The results suggest that long-term heavy metal exposure perturbed the structure and reduced the diversity of thee GM in Buforaddei (Zhang *et al.,* 2016).

***In vitro* studies:**

By using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) some authors have simulated an exposure to As and Fe in different concentra-tions suggesting that GM had a significant role in As species modification, and Fe can influence As transformation through

alterations in GM. In the evaluation of the health hazards of As in drinking water, the residual Fe should be taken into account (Yu *et al.,* 2016). Recently, always through the use of the SHIME, fresh fecal microorganisms obtained from volunteers were mixed with Fe minerals (goethite and jarosite). Total and bioaccessibleAs and Fe concentrations were determined. As bioavailability in human intestine was mainly due to FeIII dissolution in jarosite, and to bacterial community reduction of FeIII and AsV in goethite, underlining the importance of Fe minerals in human health risk evaluation associated with environmental As exposure (Yin *et al.,* 2020).

Arsenic:

As, an ubiquitous nonmetal in nature, is a human carcinogen. While it can be found as a metal in its pure form, it is typically found as a component in both inorganic and organic compartments (Coryell *et al.,* 2018). As is found in water and food worldwide, as both organic and inorganic species in dietary essentials, such as rice, and exclusively as inorganic As in water (Hoen *et al.,* 2018). As soil and water contamination has been associated with human activities, such as smelting, mining, coal and ash disposal, pesticide use (Baker *et al.,* 2018). As exposure is a public health concern worldwide; in fact, >100 million individuals are exposed to As from drinking water and it has been associated to a number of human diseases, particularly diabetes, cancer and cardiovascular illnesses (Chi *et al.,* 2016). The consumption of contaminated drinking water and/or food is the principal pathway of As exposure in humans; thus, the GM can be the most vulnerable to As exposure (Dong *et al.,* 2017).

Human studies:

Hoen *et al.* analyzed, on 204 infants in the USA, As levels in urine and microbiota composition from fecal samples (Hoen *et al.,* 2018). With higher As exposure, 8 genera were enriched. Urinary As levels were negatively associated with 15 genera, including Bacteroides and Bifidobacterium. Following stratification by sex and feeding method, associations were found in formula-fed males, but not in the other groups. In infant development, sex-specific effects on the GM can be observed even with moderate As exposure (Hoen *et al.,* 2018). GM may have an important impact, in particular among subjects exposed to higher As levels (Wu *et al.,* 2019). Other authors evaluated 16S rRNA gene sequencing from fecal samples and urine As concentration in 2 southern Nepal communities. Pathogenic bacteria were positively associated with higher As concentrations in the subjects. Lower levels of As were associated with intestinal commensal bacteria. The authors stated that As plays a role in the GM shaping through the enrichment of pathogens and the weakening of commensal bacteria (Brabec *et al.,* 2020). Previously, a study conducted among children (Dong *et al.,* 2017) made a comparison between high and low As-exposed groups. Children exposed to higher As concentrations exhibited an

abundance of Proteobacteria in their feces. In the high exposed group, a positive association between genes involved in virulence and multidrug resistance and As concentration was observed. The qPCR quantification of 2 As resistant genes (ArsB, ArsC) revealed a higher expression of these 2 operons in the children with high As exposure. These studies conducted on humans evaluated the gut microbiome composition through the 16S rRNA gene sequencing from fecal samples. All the studies, except one (Wu *et al.*, 2019), found an association between As exposure and significant variations in the gut microbiome, with an increase in the number of pathogens, underlining an important role, played by As exposure.

Animal studies:

Coryell et al exposed mice to As. As levels in feces and in organs were measured and the GM composi-tion was analyzed through the 16S rRNA gene sequencing from fecal samples (Coryell *et al.,* 2018). The lack of As detoxification enzyme (As3mt) rendered the mice hypersensitive to As. The protection deriving from humane microbiome transplant depended on microbiota stability and the presence of certain bacteria, including Faecalibacterium. In mouse model As3mt and certain microbiota bacteria are fundamental for protection against acute As toxicity (Coryell *et al.,* 2018). Previously, other authors divided mice in cases and controls, treated with sodium arsenite in their drinking water for 13 weeks. In the microbiota of mice treated with As, the genes involved in the biosynthesis of lipopolysaccharides (LPS), in DNA repair mechanisms and in multidrug resistance were increased and the genes involved in the production of vitamins (B6 and B12) were enriched (Chi *et al.,* 2016). The same authors recently conducted a study on same animals to compare GM-disrupted animals (treated with antibiotics for 72 h prior to As exposure) and non-antibiotic-treated mice. In the GM-disrupted mice, total As levels were lower in feces and much higher in urine than the levels in control subgroup mice. The disruption of the microbiota significantly altered As biotransformation by affecting one-carbon metabolism and also increased the As toxic effects (Chi *et al.,* 2019). Other authors compared Helicobacter-free mice and Helicobacter trogontum-treated animals (by oral gavage) successively treated with As. As exposure caused GM perturbation inducing variations of several metabolite path- ways, involving the metabolism of fatty acid, phospholipids, cholesterols, and tryptophan. The authors proposed that GM perturbation could worsen or cause metabolic disturbances induced by As exposure (Xue *et al.,* 2019). Chi *et al* treated mice with As for 4 weeks and evaluated variations in microbiota composition (Chi *et al.,* 2019). At the end of the 4-week treatment, it changed in both males and females, but more significantly in females than in the respective controls. The host sex determines the microbiota phenotypes, whose sensitivity

to As differs between male and female mice. Moreover, same authors compared antibiotic and non-antibiotic treated mice. As exposure suppressed gene expression in cholesterol metabolism, transportation and synthesis only in control mice. In antibiotic-treated mice, a smaller perturbation was observed on liver and serum lipid homeostasis caused by As exposure. The As-induced effects on lipid metabolism may be buffered by regulating the GM (Chi *et al.*, 2019). In another study, mice were divided into the control and As-treated groups. In As-exposed mice, the α-diversity of the GM was significantly lower than that of the controls. Following As exposure, carbohydrate metabolism was significantly perturbed due to the increase of several proteins involved in this pathway. As exposure may increase the LPS synthesis in the GM through the upregulation of UDP-N-acetylglucosamine acyltransferase (Liu *et al.*, 2019). Chiocchetti et al administered various doses of AsIII through drinking water to mice (Chiocchetti *et al.*, 2019), evaluating the expression of genes encoding inflammatory cytokines (TNFα, IL-1β, IL-2 and IL-6) and claudins (Cldn-1, Cldn-2 and Cldn-4). At As concentrations ≥50 mg/l, an increase in gene and protein expression of pro-inflammatory cytokines (IL-1β, IL-2 and IL-6) was showed. Moreover, the diversity and global composition of GM were accompanied by mucosa and submucosa moderate inflammation. These effects are suggestive of an increased intestinal permeability, resulting in a barrier function loss, particularly at the highest As concentrations. A study on the adult and developmental stages of CD1 mice (Gokulan *et al.*, 2018) evaluated the effects of a single oral exposure and of repeated exposure. In adults after a single gavage, responses in microbial composition and bacterial recovery were observed in a dose and time-dependent manner. Repeated exposure affected intestinal bacteria with a decrease in recovery and an abundance of As resistance genes. High levels of intestine-secreted chemokines were revealed in adult animals exposed to As. Both in juvenile and adult animals, it was possible to observe the gut-associated immune status and intestinal microbiota changes. Tikka et al compared mice in 2 different exposure periods (3 and 6 months) (Tikka *et al.*, 2020). As exposure not only altered the GM composition, but also increased inflammatory cytokines (TNF-α, IFN-γ and IL-17) and depleted anti-inflammatory cytokines (IL-10). Moreover, the level of β-catenin, a colon cancer marker, increased in the animals exposed to As for both 3 and 6 months. These results suggest that variations in the GM can alter inflammatory cytokine pathways, resulting in an immune system dysregulation, affecting colon cancer marker expression (Tikka *et al.*, 2020). The majority of the studies used C57BL/6 male mice as a study population; other models used were female BALB/c mice, adult and developmental stages of CD1-mice and Kunming mice. All the experiments were designed as control/case studies, exposing animals to various As concentrations in drinking water, in food or oral gavage. The

majority of the studies evaluated GM composition through 16S rRNA sequencing from DNA extracted from fecal samples, only two using metagenomic (Chi *et al.*, 2016) and (Chi *et al.*, 2017) and one metaproteomic approaches Liu *et al.*, 2019). Data on GM composition, gene expression, metabolic pathways and tissue damage were all concordant on the negative effects elicited by As exposure on animal health status. A study on the earthworm *Metaphire californica* (Wang *et al.*, 2019) evaluated the interactions between microplastics (MPs), As exposure and GM. Following As exposure, significant changes in gut bacterial composition were observed, while MPs did not significantly impact the gut bacterial communities. MPs not only decreased the As accumulation, but also the transforma-tion of AsV to AsIII. MPs may have played a role in lowering As bioavailability, reducing its effects on the GM, resulting in a lower toxicity on earthworms. Another study on the earthworm (*Metaphire sieboldin*) compared various As concentrations in the controls and cases for 28 days. The analysis of gene expression revealed that As redox and efflux genes were abundant, whereas As biotransfor-mation (methylation and demethylation) genes were very low. These results suggest that the earthworm GM has a marked capability in reducing AsV even if As exposure disturbs the GM composition (Wang *et al.*, 2019). Studies conducted on worms have demonstrated the negative effects of As exposure on the gut microbiome, resulting in bioaccumulation, reducing capability and gene expression alterations.

***In vitro* studies:**

By using the *in vitro* SHIME, Yin et al aimed to investigate the variability in the Asbioaccessibility in gastric, small intestine and colon phases in fecal microorganisms (Yin *et al.*, 2016). The results suggested that the Asbioaccessibility varied in the colon phase, due to microbial reduction activity. In order to assess hazards to human health correlated with oral exposure to soil As, joining *in vitro* methods and SHIME could increase the accuracy in risk assessment (Yin *et al.*, 2016).

The same authors using SHIME, analyzed the interactions between four soils with different As concentrations and fecal microorganisms, demonstrating that human GM can release soil-bound As. In humans subjected to soil As exposure, an precise risk assessment could be carried out by concurrently defining As transformation and intestinal absorption (Yin *et al.*, 2017). Li et al demonstrated that the bacterium Bacteroidesvulgatus possesses resistance genes to inorganic As, which can be decisive in the ability of the microbe to maintain its prevalence in the human intestine even after exposure to food-related As (Li *et al.*, 2016). *In vitro* studies, evaluating Asbioaccessibility, transformation and absorption, mediated by the human GM,

particularly in the colon, play an important role in providing insights on mechanisms involved in As metabolism.

Cadmium:

Cd, one of the most widespread toxic heavy metal pollutants, appears in soils and drinking water supplying, as a side product of human activities, such as mining, agricultural use (in fertilizers) through air deposition or industrial divisions (batteries, pigment and plastics) (Srut *et al.,* 2019; Hc *et al.,* 2020). Cd can also be found in aquatic systems. Cd exposure has become a prevalent health concern due to the access to polluted food and water. As with other heavy metals, it is associated to several toxic effects in living organisms, particularly in oxidative stress induction, DNA damage, carcinogenesis, dysregulation in immune responses and energy metabolism (Zhang *et al.,* 2015).

Animal studies: Rodent studies have been designed as control/case experiments, exposing animals to various Cd concentrations. All studies revealed GM perturbations caused by Cd exposure and several studies suggested the important role played by Cd in the alterations of both metabolic and energetic pathways. Previously, authors demonstrated that the Cd exposure induced noticeable perturbations of GM in mice, with a significant decrease in gut microbial richness and reduction in short-chain fatty acids (SCFAs) production. Moreover, Cd exposure caused changes in the expression of those genes involved in metabolic pathways, associated with amino acid and carbohydrate (Hc *et al.,* 2020). In mice, Cd exposure significantly altered the GM composition and richness, not only at the phylum level, but also at the family and genus levels. These alterations result in an increase in serum LPS levels accompanied by hepatic inflammation, as a result of dysregulation in energy metabolism (Zhang *et al.,* 2015). Ba et al evaluated metabolic effects in mice in a sex-dependent perspective, observing that early low-dose Cd exposure (100 nM) altered the GM in composition and diversity. Furthermore, Cd exposure caused metabolic effects only in males (Ba *et al.,* 2017). The only experiment concerning the evaluation of a potential protective probiotic to decrease Cd toxicity, revealed the failure of Akkermansiamuciniphila (AKK). GM alterations were observed both in acute and chronic Cd exposure; however, the oral administration of AKK influenced GM composition, particularly following acute Cd exposure (Feng *et al.,* 2019). Following Cd exposure, a previous study observed both in the liver and GM, noticeable functional and structural modifications. Moreover, Cd exposure induced variations of several metabolites, suggesting that Cd may play an important role in energy metabolism in these animals (He *et al.,* 2020). Studies on fish and crustaceans underlined the negative effects of Cd on microbial community diversity. In particular, dietary supplementation with probiotics revealed the protective role of certain

microorganisms against Cd bioaccumulation and its toxic effects, providing new insight into aquaculture and food safety. In a study conducted on common carp, it was observed that Cd exposure significantly perturbed the composition of GM in the fish, resulting in a decrease in microbial community diversity. Conversely, the abundance of AKK, decreased after Cd exposure (Zhang *et al.,* 2019). Cd exposure caused, in Procambarusclarkii, intestinal histological damage and variations in the richness, diversity and composition of the GM, not only at the phylum level, but also at the genus level. Moreover, Cd exposure may sugges-tively perturb metabolic pathways related to diseases and cellular processes (Zhang *et al.,* 2020). Wang et al highlighted the protective effect of Bacillus cereus against Cd exposure (Wang *et al.,* 2020). Cd caused manifest variations in the GM composition of Carassiusauratusgibelio; however, Bacillus cereus succeeded to inverse these effects, inhibiting perturbations in Cd bioaccumulation and antioxi-dant enzyme levels. Cd exposure was also shown to cause a marked decay in gut microbial diversity and composition in Nile tilapia. Dietary supplementation with the probiotic Lactobacillus plantarum, CCFM8610, inverted the variations and reduced Cd accumulation (Zhai *et al.,* 2017). Studies on amphibians have also disclosed Cd toxic effects, reducing GM diversity and composition. Ya *et al.* observed severe gut histopathological modifications and microbial alterations at the Cd highest doses (100 and 200 µg/l), whilst small intestine damage was observed at the 5 µg/l Cd concentration (Ya *et al.,* 2019). Even in Rana chensinensis, Cd exposure transformed the composition and reduced the microbial diversity in GM both at phylum and genus levels (Mu *et al.,* 2018). In addition, 2 studies on worms confirmed the same nega-tive effects of Cd exposure on GM composition. Notably, variations in intestinal microbial communities in earthworm could be considered as a biological indicator of soil pollution. Šrut et al revealed a perturbation in GM composition and increased levels of heavy metal resistant bacteria following Cd exposure in the earthworm (Srut *et al.,* 2019). Lee et al compared the effects of Cd exposure between Caenorhabditiselegans fed with either soil microbial community (SMB) or Escherichia Coli strain OP50; in the OP50-fed worms, microbial community alterations mediated were particularly severe (Li *et al.,* 2020).

Mercury:

Mercury is an ubiquitous and highly biopersistent pollutant. For this reason, environmental and occupational exposure has been subjected to restriction by the European Community. Generally, individuals are exposed via contaminated food consumption, particularly fish. Bioavailable organo-metallic compounds (e.g., MeHg) can cross the blood-brain and placental barriers, developing neurotoxicity (Zhao *et al.,* Lin *et al.,* 2020). The mechanisms

involved in the complex bio-physico- chemical process of Hg methylation and demethylation remain to be fully understood. However, MeHg metabolism is the core research in experiments focused on methylation/demethylation processes in GM, that is considered to be involved in the biochemical interplay responsible for its demethylation to inorganic mercury (Rothenberg *et al.*, 2019) human studies. Rothenberg *et al.* analyzed, in pregnant women, total Hg and MeHg levels in maternal blood and stool samples, aiming to investigate whether associations between biomarkers for prenatal MeHg exposure and maternal GM differed between early and late gestation. The GM differed, not only between subjects, but also between early and late gestation in the same individual. Even low MeHg concentrations (both in blood and stool) were associated with perturbations in microbiota composition only during early gestation. The results suggested that mothers ingesting a fiber-rich diet may have significantly improve MeHg elimination.

Animal studies: All in vivo studies were concordant in stating that mercury exposure is accountable for intestinal damage, dysbiosis, metabolic disorders, an increase in the expression of genes involved in apoptosis processes and neurotoxic effects. Some authors also hypothesized that different chemical forms of Hg are responsible for its accumulation in gut and dissimilar microbiota structure alterations (Zhang *et al.,* 2019; Zhao *et al.,* 2020; Zhou *et al.,* 2020). The GM influences brain activity through the gut-brain axis, by producing neurotransmitters and their precursors. MeHg can perturbations in GM structure in rodents, resulting in alterations in intestinal neurotransmitters responsible to regulate neuron activity. These results suggest a possible mechanism concerning MeHg neurotoxicity (Lin *et al.,* 2020).

Lead (Pb) :

Pb has been an ubiquitous environmental pollutant for decades and has been causally associated with well-known occupational diseases and innumerable damaging health effects, including neurological and kidney disorders, anemia and altered immune system responses (Fenga *et al.,* 2016; Eggers *et al.,* 2019). There are a number of sources of lead exposure; apart from water and food, the majority derives from human activities and industrial productions, such as paint and electronic waste (Gao *et al.,* 2019).

Human studies: Eggers et al found a significant association between the adult urinary Pb concentration and GM compo-sition, particularly as regards the extent of Proteobacteria colonization.

Animal studies: All in vivo studies were concordant in stating that Pb exposure may play a role in dysbiosis, inducing structural intestinal damage. GM structure and diversity were always perturbed by lead exposure yu *et al.,* 2021). In experimental studies performed on mice, metabolomics profiling and metagenomics sequencing revealed that a number of metabolic

pathways, including energy metabolism, oxidative stress and detoxification mechanisms, were meaningfully altered by Pb exposure (Gao *et al.,* 2017). Pb exposure caused memory impairment, perturbations in GM composition and release of oxidative stress biomarkers in serum (Cheng *et al.,* 2019). Prenatal Pb exposure may also contribute to increase adult bodyweight (Wu *et al.,* 2016) and can cause large intestine immune disorders. The same effects were confirmed in zebrafish (Kou *et al.,* 2019).

***In vitro* studies:** By SHIME simulation, Pbbioaccessibility was evaluated in soil samples through the Physiologically Based Extraction Test (PBET). The mean Pbbioaccessibility changed in gastric, small intestine and colon phases, depending on soil pH and types. The action of the human GM was important in the colon phase, when mean Pbbioaccessibility was compared between farming soils (higher) and mining soils (lower) Du *et al.,* 2020).

Zinc (Zn):

Zn is a fundamental nutrient for almost all organisms. From a clinical point of view, even mild deficiencies can reduce cellular differentiation, influence immune system developing and deeply affect growth and maturation (Reed *et al.,* 2018). Infants, also when healthy, are given additional Zn with food, beverages and nutritional supplements; consequently, it often exceeds established nutritional necessities (Podany *et al.,* 2019). Furthermore, the microbial community has its own requirements for minerals, including (Zn Ishaq *et al.,* 2019).

Animal studies: The excessive intake of dietary Zn in mice, can cause oxidative stress in the intestine, resulting in deep shifts in GM. These perturbations are associated with a microbial community enriched in pathogenic taxa. Moreover, variations in the expression of genes involved in metabolic processes, oxidative stress and protein glycosylation were observed (Podany *et al.,* 2019). Excess dietary Zn not only significantly perturbs the GM, but also worsens C. difficile-associated disease, by varying the host immune response (Zackular *et al.,* 2019). In a study conducted on chicken hatchlings (*Gallus gallus*), animals receiving a Zn-biofortified diet exhibited a significant increase in β-but not α-microbial diversity; in particular, associated intensifi- cation in Zn-dependent bacterial metabolic pathways was detected (Reed *et al.,* 2018). A research conducted on Targhee yearling rams revealed that a quantity of bacterial genera was perturbed, but only the phylum Tenericutes was significantly decreased by Zn supplementation, suggesting that Zn formulations can be employed without producing a high-level modification in the rumen gut microbiome composition, which could have adverse effects on metabolism and health status (Ishaq *et al.,* 2019). These studies demonstrated how an excess in dietary Zn, not only significantly perturbs microbiota, but also affects different metabolic pathways and gene

expression, resulting in negative effects on animal health status. 9. Copper Cu is an essential microelement (Zhang *et al.,* 2019); however, excessive Cu quantities can affect the ecosystem resulting in soil and water pollution. Disproportionate Cu exposure may cause negative effects on animal organisms, inducing respiratory, nervous and gastrointestinal diseases (Cheng *et al.,* 2020). The effects of overexposure to Cu on the GM are not yet well described in the literature (Dai *et al.,* 2020).

Animal studies: High Cu concentrations can cause liver damage in rats. Cu exposure can exert dose-dependent effects on the structure of the GM. Moreover, metabolomic analysis confirmed the effects of Cu exposure on metabolic pathways involved in hepatic injury and intestinal inflam-mation. These alterations may induce an inflammatory response, in which TNF-α could be the main respondent in Cu overexposure (Zhang *et al.,* 2017; Dai *et al.,* 2020). Furthermore, various dietary doses of Cu and fructose can interact with host microbial metabolic activities by various mechanisms, resulting in alterations in GM composition, liver damage and hepatic steatosis (Song *et al.,* 2018). In mice, some authors have demonstrated that high concentrations of Cu can cause intestinal histopathological lesions and perturb GM composition (Cheng *et al.,* 2020). In piglets, a low-Cu diet can alter the structure of the microbiota and modify bacterial metabolic pathways, including proteins, carbohydrates, the urea cycle, gluconeogenesis and amino acids, which may play a role in affecting animal health (Zhang *et al.,* 2019). In a previous study, the GM of Cu-treated fish (*Takifugu fasciatus*) exhibited pertur-bations in composition at the phylum level, revealing the role of intestinal microbial community in maintaining the health status of Takifugufasciatus for aquaculture (Wang *et al.,* 2019). In addition, in the common carp (*Cyprinus carpio* L.), Cu exposure altered the α- and β-diversity of the GM (Meng *et al.,* 2018). The authors observed that the presence of short-chain fatty acid (SCFA)-producing bacteria was significantly reduced, as well as probiotics, with an increase in pathogens, resulting in an increasing risk of pathogen invasion. Moreover, in Rana chensinensis larvae, the Cu-altered microbiota composition was observed at the phylum level, resulting in a reduction of Fusobacteria abundance, while Bacteroidetes did not exhibit significant differences (Yang *et al.,* 2020). These studies suggest that Cu exposure exerts dose- dependent effects on the structure of the GM. Changes in the composition of the microbial community may be related to alterations in several metabolic pathways, inflammatory responses (through cytokine modulation) and liver damage (Hemrajata *et al.,* 2013).

Conclusion:

Challenges, Limitations, and Opportunities

Undoubtedly, the gut microbiome does have a profound impact on the toxicity of metals and their health effects on humans and animals. Current challenges and limitations in understanding the microbiome-metal interrelationship are posed by differences in experimental design (animal model, metal dosing, mode of exposure, sequencing technologies, data analytics, etc.), quality control (QC) measures, and by the sheer overwhelming complexity of the microbiome-host interactions. Each individual's microbiome is unique and dynamic, constantly influenced by environmental, dietary, and biological factors. At present, strong efforts are underway to make microbiome research more reliable and reproducible; for example, by using mock communities and spike-in controls for QC as well as standardized sampling procedures and data analysis pipelines (Sinha *et al.,* 2017; Morton *et al.,* 2019). As briefly summarized, correlations of microbiome structure with metal toxicity are not enough - for elucidation of the mechanisms operating at the metal-microbiome-host interface, mechanistic confirmation using multiple omics such as metagenomics, transcriptomics, proteomics, and especially metabolomics will be necessary. Furthermore, the gut virome's involvement needs to be evaluated

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STYLISH AND ELEGANT LANDSCAPE: AN ART TO STYLE OUR GARDEN

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Abstract:

In this we are gonna see about importance of landscape its layout different types and styles of landscape. The international scenario of landscaping. Elegant landscape and all the gardens in India there landscape architecture along with colour combination and contrast which can be applied in landscape architecture.

Introduction:

Landscape is usually considered for aesthetic appeal, it is the visible feature of an area and how it is integrated with nature and man made features.The term comes from the Dutch word landschap, the name given to paintings of the countryside. Geographers have borrowed the word from artists. Although landscape paintings have existed since ancient Roman times (landscape frescoes are present in the ruins of Pompeii), they were reborn during the Renaissance in Northern Europe. There are lot of landscape painting from a very long time. Landscape gardening refers to arrangement of tress, shurbs, climbers and various other plants together with building , walks, artificial and natural features for the use of humanity. “Landscape gardening is the decoration of the react of landscape with plants and other garden materials so as to produce a picturesque and naturalistic effect on a limited space” (Salaria and Salaria, 2010)

Importance of landscape:

Landscapes and features are important because they contribute significantly to our well-being and quality of life. They provide the broader context within which we live our lives. Living within aesthetically pleasing and culturally meaningful landscapes enhances our sense of wellbeing. It allows us to reconnect with nature and to refresh our minds.

Within the landscape the lawn provides a perfect setting for a flower beds, a border, a shrubbery or a specimen tree or a shrub; besides this it also has a spiritual value. A lawn is the source of charm and pride it reduces tension of mind after a hard work day the economic importance include tourism, branding, attractive investment, attractive residence, productive capacity it has a important role in supporting tourism small scale gardeners, nursery bed producers and also film industry. A good landscape is very important for film industry and for

there site selection it also increases indegenous habitats, regeneration and conservation todays pollution level and all other global warming effects and hurts our environment , the negative consequence of defforestation, the factories which release toxic waste, chemical spills and emission. We cannot completely solve this problem but we can reduce the effects by providing landscape it's an oppurtunity to preserve and protect the environment.it promotes planting native flora and keeping it green and healthy.

It also helps in managing soil pollution by growing plants like alfaalfasnd sunflower because they can reduce its effect. It also purifies are air and gives us fresh and healthy air it also has a cooling effect. Towns with open spaces around the building terrace industries etc are areas that require the service of landscape architecture. Landscape architecture deals with site development, building arrangement, grading, play grounds pools and so on.

The other scopes include design of walking path, patios, walls and fences ,trellis and pergolas, pools, fountains, roads, parking lots, plantings etc.

It also involves in planning and designing of national parks, resorts, golf courses, memorials, urban plazas, shopping malls and many more.

Landscaping is closely linked to arts, architecture, planning, ecology, sociology and horticulture. As a result landscapes are quite involved in interdisciplinary projects. It integrates functionally and aesthetically, the people, building and site. It also helps to develop some useful hobby and skill. Landscaping the roadside on cities and towns is also given importance in tropical countries like India as they provide shade and beauty to that area. It serves as the purpose of public recreation and education.

Different styles of landscaping:

Formal gardens:

- A formal garden is laid out in a symmetrical or geometrical pattern. In this garden, the design is similar to one other, everything is done in a straight and narrow way and it is planned in straight line.
- Square forms and rectangular forms are repeated in the various landscaping properties which are used generally as geomentricalfroms of formal garden.
- In the planing of formal garden designs the symmetrical balance can be achieved when the same objects are placed on either side of an axis which should be as mirror image.
- For example: consider a plant topiary is kept in a shape of circular on the left side same shape is repeated on the right side and same happens for the planting in tress also.
- Arrangements of the flower beds, borders and shrubbery in done in a geometrical manner.
- The shaping of hedges, edges, ashoka trees and topiary act as a typical features of a formal garden.
- Usually formal gardens are scaped for childrens park and public parks, educational institution and government buildings so that it gives a additional beauty to them.

Informal gardens:

- Informal gardens are classified by their non-symmetrical arrangements of the plants and the tress that are allowed to grow in their own way or their own shapes but makes the garden neat.
- The site that is selected is not geometric for the devolopement of an informal garden.
- Informal way of gardens are harder to design than the formal ones as they are irregular in manner and looks natural.
- Some of the hardscaping structures are added to the garden and everything will relapse into an untrammelled wilderness.
- Diagonal or curved path work well, and boundaries between the various areas of your garden are often formed using native mixed hedging.
- The general planting of the informal gardens includes tall shrubs and taller trees which add to the vertical dimension and this will often hide the edges to create a feeling.
- The garden should reveal the colours and everything should look natural to feel the nature . In case of introducing water structers you can make some pound like structers and also fountains or natural looking falls and also pools can be added.
- When making a falls or other natural structures the fittings should not appear openly to the view as they make them look artificial and unattractive.

Wild gardens:

- The wild garden is way of landscaping where no rules are followed but makes the garden look neat and good ,it's a way of developing gardens by breaking the rules of landscaping.
- The main idea of wild gardens is to make the plants and other shrubberies to look natural.
- The grass are left undisturbed unmoved and kept as it is in nature, and other bulbous plants are kept to be grown scattered in the grass to make them look like a wild scenario which adds naturality to them.
- The garden pathway should be opened with the woodland and trees, shrubs and bulbous plants should be planted among the forest flora to fulfil the idea of a wild garden.
- The creepers are allowed to grow on the trees, so that it looks very natural as mostly this is seen in wild forest and hence can be used in the gardens also.
- Presently the wild garden are represented by the butterfly gardens, birds garden, biodiversity parks, and the Indian known NakshatraUdyan or astral garden.

Tropical

- The tropical gardens are nothing but they can be filled with some wide big leaves and also some colourfull plants that are attractive are planted which makes the area attractive.

- They can be done in the backyard of the living areas, so plants should be added according to the local climate and also the plants can be chosen that are locally grown so that they can be easly maintained.
- The palm trees are addes to the garden area as they are most suitable for tropical zones also and they cost comparatively less than the other scaping plants.
- The water pools can be added to them if the required space are available, they can also be constructed in a natural way.
- In case of very small area to manage efficiently small ponds are created around which the plants can be added less leaf sheading plants are more preferable.
- Hardscaping structures like chairs or benches can be placed to sit a wile also other features for relaxation are given importance.

Prairie

- They are comparatively cost efficient than the other types of gardens.
- They can be filled with the birds and animals which makes the garden feel alluring for the people, also adds beauty to the garden.
- They can be easily maintained and also the application of herbicides and fertilizer can be added to them.
- Native plants can be preferred which makes them easy adaptable to the environment.
- More than the annuals and shrubs the perennials are chossed for the planting in these type of garden.
- Also the shrubs and the evergreens are not much used and hence makes the garden more cost efficient.
- Stones can also be added to them so that it makes the garden complete.

Desert

- Desert gardens are also cost efficient type of gardens and undergo low cost of maintenance.
- The main factor to consider is the plants that you select or choose to plant in the gardens as they are mostly done in the water scarce areas and plants are taken to extreme temperature stress.
- Mostly plants like succulents such as cactus, golden barrel, agave, desert willow, sage, grasses, desert marigold, yellow bells, aloe vera, yucca, ocotillo, joushva tree and many other plants are available,
- Sculptured rocks can be used to make the garden more attractive and make sure that it is not much bright in colour as they distract the garden plantings, mostly the dessert plants are dull in colour so this is taken into consideration.
- Artificial lights can be added to these gardens to make the plants look detailed, even the thorns adds the beauty to the garden under a good light condidtion.

English garden

- The main role in the scaping of the English garden is taken by William kent and Charles Bridgeman.
- The lawn or the green carpet is a important component of the English garden, also the rockery works are done.
- They gave more importance to the nature so they made gardens based on the natures expressions .
- Herbaceous border is also a important one in the English gardens which are made up of perennial plants.
- The classical builidings and the ruins are kept as such which also adds beauty to the garden.
- Natural stones, cabble stones as pathway, the bridges and other hardscaping components are used.
- Some important attractive components include the bee skep, the lake, haha wall, tree grove, artificial grottos and such structures are added to the garden.

Japanese garden

- Japenese garden concept is evolved from the sayings of ‘nature in miniature’, also they were said to be as the ideas of heaven.
- The japenesebelived that unless a garden has aair of peace it is not woth of visiting. It should be a place were the mind finds some rest and relaxation.
- They had many types of gardens such as the hill garden, the flat garden, the tea garden, flat garden, passage garden.
- The main components of the japenese garden include the pagoda and other garden laterns .
- The hills, water components like water falls , ponds, the islands , bridges connecting the islands, stones whith different shapes,the trees are arranged based on their utility,and many other elements with an antique sense are added.
- The japenese gave a major importance to the tea ceremony and hence they created the tea gardens, they were made in such a way that it is mainly for the relaxation of the people.
- The tea gardens are made in such a way that they are bright outside and the inner area for the tea exchange is dark by planting the trees in such a way, also the inner area is filled with some antique look.

French

- The French type of gardcens are mostly formal in nature and also they come followed by the Italian styles, they add some extra feauters to them which makes up the the French style of scaping.

- The Garden of Versilles is a main example for the French type of gardening.
- They have a well defined order and symentry among the components of the garden and over crowsding is avoided.
- They are neat and well defined gardens, they add cool colours to the garden which are complementary to the eyes.
- The stone elements and the statues are well placed with a meaning to them.
- Symentry and the geomentry acts a hallmark for the French gardens.
- Also the lawns are build for the relaxation which are mostly square or rectangular in nature, flower beds are made, tall hedges and topiary acts as a wall for the garden.

Spanish

- The Italian type of garden is influenced by the Islamic, Persian type and the Moorish types of garden
- They are mostly meant for the hot and dry climatic areas where plants are even chossen to sustain in those areas.
- The ceramic works is the major component of the Spanish garden which makes the garden look glossy and gives a royal appeal to them, also they create a great vibrant atmosphere.
- The benches, pools, statues, arches and many other components are made up of ceramics which are shining and attractive to the eyes.
- Even after centures the Spanish gardens are appeared to be the same, this is because of the mixture of grandiose and the pastoral works by them, the curves and the columns, and the vibrant colours they used.

Gardens in India:

1. Mughal Garden, Srinagar

The Mughal Gardens in Srinagar comprise of Nishat Bagh, Shalimar Bagh, ChashmeShahi, Pari Mahal, Achabal and Verinag Garden. Jahangir was responsible for the selection of the site and planning the requirements of the paradise gardens. It is always a lot of fun in exploring the history behind these gardens. They follow a Persian style of architecture and have been influenced by the Persian gardens. The landscape of the city has evolved ever since the Mughal Gardens have been built. The concept of gardens was introduced in South Asia by Babur in the form of planned charbagh which was persistent in Farghana, his homeland as well as his kingdom Kabul. According to Babur, the Hindus did not use running water for gardens, and he considered only enclosed spaces to be gardens in the real sense. Taking into consideration all these faults, he started building gardens with all the needed waterworks to bring him back to his homeland. According to legends, Shalimar Bagh, one of the Mughal Gardens was built during the rule of Pravarsena II who had made the district of Srinagar and lived in a cottage

situated nearby. After Emperor Jahangir became the ruler, he paid a visit to this place and decided to build a beautiful garden there for his wife, Nur Jahan.

Pinjore Garden, Chandigarh

Pinjore Gardens is a beautiful Mughal Garden made in 17th Century India located in the city of Pinjore, in the district of Panchkula, Haryana. The garden expands over a massive area of 100 acres and is a beautiful place to unwind with nature in the ever-growing concrete city. The Pinjore garden is also known as YadvinderGarden which is famous all over the world for its well-maintained greenery, refreshing fountains and mesmerising water bodies. The Pinjore Garden has the Charbagh Pattern and is built in traditional Mughal style. The area is cleverly designed to have terrace gardens, grand pavilions and beautiful fountains. There are a total of seven terraces in the garden that are built descending into a distance. This feature adds a magical touch to the entire stretch of 100 acres of the garden. The flora consists of a variety of aromatic flowering plants, Mango orchards, shrubs and other trees. The lined tall trees provide cool shade for visitors walking on the pathways. There is also an open-air theatre at the end of the garden. Pinjore Garden is also adorned by a central watercourse that flows in the middle of every level, shimmering fountains, several other pools and arched balconies amidst colourful and fragrant flowerbeds and lush green lawns

Lalbagh, Banglore

Referred to as Rose or Cypress Garden up till 1856, this garden was commissioned by Haider Ali in 1760 who was a big admirer of Mughal Gardens and wanted to set up one in his own city as well. He was influenced by his father who used to work for Dilawar Khan, a viceroy of the Mughals and was especially passionate about setting up gardens. The garden is modelled on another one that existed in Sira which was situated 120 km away from Lalbagh. Over the years, many species of rare plants, trees and shrubs were added to Lalbagh and most of these were added by Tipu Sultan himself.The garden area stood at 45 acres in 1874 with major additions done in 1889 (30 acres) and 1891 (94 acres). The foundation of the famous Glass House was laid down in the year 1898 and was further built by John Cameron in the image of London's Crystal Palace.

Rose Garde, Chandigarh

Spread across a vast area of over 30 acres, Rose Garden is one of the Asia's largest Rose garden. It has several entrances and a parking facility as well. It is adorned with long stretches of green grass to walk on and several benches placed under the trees for relaxing in the shade. Long stretches and curved paths are all over the park for joggers or those exercising. A small lake-like structure along with a colorful fountain is also present in the

garden where you can relax and unwind or do a bit of bird watching. You can also find some food stalls installed outside the garden.

Brindavan Garcen, Mysore

The Brindavan Gardens, spread over 60 acres, is located at a distance of 21 km away from Mysore. Built across the notable river of India, Cauvery, it took around five years to complete the project. The well-synchronised fountain show with music, boating and well-manicured grass with flowerbeds are some of the top experiences of Brindavan Garden. Constructed in 1932 by the Diwan of Mysore, Sir Mirza Ismail, Brindavan Garden is visited by millions of tourists every year. Divided into two parts, north and south, boating facility offered by the Karnataka State Tourism Development Corporation which connects the two parts along with a walkway bridge.

Rock Garden, Chandigarh

Nek Chand started building the Rock Garden secretly near Sukhna Lake in the year 1957. It was based on his vision of the divine kingdom of Sukarni. He would collect waste materials from around the city in his spare time and use them to build structures on a forest buffer land which had been rendered infertile. His work continued for 18 years and spread over 12 acres through a complex of interlinked courtyards. Although his work was illegal, he managed to hide it from the authorities until it was discovered by the authorities in 1975. Rock Garden faced the danger of being demolished on the grounds of being illegal but was saved by popular public opinion. Nek Chand was then put in charge of the park and given a team of 50 labourers to work on his project. The park was opened to the public in 1976 and today is visited daily by around 5,000 people. Rock Garden consists of man-made interlinked waterfalls and many other sculptures that have been made of scrap and other kinds of wastes (bottles, glasses, bangles, tiles, ceramic pots, sinks, electrical waste, etc, these are placed in the walled paths. The sculptures are mainly made from recycled ceramic

Nishat Bagh, Srinagar

The Nishat Bagh is a 12 terraced garden located near Srinagar's famous Dal Lake. It is the second-largest Mughal garden in Kashmir after Shalimar Bagh. Popularly known as "Garden of Bliss", it has a splendid Mughal central water channel with several fountains, which is surrounded by tall Chinar trees. The Nishat Bagh was designed and built in 1633 by Asif Khan, elder brother of NurJehan. The then emperor of Mughal India Shah Jahan was in Kashmir in 1633, when he visited this garden. He was so impressed by the heavenly beauty of the Nishat Garden that he had wanted the garden to be gifted to him by Asif Khan. When Asif Khan failed to do so, the water supply to the garden was stopped by the emperor. Distraught, Asif Khan was heartbroken and lost interest in everything else. One day, as he was sitting beneath a tree in the garden, one of his servants was bold enough to turn the water supply from Shalimar Bagh. When Khan got to know about this, he ordered for it to

turned off immediately fearing Shah Jean's livid reaction to the breach of his orders.However, Jehan did not really get annoyed at the situation. Instead, he appreciated the servant for loyal service to his master and then ordered full restoration rights for the supply of water to the garden to Asif Khan.

Shalimar Bagh, Srinagar

Shalimar Bagh is a beautifully laid out Mughal garden, the largest of the three Mughal gardens in Srinagar (the other two being NishatBagh and ChashmeShahi). This pristine attraction was built in the year 1619 by the Mughal emperor Jahangir for his beloved wife Nur Jahan and lies overlooking the scintillating waters of the Dal Lake. It is now a public park and is known as the "crown of Srinagar".Thechinikhanas or arched niches placed behind waterfalls and the chinar trees are the highlights here. The term 'Shalimar' is a Sanskrit word meaning 'Abode of Love' and is known by several other names such as FaizBaksh and Farah Baksh. Sprinkled generously with well-trimmed gardens and exquisite architecture, Shalimar Garden is a gentle blend of natural allure and man-made structures.

Ooty Botanical Garden, Ooty

Ooty Botanical Gardens lie on the lower slopes of the Doddabetta peak, the Government Botanical Garden is a splendid garden in Udhagamandalam, near Coimbatore in the state of Tamil Nadu. Sprawled over an area of 22 hectares, the garden is divided into several sections that are trimmed beautifully to present an endearing sight. The foundation of Ooty Botanical Garden was laid down in the year 1848 and was designed so as to have a terraced terrain. The slopes of the hill lie at an ascent of 2500 metres above mean sea level, as a result of which the garden enjoys a temperate climate ideal for a number of plants and shrubs to grow. The Botanical garden in Ooty is home to many rare species of trees, chief among them being the cork tree, the only one of its kind in India. The paperbark tree, the monkey puzzle tree and an old fossilized tree estimated to be 20 million years old round out the other rare species. The monkey puzzle tree has been named so because even monkeys fail to climb it. In addition to these, a number of exotic and indigenous vegetation of plants, shrubs, ferns, trees, herbal and bonsai plants are found here as well.

International scenario of landscape:

The ecosystems of our intensively used European landscapes produce a variety of natural goods and services for the benefit of humankind, and secure the basics and quality of life. Because these ecosystems are still undergoing fundamental changes, the interest of the society is to know more about future developments and their ecological impacts. To describe and analyze these changes, scenarios can be developed and an assessment of the ecological changes can be carried out subsequently. In the project "Landscape Saxony 2050"; a methodology for the construction of exploratory scenarios was worked out. The presented methodology provides a

possibility to identify the driving forces (socio-cultural, economic and ecological conditions) of the landscape development. It allows indicating possible future paths which lead to a change of structures and processes in the landscape and can influence the capability to provide ecosystem services. One essential component of the applied technique is that an approach for the assessment of the effects of the landscape changes on ecosystem services is integrated into the developed scenario methodology. Another is, that the methodology is strong designed as participatory, i.e. stakeholders are integrated actively. The method is a seven phase model which provides the option for the integration of the stakeholders' participation at all levels of scenario development. The scenario framework was applied to the district of Görlitz, an area of 2100 sq km located at the eastern border of Germany. The region is affected by strong demographic as well as economic changes. The core issue focused on the examination of landscape change in terms of biodiversity. Together with stakeholders, a trend scenario and two alternative scenarios were developed. The changes of the landscape structure are represented in story lines, maps and tables. On basis of the driving forces of the issue areas "cultural/social values" and "political control", three scenarios were developed up to the time horizons in 2030 and 2050. They are titled "Trend", "Tradition and Ecology" and "Technology and Energy". These scenarios differ markedly in the degree of the future lignite exploitation, in the use of renewable energy and in the environmental compatibility of the agricultural production. In total, the investigation shows that the integration of the ecosystem services approach into the scenario technology has brought new aspects. However, the procedure became more complex. For the development of the scenarios a precise definition of the driving forces turned out to be essential. The experiences of the project further show that only two or at most three key driving forces (KDF) can be distinguished really sensibly or can be looked at in their interactions. It could be shown that from these results itself concrete measures can be derived which support desirable developments or counteract against undesirable effects. By the integration of stakeholders in different working steps, the scenarios can contribute to the sensitization and better perception of future problems and chances of a region.

Color combination of landscaping:

Color is usually the first thing people notice when they see a flower garden, and choosing plants in pleasing color combinations can be daunting to a new gardener. The color wheel is a helpful tool. It's based on three primary colors — red, yellow, and blue — and also depicts the colors in between.

The concentric bands show the colors in different degrees of saturation. Fully saturated colors are the most intense; as you move toward the center of the wheel, the colors become softer, more pastel.

Warm colors: Yellow, orange, red, magenta — these "warm" colors bring energy and excitement to a planting. When planted at a distance, they draw the eye in.

Cool colors: Purple, violet, and blue tend to be soothing and quieting. They can get lost at a distance and so are best for close-up viewing.

Complementary Color Scheme

In complementary color schemes, two colors on opposite sides of the color wheel are combined.

- Brings energy and excitement to a planting
- Examples: blue and orange, yellow and purple, red and green
- Use varying hues (shades of blue, for example) to keep it interesting
- If needed, tone it down with quieter colors and foliage

Analogous Color Scheme

To create an analogous color scheme:

- Select three neighbors on the color wheel (colors that are adjacent or near each other)
- Makes it simpler to choose plants that are attractive together
- Examples: yellow, orange and red; blue, purple and red

Monochromatic Color Scheme

Another option for a monochromatic color scheme is a "moon garden." Gardens featuring all white flowers are especially beautiful when viewed by moonlight. Good choices include moonflower (*Ipomoea alba*), night-blooming jasmine (*Cestrum nocturnum*), and evening stock (*Matthio laincana*).

Landscape and garden designers base their efforts on a number of principles, including form, line, texture, scale, and color. Secondary principles that rely on the five main principles include proportion, transition, and unity.

Color in Context

Your choice of colors to be used in the yard should not be considered in isolation. Always keep in mind how color interplays with the colors of other basic elements, with the other principles of landscape design, and with the overall objectives of your plan.

Remember, too, that color, along with the other essential design elements, applies not only to the overall landscape but also to garden beds and planting areas within the landscape. In an individual flower garden bed, the principles of line, form, texture, scale, color, proportion, transition, and unity also apply on an individual scale. The only difference may be that color is even more important in a garden, since this is the place where we usually want color to be the star.

The Color-Wheel Categories

Color theory in design is based on the color wheel, a standard circular illustration that shows the relationship between all the various colors of the spectrum. The spectrum of colorsis often divided into four categories:

- **Primary color**s: reds, yellows, and blues
- **Secondary colors**: greens, violets (purples), and oranges
- **Tertiary colors:** Blends of the primary and secondary categories
- **Neutral colors**: White, grays, and silvers. Gray is an unusual color for blooms or berries, but an example is to be found on bayberry shrubs.

The secondary colorsare produced by blending two primary colors in equal proportions. Thus, red and yellow combine to produce orange, yellow and blue produce green, and red and blue yield purple.

The blends known as "tertiary colors" add a further element of complexity to the color wheel. They are combinations of primary with secondary colors, producing not entirely different colors, but colors that include qualities of both:

- Yellow-green Blue-green
- Blue-violet Red-orange
- Orange yellow

Combining Colors

Using color theory as your guide, you can choose the colors you use in your landscaping so that they "go together" to produce different effects. This can be done in a number of ways.

Cool Colors vs. Warm Colors

One common way of categorizing the colors of the spectrum is by dividing them into warm colors and cool colors. This categorization is often used to influence mood and perception in a landscape. Blue purple and green are considered "cool colors" and their impact on viewers tend to be relaxing and calming. Thus, for a meditation garden, blue and/or purple flowers are logical choices. Red, yellow, and orange are considered "warm colors," and they tend to excite and invigorate the viewer. In addition to using the warm/cool qualities to influence mood, warm and cool colorscan be used for other effects:

- In a small yard, combining warm and cool colors can change the perception of depth. Place flowers with warm colors in the foreground. Behind them, position flowers with cool colors, starting with darker shades (such as purple), followed by shades that are successively lighter. This will create an illusion of depth. You can also create this illusion by placing larger plant material in the foreground, then tapering off the size of your plants as you work your way in deeper. The effect is to make the garden seem much larger and deeper than it actually is.

- Warm colors like red can make overly large spaces seem smaller and more intimate. The warm colors appear to come forward in the landscape and seem closer than they are in reality—thereby scaling down the whole landscape in the process.
- The warm colors are born attention-grabbers since they bring a mood that arouses rather than relaxes. To draw visitors into a space, create a focal point using red, yellow, or orange—or all three.

Unity and Contrast

Another application of color theory can be seen in the use of color to create either *unity* or *contrast*. Landscapers may stay within the warm-colors group or the cool-colorsgroup in order to provide unity, whether it is within one planting bed or throughout the yard. In the latter case, different parts of the yard can be tied together to form a harmonious unit.
An unusual form of unity occurs when pairs of colorsare chosen that lie exactly across from one another on the color wheel. You might expect that such colorswould be viewed as contrasting, but in reality, these pairs produce a reassuring and "right" feel to viewers. These are known as complementary pairs. There are three pairs of complementary primary colors, including:

- Yellow and blue
- Blue and orange
- Red and green

Shifting directions on the color wheel gives another set of complementary colors:

- yellow-orange and purple
- orange-red and blue-green
- red-purple (pink) and green-yellow

Any number of complementary pairs can be determined simply by shifting positions on the color wheel, but for the purposes of planning flower-color combinations, designers usually confine their discussions to the primary and secondary colors. Complementary color pairs are thought to be pleasing in part because they highlight and intensify the experience of their opposites. Thus, this is one form of contrast that works very well in the landscape.

When color pairs are used that have no discernible relationship on the color wheel, the contrasting effect can be a bit jarring, and the pairing is sometimes said to be *clashing*. But there may be occasions when you simply have a great fondness for two colors and want to use them in a garden. The tertiary colors can then serve as transitional colors in such situations. For example, if you want a garden color scheme using reds and violets because they are favoritecolors, a plant or flower with a red-violet color can help bridge the gulf between the two colors. In this situation, the addition of the third plant color makes the difference between a slightly jarring effect and a smoother, more harmonious ensemble.

Using Neutrals

Neutral colorscan also be used to soften the effect of loud color schemes or stand on their own in a monochromatic scheme. True blacks are rare in gardens and landscapes, but all-white gardens consisting of various shades of whites and creams are sometimes used in so-called moon gardens, which are designed to be enjoyed at night.

Using color theory in gardening

Though not easy, using color theory in designing the hardscape of an overall landscape is a learnable skill. Applying these skills to a garden can be somewhat more tricky, since Mother Nature doesn't always cooperate with our abstract plans for how color should work. Not all plants will automatically bloom during the same season, and foliage color also changes from season to season in some cases. And the structure of a garden will change over time, as shrubs grow and perennial plants mature and spread.

For example, the black-eyed Susans you chose for their warm deep yellow tones won't contribute that color at all in spring, but will provide it in ample amounts in late summer and fall. You may need to add spring-blooming daffodils to provide that yellow pop in the spring time; they will quickly fade and make room for other plants. Thus, there is a unavoildable element of time that enters into garden design, which is not really present for designers working on interior spaces. When designing a color scheme, always learn about blooming times in your region before buying plants.

Matters are perhaps most difficult for flower gardeners aiming at a particular color scheme for fall, since most flowering plants are naturally prone to delivering their flowers in spring or summer. Even chrysanthemums, the most popular autumn flower, have to be coaxed into attaining the familiar form by nurseries who carefully control the lighting conditions. Potted fall mums that are then planted in the ground will revert to flowering in mid-summer. Fall coloris often controlled by the selection of plants with notable fall foliage colors, or colorful berries, or both.

But with a little extra planning and work on your part, you can include flowers in your fall color schemes. This can be a matter of choosing species known to flower in fall, or those who have notable fall foliage or berries. Fall colorcan also be assisted by late planting of some species. Waiting until fall to put in salvia transplants, for example, will spare them the heat of summer that usually destroys the spring-planted salvias. Pansies are another flower that often goes away in mid-summer, but can be planted again for fall. Finally, many plants can have their bloom period extended through diligent deadheading of their spent flowers. For example, lupines that are closely cropped after the summer bloom often come back with a lesser flush of fall flowers.

Here are some suggestions for flowers in particular color groups:

Flowers for Red Blooms

- Stewartstonian Azalea
- Red Coleus (Foliage)
- Red Salvia
- Castor Bean
- Red Amaranth
- Virginia Creeper (Foliage)

Flowers for Blue Blooms

- Blue Scilla
- Grape Hyacinth
- Cornflower
- Bluebeard
- Blue iris

Flowers for Purple Blooms

- Rose Campion
- Annual Purple Lobelia
- Purple Verbena
- Jackman Clematis
- Perennial Purple Lobelia (*Lobelia x speciosa* 'Grape Knee Hi')
- Heliotrope
- Purple Iris

Flowers for "Black" Blooms or Dark Foliage

- "Black" Rose
- Castor Bean

Flowers for Yellow Blooms

- Yellow Daffodils
- Forsythia
- Marsh Marigolds
- Yellow Iris
- Stella d'Oro
- Yellow Yarrow
- Black-Eyed Susan
- Sunflower
- Goldenrod

Flowers for Orange Blooms

- Crocosmia
- Orange Canna
- Orange Zinnia
- Trumpet Vine
- Orange Impatiens
- Orange Nasturtium
- Bittersweet Berries

Flowers for White Blooms

- White Daffodil
- Star Magnolia
- Callery Pear
- Mountain Laurel
- Queen Anne's Lace
- White Cleome (often pink and white together on the same flower)
- White Allium
- Autumn Clematis

Flowers for Pink Blooms

- Pink Hyacinth
- Weeping Higan Cherry
- Mandevilla
- Pink Cosmos
- Tall Pink Garden Phlox

Plants for Silver Foliage

- Dead Nettle
- Silver Mound Artemisia
- Silver King Artemisia
- Lamb's Ears
- Dusty Miller

Flowers for Lavender Blooms

- Lavender Crocus
- Lavender Delphinium
- Lavender Rose of Sharon
- False Dragon Head

Layout of landcapes

In the early stages of design development, landscape designers are thinking in terms of form composition-the combining of lines and arcs to form geometric shapes such as circles, squares and triangles. This combination of forms is what establishes the edges of each outdoor room; from bed lines defining areas of lawn to the edge of a terrace or swimming pool. The same form used repetitively throughout the design creates a "visual theme". It is what establishes consistency, harmony and a sense of order in a garden space. Below are six layouts for various backyard landscape types that serve as a guide to landscape designers when developing a site.

Curvilinear

The curvilinear approach to design is the most typical amongst landscape designers; even on sites where another approach would be much more appropriate. Sometimes referred to as 'natural', this is a mistake because the curvilinear forms are created from an underlying geometry following very subtle guidelines. The curvilinear design approach is most appropriate for large, open sites where the large sweeping curves can lead the eye about the property. It's also the best approach for rolling terrain where the curves can accentuate and accommodate changes in grade. The curvilinear approach is often mistakenly used on small and restrictive sites where the forms created become an inefficient use of space.

Radial

A radial theme, or circular, theme is created by using concentric circles radiating from a central point (and straight lines) or by combining overlapping circles. The illustration shows a combination of concentric circles and straight lines. This approach differs from the others in that forms are created from an underlying radial grid versus a square grid. This makes for very interesting, dynamic spaces. I rarely see the radial approach used in residential settings. On the few residential sites I have seen it applied, the home is typically of a contemporary style and is itself designed from a radial grid (at least part of it). The garden simply radiates from the central point of the home following the concentric patterns.

Rectilinear

A rectilinear theme is created in plan-view through the use of vertical and horizontal lines on a square grid. Just as a homes architecture is designed in this way, the same treatment of the garden reinforces the rectangular layout of the home. The rectilinear theme is most appropriate for creating garden spaces, or rooms, that serve as an extension of the home-decks

and patios for instance. Backyard partitions can be helpful when trying to create distinct and separate spaces. This approach ensures for a much more efficient use of space. The rectilinear design theme is also ideal for restrictive sites like city gardens enclosed by fences and walls. Level ground is best for this theme although slopes can be terraced to extraordinary effect using a rectilinear approach. Because this approach is more orderly and logical, it suits formal architectural styles perfectly.

Rectilinear-45

Often referred to as the diagonal approach, forms are created in the same way as the rectilinear approach only diagonal lines are introduced; most typically 45-degree although lines of 60-degree are acceptable. Often times this theme will be utilized without the vertical and horizontal lines. These diagonal lines can make a space more dynamic and exciting as well as make a space seem larger. This approach is used on similar sites as the rectilinear approach only less with formal, traditional architecture. I've seen the rectilinear-45 approach utilized very successfully with contemporary, modernist homes designed from similar diagonal lines.

Arc-and-Tangent

The arc-and-tangent approach is a combination of the four previous approaches. Using vertical, horizontal and 45- degree lines with arcs and circles, a well-organized and dynamic space can be created. I find that the arc-and-tanget approach can be well adapted to many different types of sites and architectural styles. The straight lines provide a feeling of structure while contrasting with the more "natural" soft curves. I've seen this approach used successfully on large sites where larger and more numerous arcs are introduced as the garden moves away from the home towards a woodland or open field.

Irregular

The irregular theme combines multidirectional lines on a square grid; not restrictive to only 45-degree or 60-degree lines. This approach can make for a very bold and complex space. As with the radial theme, this is not common on residential sites, although I have seen it executed effectively with contemporarystyled homes on occasion.

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MICROBES AS GENETIC ENGINEERS

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Abstract:

The use of genetically engineered microorganisms is recently increasing in the production of modified products used in health, food industry, improvement of genomic studies etc. These modified organisms increase efficiency, resource requirements, enhancement of bioremediation, and increase in degradation of hazardous waste. The present chapter is an attempt to orview the role of some important microbes as potential genetic engineers in different fields.

Introduction:

Genetic engineering is a direct modification and manipulation of genes of any organism or microbe. The technique is basically used to change or modify the cells at gene level which includes intraspecific or interspecific gene transfer so as to produce organisms or microbes with improved and better characters. The change in gene levelin microbes is known as Microbial genetics. Different microbes are studied under microbial genetics. Bacteria and archea are the main microorganisms that are observed and studied alongwith some fungi and protozoa. The genotypes (inheritable form of microbes) and expression system of the microbes are considered for study.Recombinant DNA technology is used in microbial genetics and create recombinant DNA molecules by changing DNA sequence. Important example of genetic engineering is gene cloning.

Robert Hooke and Antoni van Leeuwenhoek studied many different processes and applications in numerous areas of study in genetics. For example:Scientists have used microorganisms in evolutionary studies due to their rapid growth rates and short generation time. Hooke and Leeuwenhoek included observations, depictions, and descriptions of microbes. *Mucor* (microfungus) is the first ever illustrated microbe which wasdescribed by Hooke.Studiesby Leeuwenhoek provided scientific observations and descriptions about microscopic protozoa and bacteria. Microbial genetics is also helpful to study the processes and pathways similar to those found in humans like drug metabolism.

Role of Microbial genetics in understanding evolution:

Scientists focused on theories and works of Charles Darwin by using natural selection theory as a source for study by using microbes. For example: Study of genetic drift or natural selection in microbes. This study involved identifying pathways, genes and their functions. After observing and studying the subject, scientist compare it to a sequence of a conserved gene. However, this way of evolutionary study does not provide the time scale of occurrence of evolution, however rates and outcomes of evolution can be predicted. The key point of microbial genetics evolution is studying the relationship between microbes and the environment.

Microorganisms described using Microbial genetics:

1. **Bacteria:** Bacteria are part of this planet since 3.5 billion years. Bacteria are further classified based on their shape. Bacterial genetics allows to study their chromosomes, heritable features, different types of plasmids, transposons, and associated phages.

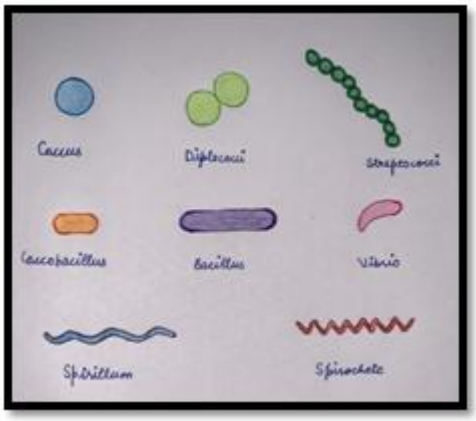

Figure 1: Different types of bacteria

Gene transfer system is a process through which genetic or genomic details of a bacteria is delivered to another one.The transfer can occur vertically or horizontally. Transfer of genes to offspring from the parent organism is vertical transfer and horizontal transfer occurs between two independent organisms.The three types of gene transfer systems are observed and studied in bacteria: Transformation, Conjugation and Transduction.

a. **Transformation**: In bacteria, first horizontal gene transfer was discovered by Fred Griffith. This process involves uptake of DNA fragment or donor DNA by a cell from surroundings and then incorporation of this donor DNA into recipient chromosome. Transformation can be natural or artificial. Natural transformation occurs occasionally and can be seen in both gram-positive and gram-negative bacteria. Competence is the ability of the recipient bacteria to take the donor DNA and incorporate it in its own DNA. Transformation is a complicated development process that require lot of energy. Transformation mechanism have

been studied deeply in *Streptococcuspneumonia* (gram-positive bacteria), *Haemophilus influenzae* (gram-negative bacteria). (Lorenz and Wackernagel, 1994)

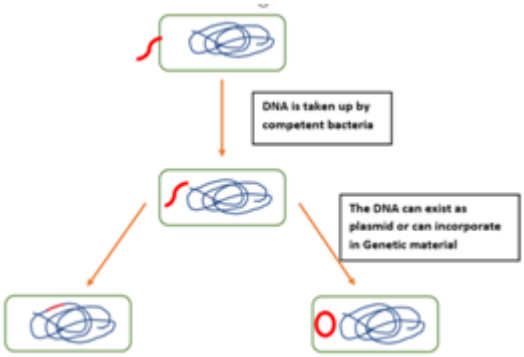

Figure 2: Uptake of DNA by Bacteria through Transformation

b. **Conjugation**: The process of conjugation was observed by Joshua Lederberg and Edward Tatum. This process includes the transfer of genetic material from one bacterium to another by establishing direct contact between cells or by forming cytoplasmic bridge between the two cells. The completion of this process requireslong contact between the donor and recipient bacteria with stability. The donor DNA fragment is then incorporated into chromosome of recipient bacteria by homologous recombination. Ability of donor is described by conjugative plasmids present in both gram-positive and gram-negative bacteria. F-plasmid is the most studied plasmid. The cell which receives donor DNA is called transconjugant.

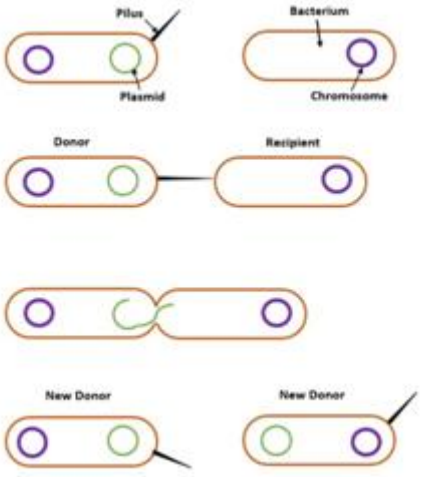

Figure 3: Uptake of DNA by Bacteria via Conjugation

c. **Transduction**: The process was discovered by Zinder and Lederberg in *Salmonella typhimurium*. Transduction process involves transfer of DNA from donor bacteria to recipient by introducing the DNA into virus or viral vector or bacteriophages. It is of two types

generalised and specialized transduction. Generalised transduction occurs in lytic cycle of virus and phage whereas in specialized transduction it occurs in lysogenic cycle. The process of transduction is practiced by molecular biologists to introduce any gene into stable host.

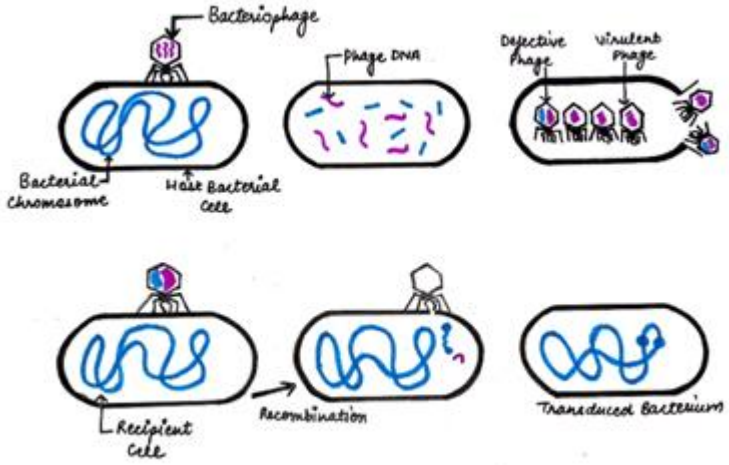

Figure 4: Uptake of DNA by Bacteria via transduction

2. **Archea**: This group of organisms was introduced on planet 4 billion years ago. They differ from bacteria in processes like replication, transcription, and translation. These can be gram-positive and gram-negative and can have different shapes like bacteria. They lack peptidoglycan in cell wall which is a characteristic feature of bacteria. Archea reproduce by binary fission (asexually). These show motility with help of flagella. Some of the archaebacteria can survive in extreme environments that increase its applications in genetics.

Archea are classified in 3 subgroups:

a. **Methanogens**: These are anaerobic organisms which get killed if exposed to sunlight. These live in swamps, marshes, gut of humans and play important role in decomposition. methanogens can act as an electron sink for anaerobic hosts.

The symbiotic methanogens may be ectosymbiotic - on the surface of ciliates of the family Entodiniomorphidasurviving in the rumen of cattle

Intracellular – *Methano bacterium formicicum* in Plagiopyla

Metopus species in rice paddies and land-fill sites

Methano brevibacter species in cellulolytic protists in termite guts.

b. **Halophiles**: These organisms can tolerate high salt concentration and present in high salt concentration areas like Great Salt Lake and the dead sea.Eg: *Halobacillus, Halococcus, Halobacterium*

c. **Thermoacidophiles or Thermophiles**: These organisms live in acidic areas which have low pH like hot springs.Eg: *Thermoplasma, Thermoproteus,Thermococci*

Archea contain single circular chromosome with multiple origin of replication. The primase used in replication is different in case of archea. It uses RNA recognition motif (RRM), a highly derived version.

3. **Fungi**: Fungi are heterotrophic, unicellular, or multicellular, spore-bearing eukaryotes. These organisms secrete some enzymes that are responsible for breakdown of organic matter. Yeast and filamentous fungi areused for research purposes including chromatin structure, cell cycle and regulation of genes. Fungi is classified on basis of reproductive structures and sexual spores.

a. **Chytridiomycota**: produce zoospores that move through liquid medium.

b. **Zygomycota**: zygospores (meiospore) are sexual spores and sporangiospores are asexual one. Example:*Rhizopus stolonife*r (Black bread mould)

c. **Ascomycota**: form ascospores as meiotic spores. Example:*Neurospora crassa*

d. **Basidiomycota**: basidiospores are sexual spores. Example: Mushrooms, smut, and rust fungi.

Neurospora crassa of ascomycetes or red bread mould is a type organism for research and study, as it is easy to grow having haploid life cycle which make the study simple and easy. This fungus grows in tropical and sub-tropical regions or on dead plant matter. Two researchers Edward Tatum and George Beadle won Nobel prize in Physiology or medicine in 1958 for their experiments conducted on *Neurospora*. The results of their experiments lead to one gene-one enzyme hypothesis and proved to be very helpful in molecular genetics.

4. **Protozoa**: These are unicellular, heterotrophic organisms with genetic importance due to flagella like flagella of human sperm.Example:*Paramecium, amoeba, Plasmodium*.*Paramecium* consists of one polyploid macro nucleus which controls non-reproductive functions and one or more diploid micronuclei which is generative and transfer its genetic material to next generation. *Parameciu*mis important for studying meiosis.

5. **Viruses**: Viruses are simple organisms containing DNA or RNA enclosed in a capsid of protein. They can assemble themselves in host's replication system after replication. These are very important not only for genetic studies as well as for pathogenic studies.

Genetic recombination: The process in which 2 or more viruses of same type infect any cell and get their genome combined with each other to form recombinant progeny. Recombination can be shown by both RNA and DNA containing viruses.

Multiplicity reactivation: When viruses having lethal genome infect same host cell together, their genome get paired and show homologous recombinational repair to get viable progeny. This process can be seen in pathogenic viruses like influenza virus, HIV-1, reovirus, herpes simplex virus and bacteriophages.

Genetically modified bacteria wereone of the first organisms to be genetically modified in laboratory conditions. In 1978, Herbert Boyer in laboratory on University of California, inserted human insulin gene in bacteria *Escherichia coli* to produce synthetic human insulin and then later on it was approved by U.S. Food and Drug Administration.

Molecular tools used in genetic engineering of microorganisms:

1. **Gene transfer method**: The process by which selected genes of interest into desired hosts. It is the method in which recipient microbe take up the plasmid DNA when they show physiological competence. Alternate method is called electroporation that is used to transform eukaryotes. The recipient cells are made electrocompetantusing high voltage pulses which make pores in cell membrane and DNA uptake is allow

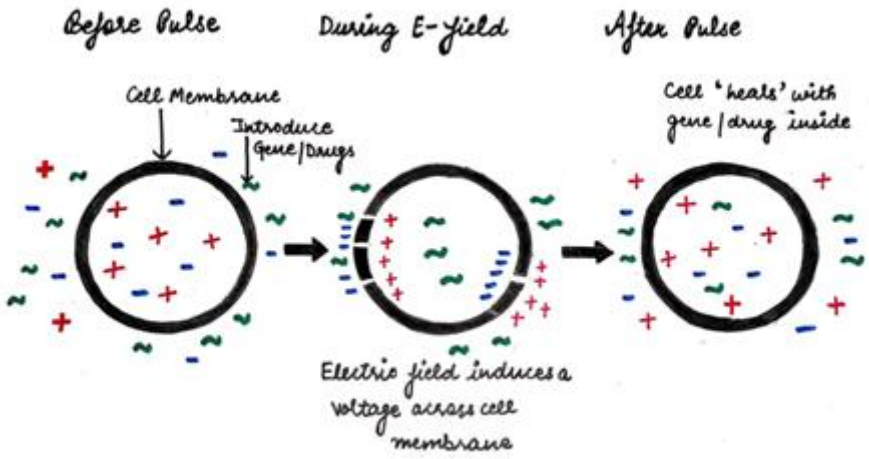

Figure 5: Gene transfer method

2. **Cloning vectors:** These are responsible for genetic manipulation depending on choice of gene to be transferred.

3. **Promoters**: A DNA segment that regulate gene expression.

- Constitutive promoters: which are continuously active
- Inducible promoters: which activate only in certain condition and involves presence of inducer.

4. **Selectable marker genes:** These are used to identify transformants. Examples: Puromycin acetyltransferase, blasticin deaminase, Bleomycin resistance gene, Glutamine synthase

Vector	Host cell	Insert Range (in kb)	Structure of vector
M13	*E.coli*	1 – 4 kb	Circular virus
SV 40	*Mammalian cells*	6.4 kb	
Plasmid (Pacyc177, Pbr322, Pbr324, pM89)	*E.coli*	1 – 5 kb	Circular plasmid
Phage lambda	*E. coli*	2 – 25 kb	Linear virus
Cosmid (pJC74, pJC720, Phc79	*E. coli*	35 – 45 kb	Circular plasmid
BAC's- Bacterial Artificial Chromosome	*E. coli*	50 – 300 kb	Circular plasmid
YAC's – Yeast Artificial Chromosome	*Saccharomyces cerevisiae*	100 – 2000 kb	Linear plasmid
Yeast episomal plasmid (YEp)	*Saccharomyces cerevisiae*	High copy number; can integrate into yeast chromosomal DNA or replicate independently	
Yeast integrative plasmid (YIp)	*Saccharomyces cerevisiae*	Stable, integrates into yeast; cannot exist independently in yeast; single copy number	
Yeast replicative plasmid (YRp)	*Saccharomyces cerevisiae*	High copy number; unstable; carries yeast chromosomal origin of replication	
Yeast centromere plasmid (YCp)	*Saccharomyces cerevisiae*	Low copy number, contain centromere sequence; stable	

Applications of genetically engineered microorganisms:

- **Human health:**

Recombinant therapeutics proteins: Mostly proteins obtained from bacteria are human proteins for medicinal use. Many out of these proteins cannot be obtained naturally.1st ever FDA approved recombinant therapeutic protein (Human insulin)was produced using genetically engineered *E. Coli* having genes of human insulin. This insulin is used to treat diabetes.After that

in 1985 Human growth hormone was produced using *E. Coli* with human growth hormone gene to treat dwarfism. Other medicines include clotting factors for treatment of haemophilia, interferons for treatment of cancers.

Recombinant vaccines: Common baker yeast (*Saccharomyces cerevisiae*) produce vaccine for hepatitis B virus using surface antigen. (Hilleman, 1987).

Bacteria as therapeutic agents: ***Lactobacillus***is modified with genes enhancing its ability to protect body against HIV. Another example is **tooth-decaying bacteria** which is altered to the form in which it does not produce tooth corroding lactic acid.

- **As Food**:

Bacteria are altered and modified for food production. They are modified to produce amino acids, enzymes, flavouring substances and many more products. Genetic engineering help to easily introduce new and useful changes in bacteria. Lactic acid bacteria are the most common example of genetically engineered bacteria for food production. It is modified for its increased efficiency, less by product, increased output and to remove unnecessary pathways. Other food products that we get from bacteria are alpha-amylase (breakdown of starch), chymosin (clot milk protein to make cheese) and pectinesterase (improves clarity of juices).

- **In Textile industry**:

Amylases are used to remove starch sizes from fabrics called desizing. Initially, amylases obtained from animals and plants were used but later on these get replaced by bacterial amylases produced by genetic engineering. Example:Alpha-amylase of *Bacillus stearothermophilus*.

- **In Agriculture**:

Used to increase the amount of nitrogen fixed by microbes. To achieve this nitrogen fixing genes are inserted in *Rhizobium meliloti* bacteria. Bt (*Bacillus thuringensis*) gene is used to protect crops from infestation and other diseases. Originally, blue pigment dye Indigo was obtained from plants but now it is produced by recombinant E. Coli with plasmid NAH7.

- **Insecticidal**:

Toxin gene of *Bacillus thuringensisisraelensis* is inserted in photosynthetic cyanobacteria *Synechocystis, Synechococcus* and aquatic bacteria *Caucobactercrescentus*. These bacteria are food source of larvae of mosquitoes which get killed after eating highly toxic insecticidal protein.

- **Bioremediation**:

The process in which pollution is reduced using biological microbes. Genetically engineered microbes can clean the oil spills.

List of the principal genera of bacteria, microalgae and fungi involved in bioremediation. (Dell' Anno *et al*., 2021)

Table 1: Applications of different microbes as genetic engineers

Sr. No.	Name of Microbes	Applications	Reference
1	*Escherichia coli*	Production of human insulin	Baeshen *et al.,* 2014
2	*Saccharomyces cerevisiae*	Production of vaccine for Hepatitis B	McAleer *et al.,* 1984
3	*Lactobacillus*	Therapeutic agent for protection against HIV	Rogers *et al.,* 2017
4	*Bacillus stearothermophilus*	Production of alpha-amylase	Yao *et al.,* 2019
5	*Rhizobium meliloti*	To increase nitrogen fixation	Zahran, 1999
6	*Bacillus thuringensis*	To protect crop from diseases	Abbas, 2018
7	*Bt gene in Synechococcus and Caucobactercrescentus*	Mosquitoes died after eating these bacteria containing Bt gene	Schnepf *et al.,* 1998
8	*Streptococcus*	Reduce cavities in mouth	Loesche, 1996
9	*Lactobacillus acidophilus*	Used in treatment of Lactose intolerance	Pakdaman *et al.,* 2016
10	*Salmonella typhimurium*	Target the tumours	Mi *et al.,* 2019
11	*Lactococcus lactis*	Treat chronic DSS induced colitis in mice	Shigemori *et al.,* 2015
12	*Alcaligenes eutrophus*	Produce polyhydroxybutyrate, a biodegradable biopolymer	Henderson and Jones, 1997
13	*Aspergillus fumigatus*	Produce enzyme that form digesting compounds that increase iron bioavailability	Wiemann *et al.,* 2018
14	*Pichia pastoris*	Produce enzymes to make bread soft	Cunha *et al.,* 2018
15	*Pseudomonas fluorescence*	Bioremediation	Ojewumi *et al.,* 2018

Examples:

Bacteria

- Alcaligens
- Bacillus
- Enterobacter
- Flavobacterium
- Pseuodmonas
- Alcanivorax
- Thallassolituus
- Cycloclasticus
- Oleispira
- Marinobacter

Microalgae/Cyanobacteria

- Spirulina
- Chlorella
- Spirogyra
- Scenedesmus
- Oscillatoria
- Chlorococcum
- Synechocystis
- Nannochloropsis
- Selenastrum

Fungi

- Aspergillus
- Curvularia
- Drechslera
- Fusarium
- Lasiodiplodia
- Mucor
- Penicillium
- Rhizopus
- Trichoderma
- Cryptococcus

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PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSES OF *PLATYCLADUS ORIENTALIS* LEAF EXTRACTS

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Abstract:

Plant research is conducted in order to discover new drugs or templates for the development of new therapeutic agents. The present study focuses and describes the phytochemical profile of the conifer *Platycladus orientalis* and its antimicrobial activity. The leaf extract were eluted from various solvents namely aqueous, petroleum ether, ethanol and acetone and the phytochemical analysis proved the presence of major secondary metabolites in every leaf extract. Antimicrobial activity was assessed by measuring the zone of inhibition against the selected bacterial and fungal isolates. The zone of inhibition proved that the antimicrobial activity was more in aqueous extract when compared to other solvent extracts and the phytochemicals present in the leaf extract of *Platycladus orientalis* performed as a good antimicrobial agent.

Keywords: *Platycladus orientalis*, phytochemical analysis, antimicrobial activity

Introduction:

Plants are used not only as a dietary supplement for living organisms, but also as a traditional treatment for a variety of health problems, and the medicinal value of many plants is still unknown. Plants contain a vast range of secondary metabolites that are employed in the pharmaceutical business either directly or indirectly (Kebede *et al*., 2021). For millennia, man has successfully employed plant components or extracts to cure a variety of ailments, including bacterial infections. Nonnutritive plant compounds with disease-preventive or protective qualities are known as phytochemicals which has its own function. These substances are produced by plants protect themselves, but research shows that many phytochemicals can also protect humans from disease. Ancient herbal plants parts namely the roots, leaves, seeds and barks contribute a major part in the therapeutic values of medicinal world (Annadurai *et al*., 2021). *Platycladus orientalis* is one such ancient medicinal plant cultivated in many parts of Asia

and the seeds and leaves were used to treat skin infections, cough, digestive ailmemts etc., *Platycladus orientalis* is a tree variety ranging to 20m tall with thin twigs and bark and it belongs to the family *Cupressaceae.* It is sparsely found in the cliffs and rocky hills and easily cultivable. The plant was used for ornamental purpose until its medicinal potentials were revealed but the leaves can be treated against asthma, chronic bronchitis, and excessive fluid secretion in lungs, persistent cough and other few respiratory problems (Ren *et al.,* 2019). The present study focuses on the importance of phytochemicals for the antimicrobial activity of *Platycladus orientalis* leaf extracts.

Materials and Methods:

Collection of plant material

The leaves of *Platycladus orientalis* was obtained from Ukkadam, Coimbatore, Tamil Nadu, India. For further investigation, the leaves were shade-dried at room temperature and stored in cotton bags. The plant specimen was validated by the Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

Extract preparation

P. orientalis leaf powder (10g) was extracted with acetone, petroleum ether, and ethanol (100ml each). The crude preparation was left overnight in an orbital shaker at 28ºC and filtered using Whatman filter paper after extraction, and the solvent was evaporated to dryness using a rotary evaporator under vacuum. The resulting crude extract was kept at 4°C for later use. After that, the leaf extract was transferred to a beaker and concentrated by evaporating the solvent at 60°C. *P. orientalis* leaf powder (10g) was boiled with 100 ml of distilled water, cooled, filtered and the aqueous extract is kept at 4°C for later use. To obtain a final concentration the crude extract was weighed and diluted in a specified volume of dimethyl sulphoxide and stored for further use.

Phytochemical analysis

The leaf extracts of *P. orientalis* obtained from various solvents were analysed for the presence of phytochemicals namely flavonoids, alkaloids, tannins, terpenoids, quinines, saponins, carbohydrates, phenols, anthacyanins, proteins and amino acids using the standard procedure by Marka *et al*. (2013).

Alkaloids

1. Wagner's test - To 1 ml of the leaf extracts 3-5 drops of Wagner's reagent were added and the formation of reddish brown precipitate indicated the presence of alkaloids.

2. Mayer's test - To 1 ml of the leaf extracts few drops of Mayer's reagent was added and formation of creamish white precipitate indicates the presence of alkaloids.

3. Dragondroff test-To 1 ml of the plant extract 1ml of drangondroff reagent was added and the formation of orange precipitate indicates the presence of alkaloids.

Flavonoids

1. Shinoda test - To 1 ml of the leaf extracts few drops of ethanol was added and placed in boiling water bath. To this solution 1 drop of concentrated hydrochloric acid and few pieces of magnesium fillings is added and kept for 10-15 minutes at room temperature. Formation of red colour indicates the presence of flavonoids.

Steroids / Terpenoids

1. Libermann - Buchard test - To 1 ml of the leaf extracts few drops of choloroform, acetic acid anhydride and 2 drops of concentrated sulphuric acid was added. Formation of rosy red colour which quickly changes to bluish green indicates the presence of steroids.

2. Salkowski test - To 1 ml of the leaf extracts 1 ml of chloroform and concentrated sulphuric acid was added. Formation of red colour in the chloroform layer and florescence in the acid layer indicates the presence of steroids.

Tannins

1. Braemer's test - To 1 ml of the leaf extracts 1 ml of water was added boiled and filtered. To the filterate 1 ml of ferric chloride was added and formation of dark green or brown colour indicates the presence of tannins.

Glycosides

1. Legal's test - To 1 ml of the leaf extracts few drops of 2% sodium nitro prusside and a drop of 20% of sodium hydroxide was added and formation of pink and deep red colour indicates the presence of glycosides.

2. Keller's Killiani test - To 5 ml of the leaf extracts 1 ml of concentrated sulphuric acid, 2 ml of glacial acetic acid and few drops of Ferric chloride was added. Appearance of blue colour indicates the presence of glycosides

Phenols

1. Ferric chloride test - To 1 ml of the leaf extracts few drops of ferric chloride was added and appearance of bluish green solution indicates the presence of phenols.

Saponins

1. Sodium bicarbonate test - To 1 ml of the leaf extracts few drops of sodium bicarbonate was added and formation of honey comb indicates the presence of saponins.

Quinones

1. Sulphuric acid test - To 1 ml of the leaf extracts was dissolved in isopropyl alcohol and to this few drops of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinines.

Carbohydrates

1. Molisch's test - To 1 ml of leaf extracts few drops of Molisch reagent was added and few drops of concentrated sulphuric acid was added along the sides of the test tubes. Formation of purple ring at the intersection of both layers confirms the presence of carbohydrates.

2. Benedict's test - To 1 ml of the leaf extracts 1ml of Benedict reagent was added and the formation of reddish brown precipitate indicates the presence of carbohydrates.

Proteins / amino acids

1. Ninhydrin test - To 1 ml of the leaf extracts few drops of Ninhydrin reagent was added and heated in a boiling water bath for 5 -10 minutes. Appearance of pink or purple colour indicates the presence of proteins / amino acids.

2. Biuret test - To 1 ml of the leaf extracts 1ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution was added. Appearance of pink or purple colour indicates the presence of proteins / amino acids.

Antimicrobial activity

To evaluate the antimicrobial activity of *P. orientalis* agar well diffusion method was used. The bacterial strains used for the study are *Vibrio cholera*, *Pseudomonas aeruginosa, Escherichia coli, Streptococcus aureus, Bacillus subtilis*, *Shigella flexneri*, *Klebsiella pneumonia.* The fungal strains used were *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* and *Rhizopus* species respectively. The bacterial and fungal cultures were swabbed on Muller Hinton agar and Rose bengal Chloramphenicol media separately. Six wells were made on each plate and to each well 20µl of the leaf extracts, amphicillin (Bacteria) and flucanozole (Fungi) the standard antibiotic which served as positive control and Dimethyl Sulphoxide (DMSO) (negative control) were also added. The plates were incubated at 37°C for 24 hours (bacteria) and at room temperature for 5 days (fungi) and after incubation, the zone of inhibition was measured.

Results and Discussion:

Phytochemical analysis

Table 1: Phytochemicals present in the leaf extracts of *Platycladus orientalis*

Sr. No	Name of the phytochemical test	Leaf extracts			
		Petroleum ether	**Ethanol**	**Aqueous**	**Acetone**
1.	Alkaloids				
	a. Wagner's test	+	+	+	+
	b. Mayer's test	+	-	-	-
	c. Dragondroff test	-	+	+	+
2.	Flavonoids				
	a. Shinoda test	+	+	+	+
3.	Steroids / Terpenoids				
	a. Libermann - Buchard test	-	+	+	-
	b. Salkowski test	-	-	+	-
4.	Tannins				
	Braemer's test	-	+	+	+
5.	Glycosides				
	a. Legal's test	-	-	-	-
	b. Keller's Killiani test	+	+	+	-
6.	Phenols				
	a. Ferric chloride test	+	-	+	+
7	Saponins				
	a. Sodium carbonate test	+	-	+	+
8.	Quinones				
	a. Sulphuric acid test	-	-	+	-
9.	Carbohydrate				
	a. Molisch test	+	+	+	+
	b. Benedict test	+	+	+	-
10.	Protein / amino acids				
	a. Ninhydrin test	-	-	-	-
	b. Biuret test	-	-	-	-

Biologically active compounds present in the leaf extracts of *Platycladus orientalis* are determined by the phytochemical analysis aid in the antimicrobial activity of the plants and phytochemical compounds like alkaloids, flavonoids and steroids are used in traditional medicine (Wintola *et al*., 2015). The therapeutic effects *P. orientalis* is due to the antimicrobial activity of

the secondary metabolites. The leaf extracts of *P. orientalis* confirmed the presence of alkaloids, flavonoids, steroids, tannins, terpenoids, glycosides, phenols, saponins, quinones, carbohydrates and proteins were absent in the leaf extract.

Plants contain phytochemical constituents that serve as a defensive mechanism against numerous pests. The chemical components in alkaloids have the capacity to impede the production of bacterial biofilms, and they have a wide spectrum of antibacterial and other therapeutic effects (García-García *et al*., 2021). Plant extracts contain phenols, tannins, and terpenoids, which have the natural ability to serve as antibacterial agents (Roy *et al.*, 2010). Flavonoids are hydroxylated phenolic subtances, which are found in abundance in fruits, vegetables, and legumes, have a wide variety of pharmacological, antioxidant, and antibacterial action, and are poisonous to many gram positive and gram negative bacteria species (Barbieri *et al*., 2017). Tannins and flavonoids have a similar mechanism in that they provide a stable free radical source and also form complexes with nucleophilic amino acids in proteins, resulting in protein inactivation and loss of function. Their potential antimicrobial effect is significant because they likely target microbial cell surface-exposed adhesins, cell wall polypeptides, and membrane bound enzymes (Suurbaar *et al*., 2017). The saponins have the ability to cause leakage of proteins and enzymes from the microbes and tannins bind with the microbial cell wall and inhibit or interfere in the protein synthesis (Murugan *et al*., 2013).

Antimicrobial activity

The aqueous extract of *P. orientalis* exhibited a maximum zone of inhibition against *Bacillus subtilis* (24mm) followed by *Streptococcus aureus* (22mm) and minimum zone of inhibition was noticed in *Vibrio cholerae* (15mm). Similarly, the zone of inhibition were seen in, *Pseudomonas aeruginosa* (21mm) *Klebsiella pneumonia* (19mm), *Shigella flexneri* (16mm) and *Escherichia coli* (17mm), against aqueous extract. The acetone extract and ethanol of the *P. orientalis* showed the moderate activity and petroleum ether extract revealed the minimum activity when compared with the standard antibiotic, amphicillin which served as positive control (Table 2). The results proved that the leaf extract of *P. orientalis* have potential to inhibit Gram positive bacteria than Gram negative bacteria. The activity of the bioactive compounds is more effective against Gram positive bacteria because it does not have lipopolysaccharides layer (Papo and Shai, 2005) and it enables the phytochemicals to penetrate into the bacterial cells, and interaction of phospholipid bilayer of the cell membrane which results in the leakage of cellular constituents, increased ion permeability or impairment of enzyme compartments (Zhao *et al.*, 2001).

The zone of inhibition of *P. orientalis* leaf extract against the tested fungal isolates depicted in table 2. The aqueous extract of *P.orientalis* leaf exhibited maximum zone of inhibition against *Aspergillus fumigates* (20mm) and minimum zone of inhibition noticed in *Aspergillus flavus*(15mm).Petroleum benzene extract of *P. orientalis* possessed minimum activity and moderate zone of inhibition was observed in acetone and ethanol extract against the tested fungal isolates when compared with the standard antibiotic (fluconazole). The antifungal properties of iron oxide nanoparticles are related to the development of oxidative stress, which results in the generation of reactive oxygen species. Iron is a potent reducing agent that helps membrane proteins and lipopolysaccharides disintegrate their functional groups (Javed *et al*., 2012). The negative control (DMSO) showed no zone of inhibition against tested bacterial and fungal isolates respectively.

Table 2: Antibacterial and antifungal activities of the leaf extract of *Platycladus orientalis*

Name of the organism	**Zone of inhibition (mm)**			
	Petroleum ether extract	**Ethanol extract**	**Aqueous extract**	**Acetone extract**
Bacterial Isolates				
Gram Negative Bacteria				
Pseudomonas aeruginosa	10	12	21	15
Klebsiella pneumonia	13	15	19	16
Shigella flexneri	9	12	16	12
Escherichia coli	11	13	17	11
Vibrio cholera	10	11	15	13
Gram Positive Bacteria				
Bacillus subtilis	13	16	24	17
Streptococcus aureus	12	13	22	19
Fungal Isolates				
Aspergillus niger	12	13	16	12
Aspergillus fumigatus	11	13	20	18
Aspergillus flavus	9	12	15	12
Rhizopus species	10	14	19	16

Conclusion:

The present study revealed the phytochemical profile of the leaf extracts of *Platycladus orientalis*. The fact that bioactive components in the plant *Platycladus orientalis* are extracted largely in aqueous extract and demonstrate promising antibacterial and antifungal inhibitory activity which is scientifically validated in this study.

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EFFICIENCY OF MICROBIAL DIVERSITY IN BIOREMEDIATION OF TEXTILE DYES

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Abstract:

Waste water from textile industries is one of the measure reasons for environmental pollution, mostly river water. Effluent generated during dyeing and rinsing steps represent the most colored fraction of textile waste waters with high COD, BOD, pH, color, odor and salinity. Various physiochemical methods are available for the treatment of such dye containing waste water. But some of these methods are economically not viable, some of them create secondary waste and some of them can not completely degrade the complex dyes. Bioremediation systems can be better option compare to available physio-chemical methods. Biotechnological approach may offer economically viable low cost biological treatment systems that can completely biodegrade and detoxify even the hard-to-biodegrade dyes. Over the past two decades a lot of focus on biodegradation of textile dyes by different bacterial species including sulphate reducing bacteria, Nitrogen fixing bacteria, Dyes adapted bacteria, Lactic acid bacteria, Halophilic bacteria and genetically modified bacteria has been observed. According to the literature survey the author finally reached at the conclusion that nature itself has protective measures to save nature. Variety of bacteria isolated from environment can easily degrade different types of dyes into simpler form without showing any harmful effects on environment.

Keywords: Bacteria, Bioremediation, Dyes, Decolorization, Environment

Introduction:

Bioremediation is defined as a process that involves the use of organisms to remove or neutralize hazardous pollutants. *Environmental Protection Agency* (EPA) defined the bioremediation as; it is a “treatment that uses naturally found organisms to break down harmful substances into less toxic or non-toxic substances”. This is a technology that can be generally classified as *in situ* or *ex situ*. *In situ* bioremediation is treatment of contaminated material at the site, while *ex situ* bioremediation is the removal of the contaminated material from location and to be treated elsewhere.

Some examples of bioremediation technologies are Phytoremediation, Bioventing, Bioleaching, Land farming, Bioreactor, Composting, Bio-augmentation, Rhizofiltration and Bio-stimulation. The emerging science and technology of bioremediation offers an alternative method for complete biodegradation and detoxification of dyes. Bacterial isolates can be effectively used in development of alternative and eco-friendly method for decolorization and biodegradation of textile dyes present in industrial effluent. It has been found that over the past two decades, interest has been focused on biodegradation methods for dyes such as, fungal decolorization, bacterial degradation, Algal decolorization and adsorption by microbial biomass (living or dead) (Siva *et al*., 2007; Mazumder, 2011; Sharma, 2012).

Microorganism used to perform the function of bioremediation is known as bioremediator. Bioremediation can be much effective only where an environmental condition allows the microbial growth and activity. Its application often involves the manipulation of environmental parameters to allow microbial growth to enable degradation at a faster rate (Vidali, 2001). The bacterial isolates originated from the dye contaminated textile wastewater of local industry can easily adapt to the prevailing local environment and can successfully be used in bioremediation. It will help in making the system ecofriendly (Karthikeyan *et al*., 2013).

A wide range of microorganisms including Bacteria, Actinomycetes, Fungi and Yeast are capable of degrading a variety of dyes (Sudha *et al*., 2014). Many authors have worked with different microorganisms like, *Staphylococcus arlettae*, Lactic acid bacteria, *Pseudomonas putida, Micrococcus luteus*, *Listeria denitrificans, Nocardia atlantica* and *Bacillus megaterium*. Basidiomycetous fungi include *Tramates pubescens, Pleurotus ostreatus, Aspergillus tamarii, Penicillium purpurogenum, Aspergillus ochraceus*, *Pleurotus ostreatu, Aspergillus niger, Fusarium oxysporum* and *Trichoderma lignorum* (Rani *et al*., 2014). Bacteria strains reduce the dyes into colorless aromatic amine under anaerobic as well as aerobic conditions (Kumar *et al*., 2006).Microorganisms reduce the dyes with the help of enzymes such as laccase, azo reductase, peroxidase and hydrogenase. The degraded forms of dyes are further mineralized into harmless compounds which are utilized by microorganisms as their energy source (Sudha *et al*., 2014).

Some microorganisms remove the dyes by Biosorption technique. Biosorption is defined as a process in which solids (e. g., Microbial cell mass) of natural origin are employed for separation of solid pollutants from the natural streams. In dyes remediation, Biosorption involves interaction between the cell wall ligands and adsorbates (dyes). This can be observed by ion exchange, complex formation, and co-ordination and micro-precipitation technique. Biosorption can be done by using living or dead biomass. Dead biomass is more advantageous than living cells because it does not require any nutrient supply for growth and can be regenerated for use.

Living cells require constant nutrient supply. Sometimes the toxicity of dyes may harm the growth of living cells (Bagewadi *et al*., 2011).

The biological modes of treatment of dye bath effluents offer distinct advantages over the conventional modes of treatment. Most importantly, they are ecofriendly. They are very efficient and cost effective methods, leading to relatively less accumulation of sludge. The basic step in the decolorization and degradation of dyes is breakdown of chromophore group. It causes mineralization of dyes to simpler inorganic compounds which are not dangerous to the other life forms (Saharan *et al*., 2011; Tripathi *et al*., 2011; Karthikeyan *et al*., 2013). The biomass can absorb the chromophores and can be reduced in low redox potential environments. Dye molecule absorbs onto the cell surface quickly and is often completed within some hours. There is no specific nutrient supplement required by the bacteria to decolorize the dyes. Dyes themselves become substrate for bacterial growth (Palamthodi *et al*., 2011). Large no. of microorganisms have been isolated in recent years which are able to degrade dyes which were in the past considered as non- degradable (Keharia *et al*., 2003).

Factors affecting on bacterial dye degradation

Research has shown that the efficiency of biological treatment systems is greatly influenced by the operational parameters. The level of aeration, temperature, pH and medium composition for the growth of microbial system must be optimized to obtain the maximum rate of dye reduction. With optimum conditions microorganisms can degrade structurally different dyes such as acid, basic, direct, disperse, metal-complex, reactive, sulphur and vat dyes.

The dye degradation rate may increase with increasing temperature, within a defined range. The temperature of decolorization depends on the bacterial isolate and it should be optimum temperature for cell growth. In most cases, optimum temperature for cell culture growth is 35–45ºC. The decline in color removal activity at higher temperatures can be attributed to the loss of cell viability or to the denaturation of the dye degrading enzyme (Chang *et al*., 2001). Most of the bacteria grow at neutral pH 7.0 and hence the pH 7.0 or a slightly alkaline pH value will be the optimum for color removal process. The rate of color removal tends to decrease rapidly at strongly acidic or strongly alkaline pH values (Willmott *et al*., 1997).

The concentration of dye substrate can influence the efficiency of dye removal. The dye decolorizing enzymes recognize the substrate efficiently at very low concentrations. As the concentration of dye increases, the rate of dye removal decreases, while time of dye removal might increase (Walker *et al*., 1971). Dyes with simple structures and low molecular weights exhibit higher rates of color removal than dyes with high molecular weight and highly substituted. In the case of the terminal non-enzymatic reduction mechanism, reduction rates are influenced by changes in electron density in the region of the azo group. For instance, the

substitution of electron withdrawing groups ($–SO_3H$, $–SO_2NH_2$) in the para position of the phenyl ring, relative to the azo bond, causes an increase in the reduction rate (Walker *et al*., 1971).

The type of waste water and its composition also affect the dye decolorization activity of bacteria. The composition of textile wastewater is varied. Variation is observed in organics, nutrients, salts, sulphur compounds and toxicants as well as the color. The pH, temperature of waste water, lots of chemicals and dyes used during dyeing process may affect the growth of bacteria. Indirectly, it may affect the textile effluent decolorization (Miao *et al*., 2011).

A microbial consortium is a group of different species of microorganisms that act together as a community. Examples of microbial consortia are found in activated sludge basins, biofilms on trickling filters and in various soil ecosystems. Microbial consortia have been widely used in cleanup of a number of pollutants in laboratory and fields under bioremediation studies (Padmavathy *et al*., 2003).

It is generally considered that microbial consortia are more effective than pure cultures in biodegradation as strains can collectively carry out complete biodegradation tasks that may not be achieved by individual pure strain. This is possibly because broader enzymatic capacity secreted by each strain and the formation of toxic intermediate metabolites is counteracted by other strain (Padmavathy *et al*., 2003). A microbial consortium is more resistant to environmental shock and can better compete and survive in the environment as compared to single microorganisms. Microbial consortia are capable of handling a wide variety of complex wastes (Mahmood *et al*., 2015; www.ebac2000.com).

Awasthi *et al*. (2014), stated that both Anaerobic and aerobic bacterial processes are required for complete azo dye mineralization. Azo bonds are reduced leading to generation of aromatic amines under anaerobic conditions. Further these aromatic amines are completely degraded under aerobic conditions. The consortium of facultative anaerobic and aerobic bacteria can be used to complete mineralization of the textile azo dyes. Thus simple and non-toxic metabolites are formed on complete degradation of dyes by consortium of bacteria.

Many researchers have reported that a higher degree of biodegradation and mineralization can be expected when co-metabolic activities within a microbial community complement each other. For e.g., complete degradation of naphthalene sulphonates by consortia of two-species, one of them is *Sphingomonas* strain BN6 is able to degrade naphthalene-2-sulphonate (a building block of azo dyes) into salicylate ion equivalents. The salicylate ion cannot be further degraded by same strain and it will become toxic. Therefore, naphthalene-2-sulphonate can only be degraded completely in the presence of a complementary organism that is capable of degrading the salicylate ion (Pearce *et al*., 2003).

Dyes degradation by bacterial isolates

Recent research has exposed the survival of a wide variety of bacteria and mixed cultures capable of decolorizing a wide range of dyes. Bacteria are capable for dye decolorization, either individually or in consortia (Viral *et al*., 2012). The bacterial isolates like *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli, Acinetobacter* spp., *Bacillus* spp. and *Legionella* spp. have potential for color removal (Saranraj *et al*., 2010).

Several other microbial cultures have been tested or are implicated in textile dye decolorization are represented in Table No. 1. Mostly all dye degrading bacteria are isolated from textile effluent or dye contaminated soil/ sludge. Such microorganisms are adapted to various dyes with various concentrations, various chemicals and pH levels of textile waste. Thus, they have more ability to withstand physical and chemical properties of textile waste water during treatment. Huge difference was observed on studying the textile effluent degradation potentialities of textile effluent-adapted and non-adapted bacteria. However, Biological decolorization processes using dyes adaptable microorganism are efficient under both anaerobic and aerobic conditions (Sriram *et al*., 2013).

Table 1: Dyes degradation shown by different bacterial species

Bacterial spp.	**Dyes**	**Reference**
Brevibacterium spp. strain VN-15,	Azo dyes - Reactive Yellow 107 (RY107), Reactive Black 5, Reactive Red 198 and Direct Blue 71.	Franciscon *et al*. (2012)
Staphylococcus aureus, Bacteroides fragilis, Bacillus subtilis, Bacillus cereus, Clostridium perfringes, Escherichia coli and *Peptostreptococcus spp.*	Indigo blue	Ajibola *et al*. (2005)
Arthrobacter spp.	Pararosaniline chloride dye (Triphenylmethane dye)	Dwivedi *et al*. (2012)
Corynebacterium spp.	Reactive Black B and Yellow 15 dyes	Aftab *et al*. (2011)
Acetobacter liquefaciens S-1, *Klebsiella pneumonia* RS 13 and *Enterobacter agglomerans*	Methyl red azo dye	Kumar *et al*. (2006)

Shewanella oneidensis	Reactive black-5	Wu *et al*. (2009)
Citrobacter spp. CK3,	Azo and anthraquinone dyes	Wang *et al*. (2009A)
Citrobacter spp	Bromophenol Blue, Crystal Violet and Methyl Red Gentian Violet, Malachite Green and Brilliant Green , Basic fuchsin	Sun-young *et al*. (2002),
Psudomonas spp. and *E.coli*	Brilliant green, Malachite green, Carbol fuchsin and Crystal violet	Sinoy et al. (2011
Rhodobacter adriaticus, R. blasticus, R. capsulatus, Rhodovulum strictum, Rhodopseudomonas palustris.	Orange II, Orange 16, Reactive black B, Remazol red 3BS and Remazol blue G133 dyes	Kim *et al*. (2003)
Aeromonas spp.	Orange 16	Shah *et al*. (2013D)
Agrobacterium radiobacter, Bacillus spp., Sphingomonas paucimobilis and Aeromonas hydrophila	Triphenylmethane dyes - Crystal violet and Malachite green	Cheria *et a*l. (2012)
Pseudomonas genus including Pseudomonas fluorescence, Pseudomonas nigificans and Pseudomonas gellucidium	Azo dyes Acid Red 151, Orange II, Sulfur Black and Drimarene Blue K2RL dyes	Aggary *et al*. (2011) Vigneeswaran *et al*. (2012 Bayoumi *et al*. (2014)
Pseudomonas aeruginosa	Navitan Fast Blue dye	Lavanya *et al*. (2014)
Pseudomonas putida MS7	Remazol Black B	Kannan *et al*. (2013)
Pseudomonas spp. AKDYE14	Wastewaters contaminated with azo dyes	Karthikeyan *et al*. (2013)

Dye degradation by sulphate reducing bacteria

Decolorization activity of sulphate reducing bacteria is due to extracellular chemical reduction of dyes with sulphite as a reducing agent. Thus, cell membrane permeability is not important for dyes decolorization by Sulphate reducing bacteria (SRB). Rate of decolorization depends on the added organic carbon source as well as dye structure. The dye reduction is seen under anaerobic conditions. SRB bacteria are non-specific as they can reduce different types of azo dyes having varied structures (Yoo *et al*., 2001).

The potential of SRB to decolorize azo dyes was studied by Yoo *et al*. (2000), employing the pure culture of *Desulfovibrio desulfuricans* with varying sulfate levels. Under sulfate-rich

conditions, the sulfides were produced from sulfate respiration with pyruvate as electron donor. *Desulfovibrio desulfuricans* decolorized the azo dyes C. I. Reactive Orange 96 and C. I. Reactive Red 120. Under sulfate-depleted conditions (≤0.1mmol/L), the decolorization of dyes occurred in correlation with the fermentation of pyruvate by *Desulfovibrio desulfuricans*. So it is suggested that the electrons liberated during oxidation of pyruvate is may be transferred via enzymes and/or coenzymes (electron carriers) to the dyes as alternative terminal electron acceptors. It leads to particular dyes decolorization.

Dye degradation by nitrogen fixing bacteria

The ability of specific microbes to convert atmospheric nitrogen into molecular nitrogen which can easily usable by the plant, a process is called as biological nitrogen fixation. Some of the naturally occurring soil bacteria are named as Plant growth-promoting Rhizobacteria (PGPR) which actively colonize plant roots and benefit plants by providing growth promotion. In view of this, use of PGPR for different pollutant's bioremediation suggests environmental friendly treatment approach (Ahmed *et al*., 2014; Perez-Montano *et al*., 2014). In line with this approach, Kadam *et al*. (2013), carried out dyes decolorization experiments by using Rhizosperic bacteria. The individual cultures of *Rhodobacter erythropholis* MTCC 4688, *Azotobacter vinelandii* MTCC 1241, *Rhizobium meliloti* NCIM 2757 and *Bacillus megaterium* NCIM 2054 showed 44%, 28%, 50% and 61% decolorization of DR73 in 48h respectively; while the consortium of tested bacteria showed complete decolorization in 48h.

Dye degradation by lactic acid bacteria

Lactic acid bacterial isolates can be used to obtain complete mineralization of textile dyes for safe metabolites in short duration under sequential anaerobic/aerobic system. The biodegradation of Reactive Lanasol Black dye was confirmed by HPLC analysis. *Lactobacillus casei, Lactobacillus paracasei* and *Lactobacillus rhamnosus showed* 75% to 100% *decolorization of* Reactive Lanasol Black B, Eriochrome Red B and 1, 2 metal complexes I. Yellow dyes within 4h (Elbanna *et al*. 2010). *Lactobacillus Delbruckii* showed good growth in agitation culture but the color removal was highest in static culture with 45% to 60% color removed in less than 72h at pH 7.0 (Siti *et al*., 2013).

Dye degradation by Halophilic bacteria

Halophiles are salt loving microbes mostly found in ocean. Most bacteria growing in halophilic conditions grow optimally within the range of 0.5–2.5M NaCl. Halophilic and Halotolerant microorganisms can be the best candidates for a practical bio-decolorization process as they are able to grow easily at high concentrations of salts. In addition, some of them can tolerate the presence of other stress factors such as toxic oxygenations and heavy metals which are very common in industrial wastewater (Amoozeger *et al*., 2011). Halophiles are

metabolically diverse and are adapted with the saline environment which is the condition of most of the industrial effluents containing textile dyes. So these halophiles can be used directly for the treatment of the effluents at the industrial sites, which will be cost effective as compared to conventional methods (Agarwal *et al*., 2012).

In recent years, several studies have been focused on halophilic and halotolerant microorganisms and their abilities for decolorization of dyes. Table no. 2 represents the halophilic microorganisms studied for textile dyes decolorization process.

Table 2: Dyes biodegradation shown by different Halophilic bacterial species

Bacterial spp.	Dyes	Reference
Shewanella putrefaciens	Reactive Black 5, Direct Red 81, Acid Red 88 and Disperse orange 3 (100mg/L)	Amoozeger *et al.* (2011
Halomonas spp. GTW	Azo dye K2BP	Zohu *et al*. (2008
Lysobacter spp. T312D9	Methyl red and Congo red dye	Barakat (2013),
Halobacillus spp. C-22	Lanaset Navy R and Lanaset Brown B. Lanaset Brown B	Demirci *et al.* (2011

Dye decolorization by genetically engineered bacteria

Genetic engineering is the direct manipulation of an organism's genome using different molecular techniques. It includes the transfer of genes within and different species boundaries to produce improved or novel organisms. Genetic material of interest will be first isolated and copied using molecular cloning methods to generate a DNA sequence or new DNA may be constructed chemically. Then it will be inserted in the genome of host organism.

An organism that is generated through genetic engineering is also called as genetically modified organism (GMO). These GMOs have wide application in food, medicine, fermentation and environment biotechnology. Genetic Engineering technology constructing such GMOs can easily deal with the environmental pollution. Contaminant-degrading genes are inserted in microbial cells to perform the function of degradation of pollutants (Jing *et al*., 2005).

GMOs can be divided into special engineered bacteria and generalized engineered bacteria. Special engineered bacteria have certain target genes with various organic compound degrading properties through its gene operations. However, generalized engineered bacteria is a mixed flora reasonably combined by the highly efficient bacterial genes which are isolated from natural environment, polluted environment and treatment systems and can degrade various organics efficiently that leads to its wide application (Ma *et al*., 2008). The first genetic

engineered bacteria *pGEX-AZR/E. coli* JM-109 possess the ability of degradation of azo dyes, especially the small molecular ones (Faldu *et al*., 2014). The Table 3 represents the GMO used for dye degradation by various researchers.

Table 3: GMO's used for dyes degradation

Fungal spp	Dyes	Reference
Penicillium simplicissimum	Reactive Blue 21, Reactive Red 198, Reactive Blue 214	Bergsten *et al*. (2009)
Acremonium kiliense	Malachite green, Crystal violet, Carbol fuchsin and Methylene blue	Youssef *et al.* (2008)
Phanerochaete chrysosporium	Congo red	Tatarko *et al.* (1998)
White rod fungi *Polyporus elegans, Trametes versicolor, Lenzites betulina* and soil fungus *Mucor mucedo*	Crystal violet and Malachite green	Motury *et al*. (2009)

There are some difficulties in the promotion of such GMOs. They have low success rate, high cost, and long adaption time for expression of gene. Also, there are a certain other problems such as hereditary, purification function and biosafety. Thus the application of GMOs in textile effluent treatment has limitations widely (Xie *et al*., 2014).

Dye decolorization by Actinomycetes

Biosorption by actinomycetes strains is also becoming a popular process of azo dyes removal from wastewaters. El-Sersy *et al*. (2011), worked on dye degradation by different species of actinomycetes. From the study, they found that, *Streptomyces globosus, Streptomyces alanosinicus, Streptomyces ruber, Streptomyces gancidicus* and *Nocardiopsis aegyptia* were capable of degrading the two tested azo dyes; Congo red and Acid fast red under static condition. Bagewadi *et al*. (2011) suggested that the enzymes such as lignin peroxidase, laccase and tyrosinase present in Actinomycetes have the ability to degrade Reactive yellow dye. In static condition, in presence of additional carbon source, Actinomycetes could luxuriantly grow and produce enzymes which are responsible for effective decolorization of dyes.

Dye degradation by Fungi

The role of fungi in the treatment of textile wastewater has been extensively researched. Fungus has proved to be a suitable microorganism for the treatment of textile effluent and dye removal, based on the mechanism involved bioaccumulation, biodegradation and biosorption.

Bioaccumulation is the accumulation of pollutants by actively growing cells by metabolism. Biosorption is defined as binding of solutes to the biomass by processes that do not involve metabolic energy or transport. Thus this treatment processes may carried out using live as well as dead biomass. Many genera of fungi have been employed for the dye decolorization; either in living or dead form are showed in table No. 4.

Table 4: Fungal species used in dyes degradation process

Fungal spp	Dyes	Reference
Penicillium simplicissimum	Reactive Blue 21, Reactive Red 198, Reactive Blue 214	Bergsten *et al*. (2009)
Acremonium kiliense	Malachite green, Crystal violet, Carbol fuchsin and Methylene blue	Youssef *et al.* (2008)
Phanerochaete chrysosporium	Congo red	Tatarko *et al*. (1998)
White rod fungi *Polyporus elegans, Trametes versicolor, Lenzites betulina* and soil fungus *Mucor mucedo*	Crystal violet and Malachite green	Motury *et al*. (2009)
Aspergillus flavus	Bromophenol blue and Congo red	Singh *et al*. (2010)
Aspergillus niger and *Phanerochaete chrysosporium*	Malachite green, Nigrosin and Basic fuchsin	Rani *et al*. (2014)
Laccase preparations obtained from *Pleurotus ostreatus, Schizophyllum commune, Sclerotium rolfsii* and *Neurospora crassa*	triarylmethane, anthraquinonic and indigoid textile dyes	(Elisangela *et al*., 2009)

Biodegradation by fungi is an energy-dependent process and involves the breakdown of dye into various by-products through the action of various enzymes. A fungus produces lignin-modifying enzymes, such as laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP), to mineralize and to decolorize dyes. These enzymes are nonspecific enzymes and thus, with the help of these enzymes fungi could degrade many dyes with different structures. There is literature available regarding the potential of these fungal enzymes to oxidize phenolic, nonphenolic, soluble and non-soluble dyes.

Fungal cultures are used as free or immobilized cultures for decolorization processes under static or agitated conditions. Free cell cultures could decolorize the dye and textile effluent, but it has some operational problems such as shear force, cell stability in agitated conditions. Immobilized fungal cells offer some advantages over free cells, which enhance decolorization efficiency, cell stability, reuse of biomass easier liquid–solid separation and minimal clogging in continuous- flow systems. Cell immobilization may also protect cells against shear force, toxic compounds and pH (Park *et al*., 2006, Kaushik *et al*., 2009).

Although stable operation of continuous fungal bioreactors for the treatment of synthetic dye solutions has been achieved, the application of fungi for the removal of dyes from textile wastewaters faces many problems such as large volume of effluent production, the nature of synthetic dyes, control of biomass, the long growth cycle and the complexity of the textile effluents that limits the performance of these fungi. In contrast, the bacterial reduction of the dyes is usually nonspecific and bacterial decolorization is normally faster (Elisangela *et al*., 2009).

Dye degradation by Algae

Algae are simple plants that can range from the microscopic (microalgae) to large seaweeds (macroalgae), such as giant kelp more than one hundred feet in length. Microalgae include Cyanobacteria (similar to bacteria), blue-green algae as well as green, brown and red algae. There are more varieties of microalgae, but these are the important ones.These Photosynthetic organisms are distributed in nearly all parts of the world and in all kinds of habitats. Algae can be grown using water resources such as brackish, sea and wastewater which are unsuitable for cultivating agricultural crops.

Algae can play an important role in the removal of dyes and aromatic amines in stabilization ponds. It has been reported that more than thirty azo compounds can be biodegraded and decolorized by *Chlorella pyrenoidosa*, *Chrorella vulgaris* and *Oscillateria tenius*, with azo dyes decomposed into simpler aromatic amines (Yan *et al*., 2004; Lavanya *et al*., 2014). Micro algae and *Cyanobacteria* are considered as an important source for decolorizing dye and textile effluent. For e.g. marine Cyanobacterium, *Oscillatoria formosa* NTDM02, decolorize the textile effluent efficiently in short period of time. Other Cynobacteria, *Phormidium valderianum* produces hydrogen and can remove dyes from the solution (Ali *et al*., 2011). *Cosmarium* spp. is a freshwater member of Chlorophyta-green algae could decolorize Malachite Green. Its decolorization ability is dependent on the dye concentration, algal concentration, pH and temperature (Daneshwar *et al*., 2007).

Conclusion:

Variety of microorganisms will offer considerable advantages in the large scale decolorization and degradation of textile wastewaters containing mixture of synthetic dyes with different structures.For the treatment of textile effluent an appropriate bioreactor can also be developed by designing a media with the help of optimum parameters obtained in this study.

The inherent advantage of microorganism, like rapid growth and cheap growth substrates etc will make this bioremediation process an efficient, environment friendly and cost effective method for treatment of textile industrial effluent. This will help in protecting the environment from the ill effects of releasing untreated effluents in the environment.

Such microbial treatment should be employed in common effluent treatment plants of dyeing units to deal with more number of dyes on a daily basis. There is a need for collaborative efforts between industrialists and microbiologists to develop such microbial treatment technologies and pilot scale plants for treatment of textile wastewater.

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PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) AS BIOFERTILIZER FOR SUSTAINABLE AGRICULTURE

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Abstract:

Some microorganisms are able to colonize the soil surrounding plant roots, the rhizosphere, making them come under the influence of plant roots. Plant growth promoting rhizobacteria (PGPR) generally refers to a group of soil and rhizosphere free-living bacteria colonizing roots in a competitive environment and exerting a beneficial effect on plant growth. Plant Growth-Promoting Rhizobacteria (PGPR) confers benefits to host plants including growth promotion and disease suppression. PGPR promote plant growth due to their abilities in phytohormone production, nitrogen fixation, and phosphorus solubilization; produce several substances which are related to pathogen control, i.e., exhibiting competition with plant pathogens, synthesis of antibiotics, antifungal metabolites and defense enzymes, and secretion of iron-chelating siderophores; and trigger induced systemic resistance (ISR). The antibiotic production induced resistance and growth promotion action mediated by PGPRs an effective management tool for plant disease control, since it is durable and environment-friendly alternative for chemical-based plant disease management.

Keywords: Rhizosphere, PGPR, nitrogen fixation, Siderophore, disease management

Introduction:

The term ‘Rhizobacteria’ was introduceb by Kloepper and Schroth (1978) to the soil bacterial community that competitively colonized plant roots and stimulated growth and thereby reducing the incidence of plant diseases. Kloepper and Schroth (1981) termed these beneficial rhizobacteria as plant growth-promoting rhizobacteria (PGPR). Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and or indirectly. In last few decades a large array of bacteria including species of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthobacter, Burkholderia, Bacillus and Serratia have reported to enhance plant growth (Kloepper *et al.*, 1989; Glick, 1995, Ahemad *et al.*, 2008). The direct promotion by PGPR entails either providing the plant with a plant growth promoting substances that is synthesized by the bacterium or

facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effect of one or more phytopathogenic micro-organisms.

The PGPR utilization is potentially increased in sustainable farming because of its eco-friendly and practical nature to substitute the increasing usage of synthetic nutrients and insecticides. Rhizobacteria produce a large number of substances that affect plant growth promotion through a direct or indirect way. The use of commercial biofertilizers containing best PGPR strains is increasing rapidly, and for this reason, the importance for the search of PGPRs and their mode of action is increasing day by day (Bhattacharyya and Jha, 2012).

Plant growth-promoting rhizobacteria as biofertilizers

Biofertilizers are the viable microbes colonizing the rhizosphere or interior plant parts. These microbes are not actually the source of nutrients however, support the plants in accessing the essential nutrients present in the root area of plant. Biofertilizers provide the potential to meet our agricultural requirements, enhance the sustainability, and improve the health of soil. They are active strains of bacterial microorganisms or combination of algae, fungi, and some other microbes, involved directly or indirectly in various actions such as increasing fertility of soil by fixing atmospheric nitrogen, mineralization of elements, and movement of nutrients like phosphorous, sulfur, potassium, zinc, and iron from soil to plant and production of growth hormones, thus improving the crop productivity in an eco-friendly manner. Biofertilizers produce siderophore, protects plants from bio-surfactants and cell wall degrading enzymes etc. (Saraf *et al.,* 2014). Biofertilizers can be supplied to fields via roots, seeds, or directly to the soil where they proliferate and translocate the inert nutrients and have proven harmless and valuable technique of enhancing yield (Vejan *et al.*, 2016). Soil microbes used as biofertilizers include free-living nitrogen-fixing bacteria like *Azotobacter*, *Beijerinckia*, *Clostridium*, *Nostoc*, *Klebsiella*, and *Anabaena*; symbiotic bacteria such as *Rhizobia*, and *Azospirillum*; phosphorus-solubilizing biofertilizers, viz., *Bacillus megaterium* var. *phosphaticum*, *Bacillus subtilis*, *Bacillus circulans, and Pseudomonas striata*; Biofertilizers are cheap and renewable sources of plant nutrients (Mushtaq, 2021).

PGPRs can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) (Martinez -Viveros *et al.*, 2010). The ePGPRs may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex; on the other hand, iPGPRs locates generally inside the specialized nodular structures of root cells. The bacterial genera such as *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas* and Serratia belongs to ePGPR (Gray and Smith, 2005). The

iPGPR includes the endophytes and Frankia species both of which can symbiotically fix atmospheric N2 with the higher plants (Verma *et al.,* 2010).

Mechanisms of plant growth promotion

PGPR affect plant growth in two different ways, indirectly or directly. The direct promotion of plant growth by PGPR entails either providing the plant with a compound that is synthesized by the bacterium, for example phytohormones, or facilitating the uptake of certain nutrients from the environment (Glick, 1995). The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms. This can happen by producing antagonistic substances or by inducing resistance to pathogens (Glick, 1995, Beneduzi *et al.,* 2012).

Direct Methods

Direct methods include nitrogen fixation; phosphorus, potassium, and zinc solubilization; siderophore production; production of phytohormones; and enzyme and vitamin production. These actions incite morphological and physiological changes in plants, thus promoting plant growth (Mushtaq *et al.,* 2021).

Nitrogen fixation

Nitrogen is an essential element for all forms of life and it is the most vital nutrient for plant growth and productivity. Although the nitrogen presents 78 % of the atmosphere, it remains unavailable to the plants. Regrettably no plant species is capable for fixing atmospheric dinitrogen into ammonia and expend it directly for its growth. Thus the atmospheric nitrogen is converted into plantutilizable forms by biological nitrogen fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase (Gaby and Buckley, 2012). Plant growth promoting rhizobacteria have the ability to fix atmospheric nitrogen and provide it to plants by two mechanisms: symbiotic and non-symbiotic. Symbiotic nitrogen fixation is a mutualistic relationship between a microbe and the plant. The microbe first enters the root and later on form nodules in which nitrogen fixation occurs. Rhizobia are a vast group of rhizobacteria that have the ability to lay symbiotic interactions by the colonization and formation of root nodules with leguminous plants, where nitrogen is fixed to ammonia and make it available for the plant (Ahemad, 2014). The plant growth promoting rhizobacteria widely presented as symbionts are Rhizobium, Bradyrhizobium, Sinorhizobium, and Mesorhizobium with leguminous plants, Frankia with non-leguminous trees and shrubs (Zahran, 2001).On the other hand, non-symbiotic nitrogen fixation is carried out by free living diazotrophs and this can stimulate non-legume plants growth such as radish and rice. Non-symbiotic Nitrogen fixing rhizospheric bacteria belonging to genera including *Azoarcus, Azotobacter, Acetobacter, Azospirillum, Burkholderia, Diazotrophicus, Enterobacter,*

Gluconacetobacter, *Pseudomonas* and *cyanobacteria* (*Anabaena, Nostoc*) (Vessey, 2003; Bhattacharyya and Jha, 2012). The genes for nitrogen fixation, called nif genes are found in both symbiotic and free living systems (Reed *et al.,* 2011). Nitrogenase (nif) genes include structural genes, involved in activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and function of the enzyme ((Kim and Rees, 1994). Since nitrogen fixation is a very energy demanding process, requiring at least 16 mol of ATP for each mole of reduced nitrogen, it would be advantageous if bacterial carbon resources were directed toward oxidative phosphorylation, which results in the synthesis of ATP, rather than glycogen synthesis, which results in the storage of energy in the form of glycogen (Glick, 2012; Ahemad *et al.,* 2014). Inoculation by biological nitrogen fixing plant growth promoting rhizobacteria on crop provide an integrated approach for disease management, growth promotion activity, maintain the nitogen level in agricultural soil (Gupta *et al.,* 2015).

Phosphate, Potassium, and Zinc Solubilization

Phosphorous is an important and vital element for growth and development of plant. It is a second macroelement which is usually restraining the growth of land plants. It plays a dynamic part in plant metabolism, synthesis of proteins, and photosynthetic process. In earth phosphorous exists in organic or inorganic forms (rock phosphate, mineral salts, and calcium phosphate) which plants cannot utilize. However, plants are able to absorb phosphorous in monobasic ($HPO4^{-}$) and dibasic ($H2PO4^{-}$) soluble forms (Glass, 1989). About 98% of Indian soil contains insufficient phosphorous and could not support plant growth and development. Phosphorus deficiency leads to poor root development, restricted growth, and low seed and yield production of plants. So, to overcome its deficiency in soil, synthetic P fertilizers are supplied to fields for better crop production. However, little portion of supplied synthetic P fertilizers are used by the plants, while the remaining portion gets converted into insoluble form. In this aspect, microorganisms present in the soil are supplied as biofertilizers which overcome the deficiency of phosphorous. These microbes convert the organic and inorganic forms of phosphorous into soluble form and are commonly called phosphate-solubilizing bacteria. Phosphate-solubilizing microbes are capable of solubilizing the phosphorous in free-living conditions in different types of soils and make it available to almost all types of crops. *Pseudomonas*, *Bacillus*, *Azotobacter*, *Agrobacterium*, *Rhizobium*, *Bradyrhizobium*, *Salmonella*, and *Thiobacillus* are few examples of phosphorus-solubilizing bacteria that have been reported to solubilize and mineralize the phosphorous in the soil (). These microbes produce the mineral dissolving compounds, viz., protons, organic acids, carbon dioxide, hydroxyl ions, nitric acid, sulfuric acid, and chelating substances (Zhu *et al.,* 2011). Besides bacteria, fungi also play an important part in solubilization of phosphorous and translocate it to the host plant (Liu *et al.,* 2012). Potassium is the third major nutrient element of plants. It plays an important role in the synthesis of protein, photosynthesis,

as well as in enzyme activation. The deficiency of potassium is a chief limitation in agricultural crops as it also exists in insoluble form that living organisms cannot utilize. PGPRs are the best alternative for keeping the quantity of potassium in soil sufficient for plant growth and productivity. Soil microbes such as *Bacillus edaphicus, Ferrooxidans species, Burkholderia species, Acidithiobacillus species, Bacillus mucilaginosus,* and *Pseudomonas species* have been testified to secrete potassium in usable state (Liu *et al.,* 2012). For better plant growth and development, several other micronutrients are also required. Among them, zinc is also an important one for optimal growth. It plays a significant role in the synthesis of biomolecules such as carbohydrates, nucleotides, and phytohormones like auxin and chlorophyll. Zinc also provides the resistance of plants against high temperature (Singh *et al.,* 2005).

Siderophore Production

PGPR are reported to secrete some extracellular metabolites called siderophores. For the first time Kloepper *et al.* (1981) reported the significance of siderophores produced by certain genera of PGPR in plant growth promotion. Siderophores are commonly referred to as microbial Fe-chelating low molecular weight compounds. The presence of siderophore-producing PGPR in rhizosphere increases the rate of Fe3+ supply to plants and therefore enhance the plant growth and productivity of crop.Further, this compound after chelating Fe3+ makes the soil Fe3+ deficient for other soil microbes and consequently inhibits the activity of competitive microbes (Singh, 2013).

Phytohormone Production

A wide range of microorganisms found in the rhizosphere are able to produce substances that regulate plant growth and development. Plant growth promoting rhizobacteria produce phytohormones such as auxins, cytokinins, gibberellins and Ethylene can affect cell proliferation in the root architecture by overproduction of lateral roots and root hairs with a subsequent increase of nutrient and water uptake (Lugtenberg *et al.,* 2009, Arora *et al.,* 2013). Different types of PGPR produce different phytohormones like cytokinins, IAA, etc. which can change the root structure and enhance the plant growth (Hayat, 2010,). PGPR producing IAA and gibberellins in rhizosphere soil play an essential function in enhancing the number of root tips and increasing the root surface area in several herbaceous plants (Han *et al.,* 2005, Jing *et al.,* 2007). PGPR producing IAA is believed to increase the root growth and length, enhance the surface area of root, and allow the plant to access more nutrients from soil. PGPRs, belonging to genera *Pseudomonas, Rhizobium, Azospirillum, Enterobacter cloacae, Bradyrhizobium japonicum, Bacillus cereus, Azotobacter, Burkholderia, and Mycobacterium* sp., have been reported to produce IAA and stimulate the plant growth. Werner *et al.* (2003) explained the increase in root surface area, root initiation, cell division, and cell enlargement through increased

growth of lateral and adventitious roots by the use of PGPR-formulated cytokinins (Bhardwaj *et al.*, 2014).

Indirect Mechanisms

The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents (Glick *et al.*, 2012).Indirect action refers to the capability of biofertilizers to diminish the harmful effects of phytopathogens on crop growth and productivity. The indirect methods include antibiotic synthesis, hydrogen cyanide synthesis, induced systemic resistance, cell wall degrading enzymes etc (Mushtaq, 2021).

Production of Antibiotics

Antibiotics are low molecular weight substances generally produced as secondary metabolites by soil microbes. Antibiotic-producing microbes are distributed widely in nature and are involved in various functions. Microbes produce a number of antibiotics; out of them, very few are nontoxic and are used in medicinal purposes. The antibiotics produced by soil microbes have biocidal and biostatic effects on soilborne phytopathogens. It was reported that *Penicillium*, *Streptomyces*, *and Bacillus* spp. Produce numerous antibiotics such as sublancin, bacilysin, chlorotetain, iturin, subtilosin, fengycin, bacillaene, phenazine-1-carboxylic acid, zwittermicin A, cepaciamide A, karalicin, pseudomonic acid, kanosamine, rhamnolipids, cepafungins, azomycin, 2,4-diacetylphloroglucinol (DAPG), aerugine, pyrrolnitrin, oomycin A etc. These antibiotics are deleterious to metabolism of pathogens and constraining their growth (Kundan *et al.*, 2015; Gupta, 2015). The synthesis of antibiotics such as pantocin, oomycin, etc. which is the primary method of PGPR acts as biocontrol agents. *Pseudomonas*, *Bacillus*, and *Rhizobium* are some significant PGPR strains identified. Antibiotics trigger to introduce systemic resistance (ISR) genes in crop plants through direct antipathogenic action. So, plant disease management is the major function of antibiotics (Fernando *et al*., 2005; Riaz *et al.*, 2020) Various strains of *Bacillus* are used in agricultural crops as biocontrol agents and suppressed the growth of other microorganisms which are responsible for various problems such as root rot caused by *Rhizoctonia solani* and *Pythium* sp. Han *et al.* (2005) have reported Delftia tsuruhatensis strain, HR4, which suppressed the growth of various plant pathogens like Pyricularia oryzae, Rhizoctonia solani and Xanthomonas oryzae.

Hydrogen Cyanide Production

It is a secondary metabolite used as biocontrolling agent of weeds in agricultural systems as it showed significant toxicity against plant pathogens (Kundan *et al.*, 2015). Large numbers of PGPR are able to produce HCN. It stops the energy supply of cells through the inhibition of electron transport chains and ultimately results in cell death. HCN have also antifungal activity as well as are responsible for the synthesis of some cell wall degrading enzymes proved that HCN produced by PGPR are able to inhibit the metalloenzymes and affect their toxicity. HCN

produced by PGPB are used as biofertilizers and were reported to promote the plant growth and productivity (Gupta, 2015; Rijavec and Lapanje, 2016)

Exo polysaccharides production or biofilm formation

Certain bacteria synthesize a wide spectrum of multifunctional polysaccharides including intracellular polysaccharides, structural polysaccharides, and extracellular polysaccharides. Production of exo polysaccharides is generally important in biofilm formation; root colonization can affect the interaction of microbes with roots appendages. Effective colonization of plant roots by EPS-producing microbes helps to hold the free phosphorous from the insoluble one in soils and circulating essential nutrient to the plant for proper growth and development and protecting it from the attack of foreign pathogens. Other innumerable functions performed by EPS producing microbes constitute shielding from desiccation, protection against stress (Qurashi and Sabri, 2012), attachment to surfaces plant invasion, and plant defense response in plant–microbe interactions (Tewari and Arora, 2014). Plant growth promoting rhizobacterial producing exo polysaccharides are highly important in promoting plant growth due to work as an active signal molecule during beneficial interactions, and provide defense response during infection process (Parada, 2006). Some plant growth promoting rhizobacterial producing exo polysaccharides can also bind cations, including Na+ suggesting a role in mitigation of salinity stress by reducing the content of Na+ available for plant uptake (Arora, 2013).

Induced systemic resistance (ISR)

Induced resistance may be defined as a physiological state of enhanced defensive capacity elicited in response to specific environmental stimuli and consequently the plant's innate defenses are potentiated against subsequent biotic challenges (Kloepper, 2004; Avis *et al.*, 2008). Biopriming plants with some plant growth promoting rhizobacteria can also provide systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal, bacterial, and viral origin and in some instances even damage caused by insects and nematodes can be reduced after application of plant growth promoting rhizobacteria (Naznin *et al.*, 2012). Moreover, induced systemic resistance involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant's defense responses against a variety of plant pathogens (Glick, 2012). Many individual bacterial components induce induced systemic resistance such as lipopolysaccharides (LPS), flagella, siderophores, cyclic lipopeptides, 2, 4-diacetylphloroglucinol, homoserine lactones, and volatiles like, acetoin and 2, 3-butanediol (Doornbos *et al.*, 2012).

Conclusion:

PGPR as biofertilizers are attractive as well as an economic approach for sustainable agriculture. The search for multifunctional PGPR is gaining importance around the world to minimize the amount of chemical agrochemical inputs required for sustainable crop production. The application of chemical fertilizers is effective as well as suitable for increasing crop productivity and disease control in agricultural practices but chemical fertilizers have several negative effects on the environment. PGPRs are considered eco-friendly alternative to hazardous chemical fertilizers. The use of PGPRs as biofertilizers is a biological approach toward the sustainable intensification of agriculture.

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BIOSURFACTANTS: APPLICATIONS AND METHODOLOGIES

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Introduction:

Surfactants are surface-active compounds that are capable of reducing the oil and water interfacial tension. Theyhave reached an indispensable presence in the pharmaceutical, agricultural, detergent, healthcareproduct industry (Zulkifli *et al.,* 2020). Chemically a surfactant molecule, also called an amphiphilic molecule, has two major parts namely, a hydrophilic head and a hydrophobic tail. The hydrophobic tail could be either a long/short hydrocarbon chain, a fluorocarbon chain or a siloxane chain. Whereas the hydrophilic head can consisteither of polyoxyethylene chains, sulfates, carboxylates, sulfonate, quaternary ammonium salts or alcohols (Gbadamosi *et al.,* 2019). A peculiar feature of surfactants is the ability to form aggregates called micelles (Bhosle *et al.,* 2020). The formation of micelles occurs once the concentration of surfactant reaches critical micelle concentration (CMC). Before entering the CMC state, the surfactants form pre-micelles and monomers which are distributed evenly in the system. The internal proton shift that occurs once CMC is achieved leads to aggregation and self-assemblyconverting pre-micelles into micelles (Cui *et al.,* 2008). It is duringthis formation of micelle, that the oil moieties get attached to hydrophobic tail. This eventually leads tothe entrapment of oil moieties in the micelle cavity facilitatingits effective dispersion or emulsification into aqueous phase (Miller, 1995).

The surfactants have mainly synthetic and biological origin. The synthetic surfactants can be categorized depending on their ionic charge as anionic, cationic, non-ionic and amphoteric (zwitterionic) surfactants (Tadros, 2014). The selection of surfactants depends on the requirement of the industry. Anionic surfactants are the most predominantly used and account for almost 50% of world surfactant consumption (Salager, 2002). Certain widelyemployed anionic synthetic surfactants include chemicals like sodium lauryl sulfate (SLS), alpha-olefin sulfonate (AOS), and alcohol propoxy sulfate (APS). In contrast, the cationic surfactants are relatively less prevalent owing to their economic concerns. However, wherever its cheap alternative is not available commonly employed synthetic cationic surfactants include cetrimonium bromide (CTAB), benzalkonium chloride (BAC), and dimethyldioctadecylammonium chloride (DODMAC). The non-ionic surfactants are the second most consumed, contributing to almost

45% of world surfactant production. Widely used non-ionic synthetic surfactants are alkyl polyglycoside (APG), Triphenylmethane (TPM), and Tridecyl Alcohol 30 Ethoxylate (TDA 30 EO). Amphoteric surfactants are relatively less toxic but face application hindrance owing to their expensive nature. Hence, their use is restricted in specialized cosmetics and areas where biocompatibility is essential. Prevalently employed synthetic amphoteric surfactants include amido sulfobetaine 16, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), and lauryldimethylamineN-oxide (Salager, 2002; Moldes *et al.,* 2021).

Surfactants and types:

Surfactants have found roles as wetting, foaming, emulsifying, and dispersing agentsin several industries like petrochemicals, pharmaceuticals, cosmetics, detergent, agrochemicals, and health care products industries (Grand Review Research, 2015). A major concern haunting this broad-spectrum application is its toxicity. Several studies undertaken to assess its safety have confirmed its environmental adverse effects at the site of release or application. Apart from environmental concerns, flora and fauna (land and water) have found to be equally susceptible. Algae,that are at the first trophic level and major oxygen suppliers in aquatic systems, are observed to be highly sensitivetowards surfactants. Sodium dodecyl sulfate (SDS) an anionic surfactant, for instance, showed toxic effect on *Lemna minor*, whereas quaternary ammonium compound showed toxicity against *Dunaliella* sp.(Dirilgen & İnce, 1995; Utsunomiya *et al.,* 1997). In yet another finding, the toxic effect of linear alkyl benzene sulfonate (LABS) on *Clarias gariepinus* (African catfish), was confirmed. The catfish was found to experience a loss of balance, respiratory damage, and irregular swimming movements with increase in concentration of LABS (Adadu and Ochogwu, 2020). Moreover, daodecylbenzene sodium sulfonate yet another anionic surfactant, was found to reduce lipid molecules located in epithelial cells, goblet cells and club cells of cat fist *Rita rita*, which further thereon affected its behavior leading to muscle spasm, erratic movements, and body torsion (Cserháti *et al.,* 2002). On the similar lines, toxicityconcerns of nonionic surfactants are equally palpable. Alcohol ethoxylates and nonylphenol ethoxylates, for example, demonstrated toxicity against exotic Australian tadpoles (Mann & Bidwell, 2001). In yet another study 4-nonylphenol exhibited dose dependent toxicityagainst African catfish *Clarias gariepinus*. Histological examinations of surfactant administered *Clarias gariepinus* revealed abnormal hepatic triad and central vein. Among the other consequences, disorganization of hepatic cords, dilation of sinusoids and enucleation of hepatocytes were noticed (Nagwanshi *et al.,* 2020). Moreover, the degradation products of alkylphenol ethoxylate, namely nonylphenols and octyl phenols, are of equal concern. They can

lead to the formation of vitellogenin in male rainbow trout fish, which under regular scenario is synthesized only in female fish under the influence of estrogen (Pedersen *et al.*, 1999). Alcohol polyoxyethylene ether-7 is reported to induce cardiac arrest, alteration in locomotion, reduction of RBCs in bone marrow, and liver damage (Al-Asmakh *et al.*, 2020). Cationic surfactant although less frequently is an equal cause of concern.Stearamidopropyl dimethylamine for instance has adverse effects on embryos of zebrafish causing edema in yolk sac and hepatomegaly (Al-Jamal *et al.*, 2020). The toxic effect of surfactants is quite noticeable in plants as well. Surfactants gaining an entry in agriculture water have demonstrated toxic effects on several plants like lettuce, cucumber, and wheat (Bubenheim *et al.*, 1997; Tot *et al.*, 2020).Moreover, a combination of salt water along with surfactants are found to work synergistically against plant well-being, causing huge damage to several coastal plants (Toscano *et al.*, 2022). Apart from the concerns raised for plants and aquatic fauna, humans are also sensitive towards surfactants. Studies have shown that cationic surfactants can damage human lymphocytes and corneal epithelial cells. Further more, they cancause irritation to skin, eyes, and respiratory systems (Johnson *et al.*, 2021). Thus, consideringthe hazardous effects of synthetic surfactants and their current industrial requirements, research has been extended exclusivelytowards search for biocompatible surfactants. Biosurfactants is one such avenue which can act as a substitute to synthetic surfactants.

Biosurfactants and types:

Biosurfactants are surfactants having amicrobial origin. Microbes produce these surface-active bio molecules as secondary metabolites. Microbes produce them either extracellularly or intracellularly. Biosurfactants possess various advantages over synthetic surfactants which include, quicker biodegradability, lower toxicity, high foaming capacity, low Critical Micelleconcentration (CMC) and eco-friendly nature. This makes them highly efficient over synthetic counterparts (Long *et al.*, 2016; Moldes *et al.*, 2021). For a biosurfactant, the hydrophilic component is either an amino acid, carbohydrate, phosphate group, proteins or alcohol moiety, whereas the hydrophobic tail isin the form of long chain fatty acids (Nayarisseri *et al.*, 2018). Based on this structural composition, biosurfactants are classified as glycolipids, lipopeptides, fatty acids, phospholipids and heavy polymeric biosurfactants (Henkel and Hausmann, 2019).

1. Glycolipids: Glycolipid biosurfactants are named after the carbohydrate present, for example, Rhamnolipid contains rhamnose sugar, Sophorolipids contain sophorose, Trehalolipids contain trehalose, etc. Yeast like *Candida apicola*, *Candida bombicola*

have been reported to produce sophorolipids, whereas *Pseudomonas* sp. are known to produce rhamnolipids (Wittgens *et al.,* 2017;Spencer *et al.,* 1970).

2. Lipopeptides: Lipopeptides biosurfactants, as the name suggests, are made up of lipids and protein moieties. *Bacillus pumilus, Bacillus stratosphericus* strain FLU5 have been reported to produce a lipopeptide called Surfactin (Hentati *et al.,* 2019). Fengycin, another type of lipopeptide biosurfactant, is expressed by *Brevibacillus borstelensis* and *Bacillus amyloliquefaciens* sp. *plantarum* F11 (Acedo *et al.,* 2018; Dong *et al.,* 2022).
3. Fatty acids: Fatty acid biosurfactantsare hydroxylated and cross linked in nature.They are a resultof microbial alkane oxidations. *Candida utilis* expressed the significant production of oleic acid, while *Aureobasidium pullulans* YTP6-14 showed the production of lactose hydroxy fatty acids. *Serratia rubidaea* on the other hand has been reported to produce rubiwettin R1 (Matsuyama *et al.,* 1990; Rahman & Gakpe, 2008; Luepongpattana *et al.,* 2017; Ribeiro *et al.,* 2020).
4. Phospholipids: Phospholipid surfactants are phosphate group containing surfactants and are a major constituent of microbial membranes. Few hydrocarbon degrading microbes when cultivated on alkanes the production of phospholipids. Phosphatidylethanolamine is seen to be produced from *Klebsiella pneumoniae* strain IVN51 and *Rhodococcus* sp. Moreover, *Bacillus azotoformans* EN16 and *Bacillus sphaericus* EN3 are also reported to be phospholipid biosurfactant producers (Kuyukina and Ivshina, 2010; Adamu *et al.,* 2015; Nwaguma *et al.,* 2016).
5. Polymeric: Polymeric biosurfactants have high molecular weight and are made up of complex molecules. Emulsan, which is a polyanionic heteropolysaccharide biosurfactant, was reported to be produced by *Acinetobacter* sp. (Jadeja *et al.,* 2019; Amani and Kariminezhad, 2016). Another polymeric biosurfactant called Liposan is produced by *Candida lipolytica* and contains 17% protein and 83% carbohydrate (Kapadia and Yagnik, 2013).

Biosurfactants and Applications:

Biosurfactants have extensive applications in diverse industries from cosmetics to pharmaceuticals. The global biosurfactant market according to Global Market Insights (2020) was valued at 1.7 billion USD in 2020 and is expected to grow with a 5.5% compound annual growth rate.

Biosurfactants in pharmaceutical industry:

Biosurfactants in the pharma industry was valued over 27.0 million US dollars in 2020 and is estimated to grow at 9% CAGR by the year 2027 (Global Market Insights, 2022).Certain prominent beneficial outcomes are described. Lipopeptides (LPs) for instance, isolated from bacteria like *Bacillus methylotrophicus*, exhibit antioxidant activity against free radicals. This antioxidant activity in turn prevents inflammation, leads to regeneration and differentiation of epidermis (Jemil *et al.,* 2017). Furthermore, another lipopeptide, isolated from *Bacillus subtilis* SPB1 used in toothpaste formulation, successfully exhibited antimicrobial activity against *S.typhimurium* and *Enterobacter* sp. (Bouassida *et al.,* 2017). Glycolipid, yet another biosurfactant, isolated from *Bacillus licheniformis* SV1 was incorporated in an ointment and was used for wound healing on rat tissue. The outcome of application was significantly positive and was recorded in the form of wound healing, proliferation of fibroblast cells and re-epithelialization. The ability of glycolipid in reduction of oxidative stress is anticipated to be the etiology towards wound healing (Gupta *et al.,* 2017). Certain biosurfactants are also found to express the anti-adhesion activity, which can be employed to inhibit the biofilm development of human pathogens at site of infection (Sun *et al.,* 2018). Surfactin on the other hand expressed antiviral properties against enveloped viruses like herpes virus, by preventing interaction between host cell membrane and virus (Yuan *et al.,* 2018). Moreover, surfactin derived from *Bacillus subtilis*induced deactivation of gastroenteritis coronavirus (Wang *et al.,* 2017). Lastly biosurfactants have also established their role in working of the immune system. Certain biosurfactants for example can play a significant role in treatment of autoimmune diseases. Probable mechanismslike downregulation of immune response by reducing the expression of CD54, CD40, MHC class II and CD80 have been put forward (Sajid *et al.,* 2020). Moreover, rhamnolipids isolated from *Burkholderia plantarii* were able to induce secretion of TNF-α by stimulating mononuclear cells in humans (Thakur *et al.,* 2021). To add on to the list, iturin produced by *Bacillus subtilis*, revealed in vitro ability to inhibit chronic myelogenous leukemia along with autophagy inhibition, apoptosis and paraptosis (Zhao *et al.,* 2018). Lastly sophorolipids exhibited inactivation of cancer cells in mice having tumors (Li *et al.,* 2017). Thus, examination of many such biosurfactants and their mechanisms are still understudy and await exploration.

Biosurfactants in agricultural industry:

A primary role played by biosurfactantsin the agricultural field includes the ability to increase the bioavailability of trace metals in soil by decreasing the surface tension between soil and metal cations (Wang and Mulligan, 2004). Besides this primary role, certain biosurfactants

have shown to exhibit plant growth promoting properties. Lipopeptides for instance derived from *Bacillus methylotrophicus* have been reported to show antifungal activity against phytopathogen *Botrytis cinerea.*Moreover, it is also seen to stimulate seed growth (Toral *et al.,* 2018). On a similar scale, cyclic lipopeptide and phenazine from *Pseudomonas* CMR12a aided in prevention of root rot in beans caused by *Rhizoctonia* (D'aes *et al.,* 2011). Furthermore, biosurfactant from *Bacillus subtilis* showed bio-nematocide activity against *Meloidogyne incognita*, which is a causative agent of root gall (Hussain *et al.,* 2020). To add on, Hultberg et al. reported anti-zoospore activity of biosurfactant produced by *Pseudomonas koreensis* on *Phytophthora infestans* which causes late blight disease in potato (Hultberg *et al.,* 2010).Biosurfactant isolated from Bacillus sp. has demonstrated to promote plant growth in different plants like tomato, canola maize and sudan grass (Sheng *et al.,* 2008).

Biosurfactants in detergent and cleaning industry:

Detergent industries play a vital role in our day-to-day life by supplying household cleaning products like laundry soaps and floor cleaners. The detergent industry is considered as the workhorse driving the biosurfactant market.Several biosurfactants have proved their worth, Bouassida et al. for instance showed the role of lipopeptide isolated from *Bacillus subtilis* SPB1 in laundry detergent formulation (Bouassida *et al.,* 2018). Furthermore, comparative study performed by Jimoh and Lin, demonstrated that lipopeptide biosurfactant isolated from *Paenibacillus* sp. D9 showed more than 60% efficiency in removing coffee and tomato sauce stains as compared to commercial surfactants (Jimoh and Lin, 2020).To add on to the scale, biosurfactant from *Pseudomonas aeruginosa* ATCC 10145 was able to remove 100% of special B1 fuel oil along with dispersion of 98% of motor oil fabric stains (Farias *et al.,* 2021). Moreover, rhamnolipid from *Pseudomonas aeruginosa* was able to remove stains of chocolate, ketchup, and blood from fabric at a similar efficacy as commercial detergents (Suryawanshi *et al.,* 2021). To upgrade further, biosurfactants produced by *Psychromonas ingrahamii* wereemployed along with lipase enzyme to prepare detergent formulations, that can work at lower temperatures (De Rose *et al.,* 2019).

Apart from these predominant applications, tremendous research is being undertaken on exploring and understanding the potential of biosurfactants in oil recovery and oil bioremediation from sea andland.

Methodology for examining biosurfactants:

There are a wide range of methods that can be employed to detect biosurfactant production by any microorganisms. These methods predominantly rely on direct measurement of

reduction in surface tension or their outcome. The choice of detection method depends on several factors of which the concentration of biosurfactant is a dominant one.

1. Drop collapse assay:

This first method is based on the principle that drop collapses/spreads on a hydrophobic surface in presence of biosurfactant. The surfactants which possess the ability to reduce surface tensions are anticipated for the collapse recorded.In this method a glass surface is coated with oil and is left standing for 24 hrs. This step ensures an even hydrophobic surface is created on the glass. On this hydrophobic surfacea drop of cell free supernatant (CFS) expected to contain biosurfactant is added. If the drop collapses instantly it's an indication of the presence of biosurfactant.This assay is sensitive, reliable and requires less volume of sample. However, at the same time effective results can be recorded only if significant concentration of surfactant is present **(**Jain *et al.,* 1991; Walter *et al.,* 2010).

2. Oil displacement assay:

As the name suggests, in this method we try to examine the displacement of oil in the presence of surfactant. Here, approximately 20 ml of water is transferredto a petri plate, across the water layer, oil is poured gently in such a manner that it results in the formation of thin surface layer. Following this, cell free supernatant (CFS) expected to contain biosurfactant is added.If the surfactant is present, the developed oil layer breaks resulting in a hollow zone of clearance. The size of the clear zone is in agreement with the concentration of biosurfactant present. With no specialized instrument required and lower detection limits in a reliable manner, makes this assay a preferred choicefor screening biosurfactant producers (Morikawa et al., 2000, Walter et al., 2010).

3. Microplate assay:

The wetting optical distortion property of the biosurfactant is the principal assessment parameter in the microplate assay. For this assay, a 96-well microtiter plate is loaded with cell free supernatant (CFS). Following the loading, a gridded paper like graph paper is placed under the plate. If biosurfactant is present, then the fluid surface will take the shape of a diverging lens (concave) and will result in optical distortionof the grid. The fluid surface is flat in case of distilled water. This simple, easy, and qualitative assay can be employed for screening of surface-active compounds (Cottingham *et al.,* 2004; Walter *et al.,* 2010).

4. Emulsification assay:

This assay measures the emulsifying competence of a biosurfactant making it a popular choice for activity detection. For this assaycell free supernatant (CFS) is vortexed at high speed along with oil/kerosene/hydrophobic compounds in equal volumes. Following the vortex, the

mixture is allowed to stand for 24 hours. If biosurfactant is absent two separate layers of CFS and oil can be visualized, whereas, in presence of biosurfactant three different layers can be recorded. The additional third layer stationed in the middle corresponds to the emulsified layer. This layer develops owing to biosurfactantsoil emulsifying capability. Further the height of the emulsified layer can be determined and be used to calculate emulsification index (EI_{24}). Emulsification index (EI_{24}) is calculated in percentage form, using the ratio of the emulsification layer height to total height of solution column. As the emulsification index may not correspondwith surface activity, the above assay only detects (does not measure) the presence of biosurfactant (Cooper and Goldenberg, 1987, Walter *et al.,* 2010).

5. BATH (Bacterial Adhesion to Hydrocarbon) assay:

This assay is based on the consideration that biosurfactant producing microorganisms can adhere to the oil surface. It is aneasy photometric assay that records the adherence of biosurfactant producing bacteria to hydrocarbons. For this assay, first the suspension of washed microbial cells is mixed with oil/hydrocarbons. After appropriate mixing of the two phases are allowed to separate. On separation, the biosurfactant producing cells will be carried above along with oil.This layer is then separated from the surface. Following separation, the turbidity of residual aqueous phase is recorded. and those which cannot produce will remain in aqueous phase. The reduction in turbidity can be directly corresponded to the amount of biosurfactant (Rosenberg *et al.,* 1980; Van der Vegt *et al.,* 1991).

6. Orcinol method:

The Orcinol method is specifically used for detection and quantification of rhamnolipids. For this assay, the diluted cell free supernatant (CFS) is mixedwith freshly prepared orcinol reagent. This mixture is then kept at 80°C for 30 min in a water bath followed by cooling at room temperature. The absorbance is recorded which on extrapolationof a standard graph of rhamnose determines the concentration of rhamnolipid (Chandrasekaran, 1980).

For the quantitative and characterization analysis of biosurfactants various advanced techniques like high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas chromatography mass spectrophotometry (GC-MS) and fourier transform infrared spectroscopy (FTIR) can be used. The underlying principle of this technique is that retention time in case of analysis and fragmentation patterns according to mass are unique for a given compound and thus can be used as a fingerprint to identify and quantitate the biosurfactant (Eslami *et al.,* 2020; Trindade *et al.,* 2021).

Overall, we can conclude the exploration of true potential of biosurfactants in terms of source and application is still underway. Considering the microbial diversity an equitable diversity in biosurfactants is expected making it the most vouched microbial secondary metabolite.

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FUNGI: A REVIEW

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Introduction:

Mycology is branch of science which deals with the study of fungi. The fungi are achlorophyllous, heterotrophic, eukaryotic, spore bearing, unicellular or multicellular, septate, aseptate or coenocytes branched. They are found in the water, in the soil, on seed, in the air, plants, animals on starchy material and on hide leather. The fungi are cosmopolitan in distribution. A bunch group of hyphae called mycelium. A single thread of mycelium called hyphae. The cell wall composed of fungal cellulose or Chitins. They may be parasites, saprophytes symbionts or predators. Some fungi grow on our food materials like bread, jams, prickles, fruits, vegetables and crops seeds. There is a food material is glycogen and fat they have complete history topic mode of nutrition saprophyte, parasitic, symbiotic or predators.

i. **Parasitic:** obtain food from other living organisms. Example *Puccinia, Albugo, Fusarium, Claviceps, Uncinula.*

ii. **Saprophytic:** obtain food from dead and decaying organic matter. Example *Rhizopus*, *Mucor* and *Aspergillus.*

iii. **Symbiotic:** Some fungi as symbionts in lichen thalli. Example, *Corella*, *Dictoyonema, Cora, Pavoniaetc.*

iv. **Predaceous:** which tab small worms, protozoa. Example *Atrobotrys.*The reproduces by vegetative, asexual and sexual. The vegetative reproduction takes place by formation of fragmentation, fission, and budding. Asexual reproduction takes place by formation of spores, conidia. Sexual reproduction takes place by isogamous, on isogamous and oogamous. Sexual reproduction takes place gamates sex organ called gametangia. Male gametangia are called Antheridia and female gametangia are called oogonia. Sexual reproduction involves karyogamyie. Union of male and female nuclear. Plasmogamyie. Fusion of protoplasm of two gametes. Meiosis after which applied condition restore producing spores (Johri *et al.*, 2011; Vashishta and Sinha, 2016).

Economic importance of fungi:

Fungi include hundreds of species which are of tremendous economic importance to man. In fact our lives are intimately linked with those of fungi. They play an important role in medicine yielding antibiotics, in agriculture by maintaining the fertility of the soil and causing crop and fruit diseases, forming basis of many industries and as important means of food (Gutiérrez *et al.*, 2011). The important activities of fungi are:

1. Useful activities
2. Harmful Activities

1. Useful activities:

Several species of fungi are useful to us in many ways. They provide food,, organic acid, industry, production of enzymes Biomedical and Pharmaceuticals products

- **Fungi as food:**

Numerous species of fungi are edible, about 2000 species of them have been reported from all over the world. Among these, about 200 are said to occur in the Western Himalayas. They include mushroom and morels such as mushroom *Agaricus bisporus (White button mushroom), Volvariella volvacea (Paddy straw mushroom), Pleurotussajor-caju (Indian oyster mushroom), Agaricus campestris* (dhingri), morels (*Morchella, guchhi*), and truffles are edible. They are excellent source of proteins, carbohydrates, fats, vitamins, minerals, energy and rich amino acids. Mushroom protein is comparable with animal protein.

- **Fungi in industries:**

Many species of fungi are used as source organism in industries to manufacture some valuable products. Some importance uses of fungi in industries are baking industry, Brewing industry, Dairy industry, production of organic acids and production of enzymes.

- **Application in Brewing and Baking Industry:**

Fermentation of sugar by yeast under anaerobic condition is the basis of production of great variety of alcoholic beverages Products commercially in high demand such as Beer, Wine, Gin, Vodka and Whisky. *Saccharomyces cerevisiae, Saccharomyces carlsbergensis and Saccharomyces uvaum* are used for production of alcohol and wine (Meyers *et al.*, 1967).

Application in Dairy industries:

Penicillium camemberti is used for making Camembert type (soft cheese) and *Penicillium roqueforti* is used for making Roquefort type cheese (Hard cheese).

- **Organic Acids: Fungal Source and Application:**

Various species of fungi are used in the commercial production of organic acids. The organic acids are metabolic intermediates produced by the biochemical activities of cells.

Sr. No.	Organic acid	Sources	Uses
1	Citric acid	*Aspergillus niger*	Medicine, Food preservation, soft drink, powerful cleaning agent, cosmetic product
2	Fumaric acid	*Rhizopus nigricans*	Used in food and beverage products, oral pharmaceutical formulations
3	Kojic acid	*Aspergillus oryzae*	Used in food and cosmetic industry, Antiseptic and industrial raw material.
4	Itaconic acid	*Aspergillus terreus*	To prepare acrylic fibres and rubbers, artificial diamond and lens

- **Fungal enzymes: Industrial Use:**

Some fungi produce enzyme and their uses given table

Sr. No.	Enzyme	Fungus	Uses
1	**Amylase**	*Mucorrouxii, Rhizopus japonicus, Aspergillus oryzae*	Beer making, corn syrup, fruit juice extraction, starch modification, f cleaning compounds, and textile processing, animal feed supplements.
2	**Protease**	*Aspergillus oryzae, A. niger, A. flavus, A. sojae*	Meat tenderization, Bakery
3	**Cellulase**	*Trichoderma koengi*	Paper industry, detergent, coffee
4	**Diastase**	*Aspergillus oryzae*	Acid reflux, food supplement
5	**Pectinase**	*Sclerotinia libertina, Aspergillus niger*	Clarification of Fruit juice, wine making,
6	**Ivertase**	*Saccharomyces cerevisiae*	Paper industry , confectionary
7	**Lipase**	*Rhizopus spp.*	Baking, Pancreatic disorder

- **Fungi in biomedical field as pharmaceuticals:**

Many fungal species produces antibiotics, steroids, vitamins and protein supplements.

- **Antibiotics:**

Sr. No.	Antibiotics	Fungus	Useful against
1	Griseofulvin	*Penicillium griseofulvum*	Antifungal, used against mycosis, ring worm, athlete's foot
2	viridin, glycodin	*Trichoderm aviridi*	Pathogenic fungi
3	Cephalosporin	*Cephalosporium acremonium*	For treatment of Gram '+' and Gram '-' bacterial diseases
4	Fusidin	*Fusidium coccinieum*	Antibacterial
5	Penicillin	*Penicillium chrysogenum*	For treatment of Gram '+' bacterial diseases
6	Clavicin	*Aspergillus clavatus*	Antifungal, used to treat fungal infection

Steroid: Cortisone is steroid produced by *Aspergillusniger and Rhizopusnigricans*.Cortisone are used as Rheumatic arthritis, allergy and some other diseases.

Gibberellins: These are plant hormones produced by the fungus *Gibberellafujikuroi*which cause Bekane disease of rice accompanied by abnormal elongation. Gibberellin is used to accelerate growth of several horticultural crops.

- **Vitamins:**

Yeast (*Sachharomyces sake*), *Fusarium oxysporum* and *Ashbyagossypii produces vitamins (Vitamin B- complex, Riboflavin ie. Vitamin B2* and *Panthothenic acid ie.Vitamin B2).* Vitamin are used increasing energy level, boosting immune system function, maintaining healthy hair and rheumatoid, premenstrual syndrome, arthritis and enlarge prostrate.

- **Fungi as biocontrol agents:**

Many fungi can antagonize, including plant pathogenic fungi. They can be readily grown in culture in large quantities for release, into the environment as biocontrol agent. For example *Trichoderma* spp. can act as biocontrol agent for pathogenic fungi (*Alternaria, Fusarium, Aspergillus, Helimenthosporium, Pyricularia*) etc.

Harmful activity/fungi as Foe:

Several fungi are found to be harmful to man, animals, crops plants, Spoilage of Food, Essential commodities and Destruction of Timber

- **Fungi cause animal diseases: S**everal fungi cause diseases in animal. Eg. *Aspergillusfumigatus*causes mastitis disease in cattle.
- **Fungi cause human diseases:** Many fungi cause diseases in human eg. *Microsporum furfur* causes Dandruff in human, *Aspergillusfumigatus* causes lung diseases in human.
- **Fungi causes plant diseases:** Many parasitic fungi cause disease in crop plants for eg. Late blight of Potato caused by *Phytophthora infestans,* Black stem rust of wheat caused by *Puccinia graministritici,* Blast of Rice caused by *Pyricularia oryzae,* Downy mildews of grapes caused by *Perenospora viticola,* Damping off Many vegetable crops in including cucurbits; brassicas; brinjal, lettuce caused b*y Pythium sp , Rhizoctonia sp, Phytophthora spp.*and Red rot of sugarcane caused by *Colletotrichum fulcatum.*

Spoilage of Food and Essential Commodities:

- **Food articles** like jam, jelly, pickles, fruits and vegetables are degraded by fungi- *Aspergillus, Penicillium, Rhizopus.*
- **Wood decay**: Main culprits are *Polyporus*spp, *Fomes*spp, *Ganoderma*spp,*Serpulalacrymans*
- **Destruction of articles**: Quality of leathers, textiles, plastics, rubber, wool, painted surfaces are degraded by different spp of*Aspergillus, Penicillium, Alternaria, Cladosporium, Rhizopus*etc.

Mycotoxins:

Food poisoning due to fungal toxins called mycotoxins. For example Aflatoxins produces by *Aspergillus flavus and A. parasiticus, Ochratoxin* Produces by *Aspergillus ochraceus, Penicillium verrucosum* and *Patulin produces* by *Penicillium expansum, Aspergillus gigantes,* other *Penicillium* and *Aspergillus spp.*

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