Microbial Analysis of Soil and Water Samples from Koel River in Rourkela, Odisha

PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF MASTER OF SCIENCE IN LIFE SCIENCE

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CERTIFICATE

This is to certify that the thesis entitled "**Microbial analysis of Soil and Water samples from Koel River in Rourkela, Odisha**" submitted to National Institute of Technology, Rourkela for the partial fulfillment of the Master degree in Life science is a faithful record of bonafide and original research work carried out by Priya Monalisha Burh under my supervision and guidance.

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DECLARATION

I, Miss. Priya Monalisha Burh, M. Sc. Life Science, 4th semester, Department of Life Science, NIT, Rourkela declare that my project work titled " **Microbial Analysis of Soil and Water Samples from Koel River in Rourkela, Odisha"** is original and no part of this work report has been submitted for any other degree or diploma. All the given information and works are true to my sense and knowledge.

(Miss. Priya Monalisha Burh)

Date:

Place:

ACKNOWLEDGEMENT

This project is by far the most significant accomplishment in my life and it would have been impossible without people who supported me and believed in my caliber.

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TABLE OF CONTENTS

LIST OF SYMBOLS AND ABREVIATIONS USED

-	Negative
+	Positive
%	Percentage
°	Degree
µl	Microlitre
C	Centigrade
CFU	Colony Forming Unit
gm	Gram
hr	Hour
ISSS	International Soil Science Society
l	Litre
LB	Luria Bertani
Min	minute
ml	milli litre
NA	Nutrient Agar
No.	Number
ppm	Parts per million
TNTC	Too numerous to count
USDA	United State Department of Agriculture

LIST OF TABLES

LIST OF FIGURES

16	*Oscillatoria*	50
17	*Nostoc*	51
18	*Scenedesmus dimorphs*	52

ABSTRACT

Koel River from its early existence plays a significant role in molding the physical, economic and social development of Rourkela. It is acknowledged as one of the important elements in environmental aspect for its crucial function as water resources. As this project entitled "Microbial analysis of Soil and Water samples from Koel River in Rourkela, Odisha" deals with the water and soil quality of Koel River, the water and soil quality here were found out to be less polluted. The texture of the soil was analyzed to be clayey type which is appropriate for irrigation. The microorganisms isolated from the soil were of staphylococcus strain and were gram positive, aerobic, coccus shaped bacteria. The phytoplankton species identified from this river were *Spirogyra, Oscillatoria ,Chromolina ovalis, Tetrapedia gothica, Diatoms, Scendusmus dimorphus*, *Chrysococcus cornuta, Penium sylvanigra of which Spirogyra and Oscillatoria were dominant, followed by diatoms, scendusmus dimorphus* as sub dominant and the rest two*, i.e., Chrysococcus cornuta, Penium sylvanigra* come under rare species. This project aims to find out the water and soil quality of Koel River and as it is flowing through an industrial area, to find out if it is getting affected by the Industrial pollutants.

1. **INTRODUCTION**

Koel River originates from the Palamu Tiger Reserve and flows in the western portion of Palamu. The river splits into two-the North Koel River and the South Koel River. South Koel River runs across Jharkhand and Odisha states in India. The south Koel originates on the Ranchi plateau a few miles east of Ranchi. It enters Odisha and joins Sankh River at Vedveyas near Rourkela from where it is named Brahmani.

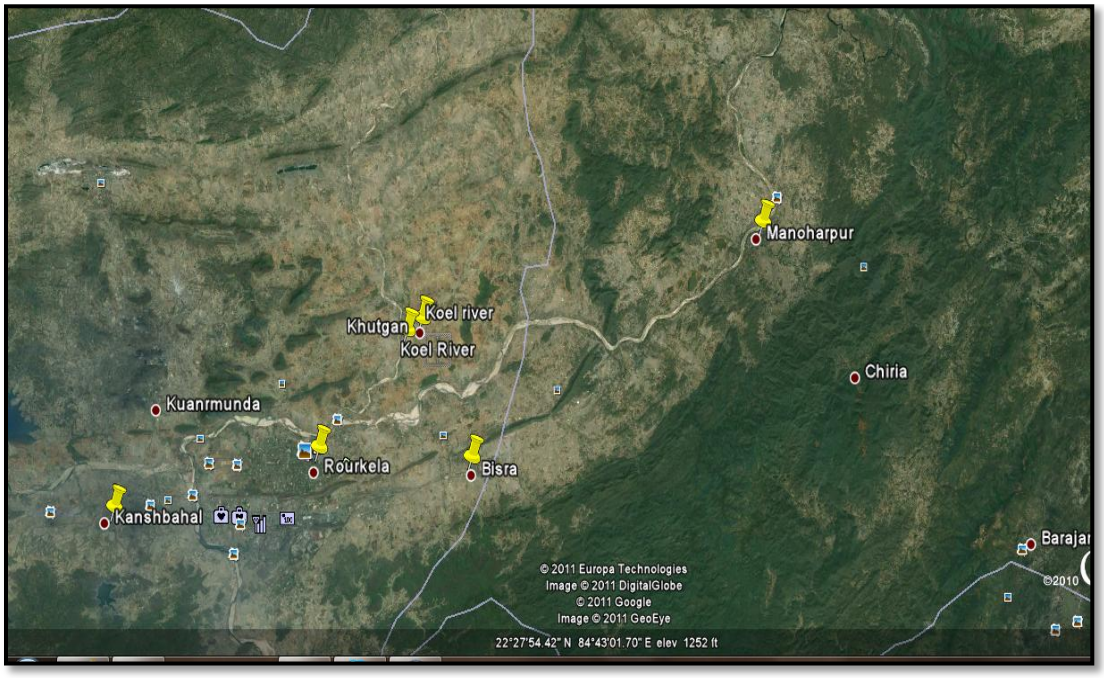


Fig1: Site map of Koel River

Classification of rivers:

Rivers can generally be classified as either alluvial, bedrock, or some mix of the two.

Alluvial rivers:

Have channels and floodplains that are self-formed in unconsolidated or weakly consolidated sediments. They erode their banks and deposit material on bars and their floodplains.

Bedrock Rivers

Form when the river down cuts through the modern sediments and into the underlying bedrock. This occurs in regions that have experienced some kind of uplift (thereby steepening river gradients) or in which a particular hard litho logy causes a river to have a steepened reach that has not been covered in modern alluvium. Bedrock Rivers very often contain alluvium on their beds; this material is important in eroding and sculpting the channel. Rivers that go through patches of bedrock and patches of deep alluvial cover are classified as mixed bedrock-alluvial.

Classification based on age of rivers:

- **Youthful River:** a river with a steep gradient that has very few tributaries and flows quickly. Its channels erode deeper rather than wider. E.g. the Brazos , Trinity and Ebro rivers.
- **Mature river**: a river with a gradient that is less steep than those of youthful rivers and flows more slowly. A mature river is fed by many tributaries and has more discharge than a youthful river. Its channels erode wider rather than deeper. E.g. the Mississippi, Saint, Danube, Ohio, Thames and Paraná rivers.
- **Old River:** a river with a low gradient and low erosive energy. Old rivers are characterized by floodplains E.g. the Yellow Ganges, Tigris, Euphrates, Indus and rivers.
- **Rejuvenated river**: a river with a gradient that is raised by tectonic uplift.

Uses:

- Rivers have been used as a source of water for obtaining food, transport, defensive measure, hydropower to drive machinery, for bathing, as a means of disposing waste.
- Have been used for navigation since thousands of years. Riverine navigation provides cheap means of transport.
- Since river boats are often not regulated, they contribute a large amount to global green house gas emission.

They also provide a rich source of food, like fish and other edible aquatic life and are a major source of fresh water which can be used for drinking and irrigation purpose.

Soil is a natural body consisting of layers (soil horizons) of mineral constituents of variable thicknesses, which differ from the parent materials in their morphological, physical, chemical, and mineralogical characteristics (Birkeland and Peter**,** 1999). It's composed of particles of broken rock that have been altered by chemical and environmental processes that include weathering and erosion. Soil differs from its parent rock due to interactions between the lithosphere, hydrosphere, atmosphere, and the biosphere (Chesworth, 2008). It is a mixture of mineral and organic constituents that are in solid, gaseous and aqueous states (Voroney et al., 2006). Soil is also known as earth: it is the substance from which our planet takes its name. Little of the soil composition of planet Earth is older than the Tertiary and most no older than the Pleistocene (Buol et al., 1973). In engineering, soil is referred to as regolith, or loose rock material.

Water covers 70.9% of the Earth's surface ("CIA- The world fact book". Central Intelligence Agency), and is vital for all known forms of life ("United Nations". Un.Org. 2005-03-22). On Earth, it is found mostly in oceans and other large water bodies, with 1.6% of water below ground in aquifers and 0.001% in the air as vapor, clouds (formed of solid and liquid water particles suspended in air), and precipitation (Water Vapor in the Climate System, Special Report, [AGU], December 1995). Oceans hold 97% of surface water, glaciers and polar ice caps 2.4%, and other land surface water such as rivers, lakes and ponds 0.6%. A very small amount of the Earth's water is contained within biological bodies and manufactured products. Liquid water is found in bodies of water, such as an ocean, sea, lake, river, stream, canal, pond, or puddle. The majority of water on Earth is sea water. Water is also present in the atmosphere in solid, liquid, and vapor states. It also exists as groundwater in aquifers. Water plays an important role in the world economy, as it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation. Approximately 70% of freshwater is consumed by agriculture. (Baroni et al., 2007).

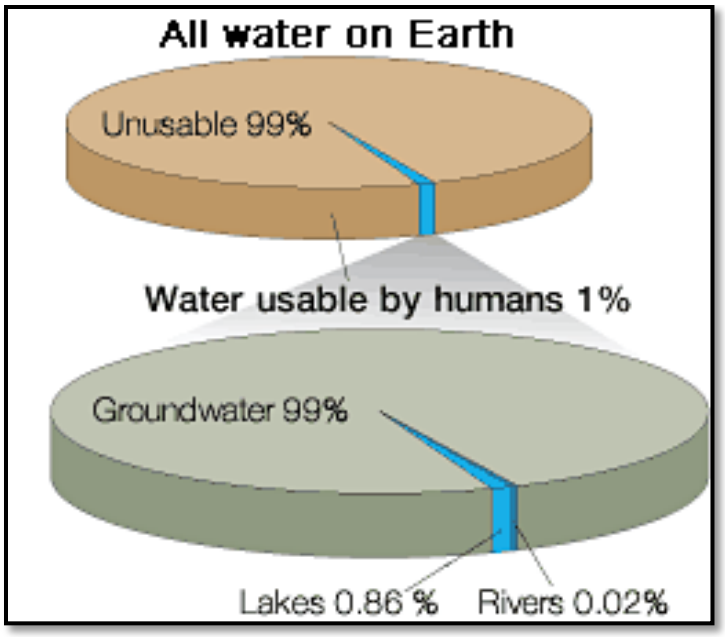


Fig2: pie chart showing percentage of water on earth

A river is a natural watercourse, usually freshwater, flowing towards an ocean, a lake, a sea, or another river. Small rivers may also be called by several other names, including stream, creek, brook, rivulet, tributary and rill; there is no general rule that defines what can be called a river, although in some countries or communities a stream may be defined by its size. A river is part of the hydrological cycle. Water within a river is generally collected from precipitation through surface runoff, groundwater recharge, springs, and the release of stored water in natural ice and snow packs (e.g., from glaciers). Potamology is the scientific study of rivers.

A fresh water ecosystem consists of natural vegetation, plants, minerals, micro-organisms, fish, some algae growth, plenty of sunshine and a natural undisturbed, unpolluted environment. A fresh water spring flows naturally through the mountains and rolling hills and can be enjoyed by many for drinking purposes. A good sign of any fresh water is abundant life and natural growing vegetation. Fish, insects, algae, and a rich natural growing plant system is all part of the natural fresh water and all thrive and survive as long as the water isn't polluted or disturbed.

Phytoplankton is the autotrophic component of the plankton community. The name comes from the Greek words (*phyton*), meaning "plant", and (*planktos*), meaning "wanderer" or "drifter". Most phytoplankton is too small to be individually seen with the unaided eye. However, when present in high enough numbers, they may appear as a green discoloration of the water due to the presence of chlorophyll within their cells (although the actual colour may vary with the species of phytoplankton present due to varying levels of chlorophyll or the presence of accessory pigments such as phycobiliproteins, xanthophylls, etc.). Extracellular organic substances are known to serve as nutrients for bacteria, algae, and invertebrates (Collier et al. 1953; Wright and Hobbie 1966).

The aim of this project is to find out the soil and water quality of Koel River and to find out if it is getting affected by the industrial pollutants, since the river flows through Rourkela which is the industrial capital of Odisha.

2. REVIEW OF LITERATURE

2.1 Sediment Characteristics:

Regardless of the source of particular sediment, certain physical characteristics are important in describing what the sediment "looks like." Primary among these are size, sorting, shape and color. The physical measurement and statistical treatment of grain-size data are basic tools in the investigation of marine sediments.

Texture is the general physical appearance or character of sediment. It is used to describe the geometric aspects and mutual relationships among the component particles or crystals and as emphasized by Lewis (1984), is one of the three basic descriptors of a sedimentary deposit, the other being structure and composition. Within the sedimentary realm of Earth Science, the term “Texture” usually includes the fundamental attributes of: (a) size, (b) shape and (c) arrangement or fabric, also defines texture as essentially the minogeometry of sediment a definition which emphasizes the characteristic of individual grains and grain-to-grain relations, versus large scale features of the deposit, such as sedimentary structures, bedding character or stratigraphic sequence.

Much of the importance of texture, as with composition and structure, lies in its inherent role as a mean of description and communication. Just as we might describe lacustrine deposit as black, organic-rich sediment or finely laminated siliciclastic-dominated sediment, we also routinely use the textural aspects of the material for classification and communication. Fine sand, well sorted silt, poorly sorted mud, matrix supported gravel all convey an explicit and quantitative meaning. Secondly in addition to fundamental description, classification and communication, careful study of the various textural parameters can lead to important clues about: - (i) source (provenance), (ii) the mechanism responsible for the transport of the material, (iii) past physical and chemical environmental and chemical environmental and limnological conditions at the depositional site within the basin, and (iv) paleoclimatic and paleohydrological conditions within the surrounding water shed. Thirdly, knowledge of the texture of a lacustrine deposit is important because of its influence on other key properties of the sediment.

Size limits of sediment particle size in the United State Department of Agriculture (USDA) and International Soil Science Society (ISSS) Schemes (Table1). Another well known classification of "detrial particles" was published by C.K.Wentworth in 1922 (Table2).

Table.1.**Size limits of sediment particle size in USDA and ISSS scheme**

USDA Scheme		ISSS Scheme	
Name of particle size	**Diameter Range (µm)**	**Name of particle size**	**Diameter Range (µm)**
Very coarse sand	**2000-1000**	**Coarse sand**	**2000-200**
Coarse sand	**1000-500**	**Fine sand**	**200-20**
Medium sand	**500-250**	**Silt**	**20-2**
Fine sand	**250-100**	**Clay**	**< 2**
Very Fine sand	**100-50**		
Silt	**50-2**		
Clay	**< 2**		
Coarse Fragments			
Gravels	**2000-75,000µm(2-75mm)**		
Cobbles	**75,000-25,400µm(75-254mm)**		
Stones	**> 254,000µm(>254mm)**		

Table.2. **Size limits of sediment particle size of detrial particles**

Name	Diameter in mm
Boulder	**›256**
Cobble	**64 - 256**
Pebble	**2 - 64**
Sand	**0.0625 - 2**
Silt	**0.0039 – 0.0625**
Clay size particle	**0.0039**

2.2 Characteristics of soil:

Soil colour:

Soil colour does not affect the behavior and use of soil; however it can indicate the composition of the soil and give clues to the conditions that the soil is subjected to (Brady et al., 2006). Soil can exhibit a wide range of colour; gray, black, white, reds, browns, yellows and under the right conditions green (Brady et al., 2006). Varying horizontal bands of colour in the soil often identify a specific soil horizon. The development and distribution of color in soil results from chemical and biological weathering, especially redox reactions. As the primary minerals in soil parent material weather, the elements combine into new and colorful compounds. Aerobic conditions produce uniform or gradual color changes, while reducing environments result in disrupted color flow with complex, mottled patterns and points of color concentration.

Soil texture

It refers to sand, silt and clay composition. Soil content affects soil behavior, including the retention capacity for nutrients and water (Brown, 2003). Sand and silt are the products of physical weathering, while clay is the product of chemical weathering. Clay content has retention capacity for nutrients and water. Clay soils resist wind and water erosion better than silt and sandy soils, because the particles are more tightly joined to each other. In medium-textured soils, clay is often translocated downward through the soil profile and accumulates in the subsoil. Soil texture is the capacity of soils to store water and nutrients increases when their clay percentage increases since clay has a large surface area/unit volume and they can absorb large amounts of nutrients and water. A soil with sandy texture has difficulty in retaining water and thus nutrients are not made available to the plants growing in such a soil. A clayey soil is poorer in aeration (due to stagnation of water) thus debilitating the plant growth. Loamy soil (a mixture of clay and sand) is the best textured soil for crop cultivation since it has all the beneficial aspects not found in the sandy and clayey soil. Thus we can see how texture affects the plant growth. Importance: Soil texture is one of the most important properties of soil particularly when dealing with water

movement through the soil. Soil mechanical and engineering properties are also influenced by soil texture.

Soil structure:

It is the arrangement of soil particles into aggregates. These may have various shapes, sizes and degrees of development or expression (Soil Survey Division Staff, 1993). Soil structure affects aeration, water movement, resistance to erosion and plant root growth. Structure often gives clues to texture, organic matter content, biological activity, past soil evolution, human use, and chemical and mineralogical conditions under which the soil formed. If the soil is too high in clay, adding gypsum, washed river sand and organic matter will balance the composition. Adding organic matter to soil that is depleted in nutrients and too high in sand will boost the quality (http://www.usaweekend.com).

Soil resistivity

It is a measure of a soil's ability to retard the conduction of an electric current. The electrical resistivity of soil can affect the rate of galvanic of metallic structures in contact with the soil. Higher moisture content or increased electrolyte concentration can lower the resistivity and increase the conductivity thereby increasing the rate of corrosion. Soil resistivity values typically range from about 2 to 1000 Ω m, but more extreme values are not unusual (Edwards, 1998).

Types of soil:

Soil is basically of 5 types, these are:-

- **Sandy Soil**: Sandy soil is light and dry in nature. It does not have moisture content and warms up quickly in the spring. Thus, it is good for the production of early crops. Sandy soil is fit for cultivation any time of the year and is comparatively easier to manage. Since it absorbs water quickly, the plants rooted in it need to be watered frequently.(fig a)
- **Clay Soil**: Clay soil is also called ‘late’ soil, because its wet nature makes it apt for planting seeds in late autumn. The soil serves as an excellent retort for the dry season,

as it has a high water retention quality. It is necessary to drain clay soil frequently, for improving its texture. The soil becomes unmanageable during rainy season, as it becomes ‘sticky’. On the other hand, during draught, it becomes ‘rock solid’ (fig b).

- **Loam Soil**: Given the tag of being the perfect soil, Loamy soil is a combination of all the three - sandy soil, clay soil and silt soil, in the ratio of 40:40:20. It is suitable for any and every kind of crops. An amalgamation of three soils, loam soil has best of the characteristic of all. It has high nutrients content, warms up quickly in summers and rarely dries out in the dry weather. It has become the ideal soil for cultivation.(fig c)
- **Peaty Soil**: Peaty soils are acidic in content, which makes them sour. This is the most exceptional feature of Peaty soils. Usually found in low-lying areas, these soils require proper drainage, as the place is accustomed to a lot of water clogging. Though peaty soils have less nutrient content, they warm up quickly in the spring, making them excellent if right amount of fertilizers are added.(fig d)
- **Chalky Soil**: Chalky soil is alkaline in nature and usually poor in nutrients. It requires nourishment, in the form of additional nutrients and soil improvers, for better quality. The soil becomes dry in summers, making it very hard, and would require too much of watering for the plants to grow. The only advantage which such a soil has is its lime content. When deep-rooted, Chalky soil becomes excellent for plant growth and favors good growing conditions as well.(fig e)

Fig a: Sandy soil

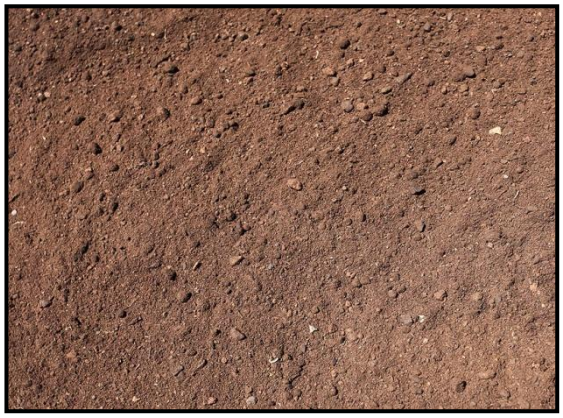


Fig b: Clay soil

Fig c: loamy soil

Fig d: Peaty soil

Fig e: Chalky soil

Fig3 (a-e): Different types of soils

2.3 Bacterial enumeration

Advances in microbial methods have demonstrated that microorganisms globally are the dominating organisms both concerning biomass and diversity. Their functional and genetic potential may exceed that of higher organisms. Studies of bacterial diversity have been hampered by their dependence on phenotypic characterization of bacterial isolates. Molecular techniques have provided the tools for analyzing the entire bacterial community including those which we are not able to grow in the laboratory. Reassociation analysis of DNA isolated directly from the bacteria in pristine soil and marine sediment samples revealed that such environments contained

in the order of 10, 000 bacterial types. The diversity of the total bacterial community was approximately 170 times higher than the diversity of the collection of bacterial isolates from the same soil. The culturing conditions therefore select for a small and probably skewed fraction of the organisms present in the environment. Environmental stress and agricultural management reduce the bacterial diversity. With the reassociation technique it was demonstrated that in heavily polluted fish farm sediments the diversity was reduced by a factor of 200 as compared to pristine sediments. Here we discuss some molecular mechanisms and environmental factors controlling the bacterial diversity in soil and sediments.

A study undertaken in a tundra area in southwestern Alaska showed that there were considerably greater numbers of bacteria in the soil than in the water. The highest plate count of a soil sample was 15,000,000 per gm, while of water it was 4,400 per ml. These counts were made of samples incubated a t 20°C. Progressively lower counts were obtained a t 37", 3’-5', 45", and 55%. While fewer kinds of bacteria were found in the soil, the difference between soil and water was not great. It was noted that mesophiles predominated both in numbers and in kinds, followed by psychrophiles and thermophiles, in that order. (Fournelle.H.J, 1958).

2.4. Phytoplankton analysis

Phytoplankton obtain energy through the process of photosynthesis and must therefore live in the well-lit surface layer (termed the euphotic zone) of an ocean, sea, lake, or other body of water. Phytoplankton accounts for half of all photosynthetic activity on Earth. Thus phytoplanktons are responsible for much of the oxygen present in the Earth's atmosphere – half of the total amount produced by all plant life. Their cumulative energy fixation in carbon compounds (primary production) is the basis for the vast majority of oceanic and also many freshwater food webs (chemosynthesis is a notable exception). Since the 20th century, phytoplankton has declined by roughly 1% yearly, possibly linked to warming oceanic temperatures - as of 2010 this means a decline of 40% relative to 1950. As a side note, one of the more remarkable food chains in the ocean – remarkable because of the small number of links – is that of phytoplankton feeding krill (a type of shrimp) feeding baleen whales.

Plankton abundance in relation to physicochemical features of Mancharibele reservoir in Bangalore district was studied by Sukumaran and Das (2002). Zooplankton population was found to comprise of four major groups, which include protozoa, rotifera, cladocerans and copepods. Protozoa were represented by *Arcella*, *Centrophyxis* and *Difflugia* species. However, the numbers were found to be less. Rotifers were found to be the second dominant group and were represented by a large number of species and genera. Cladocerans density was less when compared to rotifers and six species were observed. Copepods were found to be represented mainly by *Diaptomus* and *Cyclops*. They were found to be the dominant group among the zooplankton. The optimal temperature requirement varied for different groups of zooplankton suggesting their abundance indifferent seasons. The high chloride content and temperature were also found to favor zooplankton abundance.

A study on plankton and a few physicochemical features of Milghatta and Hutcharayangere tanks in the Malnad region of Karnataka was done by Sukumaran *et al.*, (1984). The zooplankton encountered includes Protozoa (3.54%), Rotifera (2.23%), Cladocera (3.15%), and Copepods (15.41%) in Milghatta tank. The population in the Hutcharayankere tank was very low compared to that of Milghatta tank. In Hutcharayankere tank, Copepods where the dominant groups (16.54% of the total plankton), whereas other groups were rare in occurrence. Since the tanks were low in nutrients these tanks were oligotrophic and hence less productive. The low nutrient content in these tanks to the red soil present in the region, which had low nutrient and organic matter, and also due to aquatic vegetation that takes up nutrients.

Sunkad and Patil (2004) assessed the water quality of Fort lake Belgaum, Karnataka. Zooplanktons were represented by four groups, which include Rotifers, Cladocerans, Copepods and Ostracods. Rotifers were found to contribute to the zooplankton richness of the Fort Lake accounting 52.38% followed by copepod 26.5%, Cladocerans 16.45% and Ostracods 4.67%. The dominance of rotifers in the lake was due to the continuous supply of food material which in turn indicates the eutrophic nature of the lake. The level of phosphates in the lake was high (7.2 to 13.6 mg/L) due to the entry of sewage into the lake and hence supported the cause of eutrophication. Hence the lake was highly unsuitable for drinking water purposes.

Microcystis is common in plankton of mesotrophic to hypertrophic lakes, rivers and ponds and may dominate as nuisance blooms in slow-flowing or stratified waters (Paerl, 1991). It is known that *M. aeruginosa* needs water with high retention times and low salinity to develop

(Paerl *et al.,* 1984). It is unlikely that this species develops close to the mouth of the river, where considerable salinity values are easily reached.

From average phytoplankton density values, River Minho may be considered as eutrophic, according to Margalef (1983).However, if we consider the Trophic State Index (TSI; Carlson, 1977) the TSI values for chlorophyll *a* obtained at the INAG (Portuguese Water Institute) site classify River Minho as mesotrophic. In fact the TSI chlorophyll a values vary in average between 3.5.6 in Valenqa (n=34), 39.7 in Monqiio (n=32) and 46.1 in Melgaqo (n=19). These values were calculated from data obtained between 199.5 and 1998 (Vasconcelos *et al.,* 2000).

The phytoplankton community of the international section of river Minho was studied during the spring-summer periods 1989 and 1999. Samples were collected not on a regular basis from **S.** Gregdrio (mostly in the upstream international section) to Caminha, at the Minho estuary. According to our results, eutrophication seems to decrease from upstream to downstream sites. The occurrence of cyanobacteria blooms, especially of *Microcjstis aeruginosa,* in the Minho River mainly in August suggests that they may be formed in Spanish reservoirs and then float downstream into the studied section. These data clearly show that the monitoring of cyanobacteria in Minho River is important. Data can he used to prevent human health risks, as this water is used for drinking, recreation and shellfish. A total of 79 phytoplankton species were recorded in the international section of River Minho, without taking into account other phytoplankton organisms which could not be identified to species level. The groups more represented were Chlorophyta and Bacillariophyta, although Cyanobacteria were also present in significant numbers. The species richness seemed higher in the medium section (two sites) of the studied area (Monqio-Valenga), although this may be a spurious effect of more samples being analyzed for these two sites than for the others. Nevertheless, the upstream site Melgago, presented a significant number of diatom species when compared to that of chlorophytes harvest.

3. OBJECTIVE

This project deals with the Microbial Analysis of Soil and Water of Koel River. The Koel river is the source of water supply to the nearby sectors (sector-1 & 2), colonies and institute including NIT, Rourkela. So I was greatly interested in working on this river. During rainy season fishery becomes the main occupation of the people of Mitkundri Village (the village situated along the shore of the river) while during the rest of the season of the year, the water of this river is used for irrigation and vegetation purpose, the soil too here is very productive.

The research project was conducted with following aims and objectives:

- To check the quality of river, whether it is safe for drinking purpose or is prone to pollution leading to some water borne diseases.
- To know about the type of soil found here and the microorganisms loaded here from which we can know the kind of vegetation it can support.
- To identify the phytoplanktons, which are the primary producers of freshwater ecosystem and the type of phytoplankton species dominant here.

4. MATERIALS AND METHODS

4.1 Collection of sample:

Soil and Water: Samples were collected in the month of January of from the coastal side of Koel River from different areas of the river. The samples were collected from top 4cm soil profile where most of the microbial activity takes place, and thus where most of the bacterial population is concentrated. Soil samples were collected (approx 100g) in clean, dry and sterile polythene bags using sterilized spatula and water sample were collected in 50ml sterilized falcon tubes, reducing the chances of contamination as far as possible, and were carried to the laboratory for further analysis.

Fig4: Koel River

4.2 Physiochemical analysis:

pH:

Principle: pH is defined as logarithm to the base 10 of the inverse of the hydrogen ion concentration (or preferably H^+ ion activity). It is also the negative logarithm to the base 10 of H^+ ion activity. pH is very important in determining the alkalinity and acidity of soil and it also determines the capacity of sediments for the growth of phytoplankton, availability of nutrients, bacterial activity and physical condition of sediment.

Procedure:

The pH electrode is placed inside the beakers where samples are collected and the constant value is recorded.

4.3 Analysis of Sedimentary Texture

Principle:

Texture refers to the relative proportions of sand, silt and clay and specifically texture pertains to the sediment particles of less than 2mm in diameter (Table3).

Table 3: Size of soil particles in mm

Fraction	Diameter
Sand	**2-0.05 mm**
Silt	**0.05-0.002 mm**
Clay	**‹ 0.002 mm**

Procedure:

The soil samples are dried in hot air oven and then ground to remove the clumps.Then the samples are sieved through sieves of different mesh size. Then the relative percentage of sand, silt and clay is determined and is plotted in the the trigon graph.

Calculation:-

Sand%=sand wt/total wtx100

Silt % =silt wt/total wtx100

Clay%=clay wt/total wtx100

Gravel%=gravel wt/total wtx100

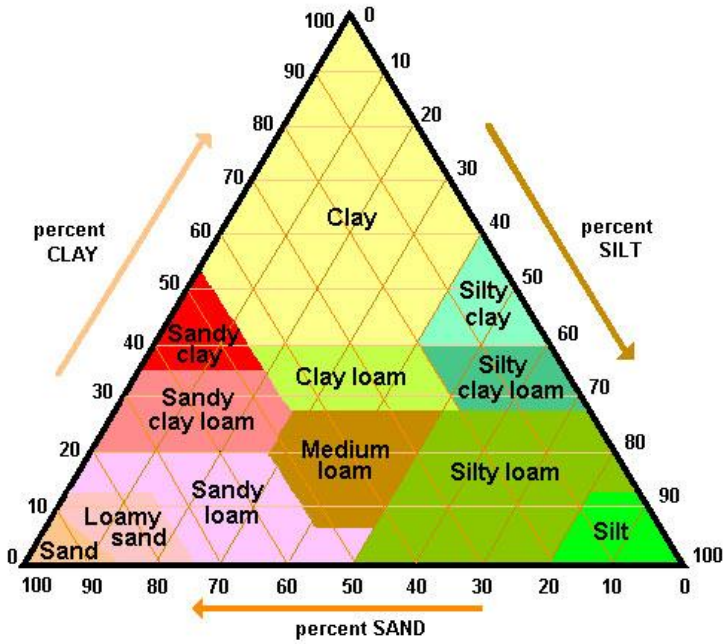


Fig5: Trigon plot

4.4 Total count of Bacteria:

Principle: Microorganisms present in water are very high in number. The exact number cannot be found out when their discrete colonies are not growing in the medium. The principle of this

method is that when micro-organisms are grown through this method possibly all live propagules will grow and develop individual colony. In this method a known amount of sample is added to a known volume of sterilized distilled water so that volume of microbial suspension is transferred to additional flasks containing 9ml to get 10 fold serially diluted suspension of 10^{-1} up to 10^{-3}

Adequate amount of 0.1ml of suspension is poured into the surface of medium and is spread by 'L' rod. The bacteria can thus be isolated and counted by calculating C.F.U. i.e, Colony Forming Unit.

C.F.U. = no of colonies/inoculums size (ml) X dilution factor C.F.U/ml

If the C.F.U. has a range of 30-300 in a plate it is considered as normal. Below 30 it is called TLTC (too low to count) and TFTC (too few to count).above 300 it is called TNTC (too numerous to count).

Procedure (from water sample):

Requirements for the preparation of media:

NA: 4.48g

Agar: 1g

Distill water: 250ml

Four different test tubes were taken and 9 ml of sterile distilled water was added in four test tubes and 10ml of water was added in one test tube.1ml of water was collected from koel river and was added to 9ml sterile distilled water flask.1ml of the suspension from the above solution was taken and added to 9ml of distilled water containing test tube.1ml of solution from 10^{-1} dilution was transferred into flask containing 9ml of distilled water to get dilution of 10^{-2}.similarly 1ml of solution was serially transferred from 10^{-2} to 3rd test tube containing 9ml water to get dilution of 10^{-3} .The process was followed repeatedly to get a dilution of 10^{-4}. 100ml of solution each from 10^{-4} to 10^{-3} dilutions were taken and spread into two different petriplates containing nutrient agar medium. The petriplates were then incubated at 37^{o}c for 24hrs.the colonies were taken observed and counted.

Procedure (from soil sample):

Five different test tubes were taken and 9 ml of sterile distilled water was added in four test tubes and 10ml of water was added in one test tube.1g of soil sample was collected from the coastal sides of Koel River and was mixed in 10ml of sterile distilled water. 1ml of the suspension from the above solution was taken and added to 9ml of distilled water containing test tube.1ml of solution from 10^{-1} dilution was transferred into flask containing 9ml of distilled water to get dilution of $10^{—2}$. Similarly 1ml of solution was serially transferred from 10^{-2} to 3rd test tube containing 9ml water to get dilution of 10^{-3} .The process was followed repeatedly to get a dilution of 10^{-4}. 100ml of solution each from 10^{-4} to 10^{-3} dilutions were taken and spread into two different petriplates containing nutrient agar medium. The petriplates were then incubated at 37^{o}c for 24hrs.the colonies were taken observed and counted.

Bacterial Enumeration: Water samples were diluted to 10^{-1} and sediment samples was diluted upto 10^{-3} dilution, 0.1ml of each dilution were inoculated in triplicate plates of 12 different specified media. After incubations all plates were incubate at 37^{0}C in the incubator for 48 hours. After the incubation different cultures were seen on the Petri plates. Counting was done for each plate. Different microorganisms were identified on the basis of their colour and growth. Three microorganisms were selected for pure culture. Pure culture is obtained by streak plate method. The strains were K1, K2 & K3.Broth culture was prepared in the nutrient broth. Biochemical test was by the specific media. Results were interpreted. Casein hydrolysis and Starch hydrolysis was done by streak plate method. And incubation was done by stab and streak culture method. Citrate utilization, urease test and litmus milk reaction was done by preparing broth culture in test tube. The entire Biochemical test were analyzed and interpreted.

4.5 Biochemical analysis

GRAM STAIN

The most important differential stain used in bacteriology is the Gram stain, named after Dr. Christian Gram. It divides bacterial cells into two major groups, gram-positive and gram-neegative, which makes it an essential tool for classification and differentiation of microorganisms. The Gram stain reaction is based on the difference in the chemical composition of bacterial cell walls. Gram-positive cells have a thick peptidoglycan layer

Whereas the peptidoglycan layer in gram negative cells is much thinner and surrounded by outer lipid-containing layers. Peptidoglycan is mainly a polysaccharide composed of two chemical subunits found only in the bacterial cell wall. These subunits are N-acetylglucosamine and N-acetylmuramic acid. As adjacent layers of peptidoglycan are formed, they are cross linked by short chains of peptides by means of a transpeptidase enzyme, resulting in the shape and rigidity of the cell wall. Early experiments have shown that if the gram positive cell is denuded of its cell wall by the action of lusozyme or penicillin, the gram-positive cell will stain gram negative.

PRIMARY STAIN

Crystal violet is used first and stains all cells purple. Its function is to impart its color to all cells in order to establish a colour contrast.

MORDANT

Gram's iodine, this reagent is not only a killing agent, but also serves as a mordant a substance that increases the cells' affinity for a stain. It does this by binding to the primary stain, thus forming an insoluble complex. The resultant crystal-violet-iodine complex serves to intensify the colour of the stain. At this point, all cells will appear purple black.

DECOLORIZING AGENT

Ethyl alcohol, 95%- This reagent serves as a dual function as a protein-dehydrating agent and as a lipid solvent. Its action is determined by two factors, the concentration of lipids and the thickness of peptidoglycan layer in bacterial cell walls. In gram negative cells, the alcohol increases the porosity of the cell wall by dissolving the lipid in the outer layers. Thus the CV-1 complex can be more easily removed from the thinner and less highly cross linked Peptidoglycan layer. Therefore, the washing out effect of the alcohol facilitates the release of the unbound CV-1 complex, leaving the call colourless or unstained. The much thicker peptidoglycan layer in gram positive cells is responsible for the more stringent retention of the CV-1 complex, as the pores are made smaller due to the dehydrating effect of the alcohol. Thus the tightly bound primary stain complex is difficult to remove, and the cells remain purple.

COUNTERSTAIN

Safranin is used to stain red those cells that have been previously decolourized. Since only gram negative cells undergo decolourization, they may now absorb the counterstain. Gram positive cells retain the purple colour of the primary stain.

PROCEDURE

- One clean glass slide was taken.
- A smear was prepared by placing a drop of water on the slide and then transferring microorganism to the drop of water with a sterile cooled loop. It was mixed and spread by means a circular motion of the inoculating loop.
- Smear was air dried and heat fixed.
- Smear was gently flooded with crystal violet for 1min.
- Gently washed with tap water.
- Smear was gently flooded with Gram's iodine and left for 1 min.
- Gently washed with tap water.
- Decolourized with 95% ethyl alcohol reagent. It was added drop by drop until no further violet colour comes out.
- Gently washed with tap water.
- Counterstained with safranin for 45 seconds.
- Gently washed with tap water.
- It was dried with bibulous paper and examined under oil immersion.

HI CARBOHYDRATE KIT

This kit is a standardized colorimetric identification system utilizing thirty five carbohydrate utilization tests. Tests are based on the principle of pH change and substrate utilization.

a) Carbohydrate fermentation test

- Colour of the medium changes from red colour to yellow colour due to acid production, if the test is positive.

b) ONPG test

- Medium changes from colourless to yellow is the positive test.
- Detects β-galactosidase activity.

c) Esculin hydrolysis

- Colour of the medium changes from cream to black if the test is positive.

d) Citrate utilization

- Colour of the medium changes from yellowish green to blue if the test is positive.
- Detects capability of organism to utilize citrates as a sole carbon source.

e) Malonate utilization

- Colour of the medium changes from light green to blue if the test is positive.
- Detects capability of organism to utilize malonate as a sole carbon source.

Preparation of inoculum:

1) The organisms to be identified were first isolated and purified. Only pure cultures were used.

2) Kit was opened aseptically aseptically. Each well was inoculated with 50µl of the prepared inoculum by surface inoculation method.

3) The kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

INCUBATION:

- Temperature of incubation 35±2^{o}c. Duration of incubation 18-24 hours.

HI MOTILITY TEST

This kit is a satndardized colorimetric identification system utilizing seven conventional biochemical tests including motility and four carbohydrate utilization tests. The tests are based on the principal of pH change and substrate utilization.

INTERPRETATION OF TEST:

a) Motility

- Motility is seen as movement of bluish green growth from 1st well to 2nd well.

b) Indole test

- 1-2 drop of kovac's reagent was added.
- Development of reddish pink colour within 10sec indicates positive reaction.

c) Nitrate reduction test

- 2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Napthylamine reagent were added.
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.

Preparation of inoculum:

- The organisms to be identified were first isolated and purified. Only pure cultures were used.
- Kit was opened aseptically. 1st well was inoculated by stabbing and 2nd well was not inoculated.
- Remaining strip was inoculated by stabbing each individual well with a loopful of inoculums.

INCUBATION:

Temperature of incubation is 35-37^{o}c and duration of incubation is 18-24 hours.

Table-2: Result interpretation chart for HI CARBOHYDRATE KIT

Sl. No	Test	Reagent to be Added After incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Motility	-	-	Colourless	Bluish green growth seen	**No growth seen**
2	Motility	-	Detects motility	Colourless	Movement of bluish green growth from 1st well to 2nd well	**No growth seen**
3	Indole	1-2 drops of Kovac's red reagent	Detects deamination of tryptophan	Colourless	Reddish pink	**Colourless**
4	Citrate Utilization	-	Detects capability of organism to utilize citrate as a sole carbon source	Yellowish green	Blue	**Yellowish green**
5	Glucorinidase	-	Detects glucorinidase activity	Colourless	Bluish green	**Colourless**
6	Nitrate Reduction	1-2 drops of sulphanilic acid and 1-2 drops of N,N Dimethyl -1-Napthylamine	Detects nitrate reduction	Colourless	Pinkish red	**Colourless**
7	ONPG	-	Detects β-galactosidase activity	Colourless	Yellow	**Colourless**
8	Lysine decarboxylase	-	Detects lysine decarboxylation	Olive green	Purple	**Yellow**
9	Lactose	-	Lactose utilization	Red	Yellow	**Red\pink**
10	Glucose	-	Glucose utilization	Red	Yellow	**Red\pink**
11	Sucrose	-	Sucrose utilization	Red	Yellow	**Red\pink**
12	Sorbitol	-	Sorbitol utilization	Red	Yellow	**Red\pink**

HilMvic Biochemical test kit

HilMvic biochemical test Kit can be used for used for screening pathogenic organisms from urine, enteric specimens and other relevant clinical samples. It can also be used for validating known laboratory strains.

Principle: Each HilMvic kit is a standard colorimetric identification system utilizing four conventional biochemical tests and eight carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated as a colour change in the media that can be either interpreted visually or after addition of the reagent.

a) INDOLE TEST

1-2 drops of Kovac's reagent was added. Development of reddish pink colour within 10 seconds indicates positive reaction.

b) METHYL RED TEST

1-2 drops of Methyl red reagent was added. Reagent remains red colour if the test is positive.

c) VOGES PROSKAEUR'S TEST

2-3 drops of Barrit reagent A & 1-2 drops of Barrit reagent B were added. Pinkish red colour development within 5-10 minutes indicates a positive test.

Preparation of inoculum:

- The organisms to be identified were first isolated and purified. Only pure culture was used.
- Kit was opened aseptically. Each well was inoculated with 50µl of the prepared inoculm by surface inoculation method.
- The kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

INCUBATION:

- Temperature of incubation 35±37^{o}c. Duration of incubation 18-24 hours.

Table-3: Result interpretation chart For Hilmvic Biochemical Test Kit

Sl.no	Test	Reagents added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Indole	1-2 drops of kovac's red reagent	Detects deaminatio n of tryptophan	Colourless	Reddish pink	**Colourless**
2	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	**Yellow**
3	Voges proskaeur' s	1-2 drops of Barrit reagent A & 1-2 drops of Barrit reagent B.	Detects acetoin production	Colourless	Pinkish red	**Colourless \slight copper**
4	Citrate utilization	-	Detects capability of organism to utilize citrate as a sole carbon source	Yellowish green	Blue	**Yellowish green**
5	Glucose	-	Glucose utilization	Red	yellow	**Red**
6	Adonitol	-	Adonitol utilization	Red	yellow	**Red**
7	Arabinose	-	Arabinose utilization	Red	yellow	**Red**
8	Lactose	-	Lactose utilization	Red	yellow	**Red**
9	Sorbitol	-	Sorbitol utilization	Red	yellow	**Red**
10	Mannitol	-	Mannitol utilization	Red	yellow	**Red**
11	Rhamnose	-	Rhamnose utilization	Red	yellow	**Red**
12	Sucrose	-	Melibiose utilization	Red	yellow	**Red**

HI ASSORTED BIOCHEMICAL TEST KIT

This kit is a test system that can be used for identification of gram negative rods. Hi Assorted Biochemical test kit cab is used for screening pathogenic organisms from urine, enteric specimens & other relevant clinical samples. It can also be used for validating known laboratory strains.

Principle:

Each Hi Assorted test kit is standardized calorimetric identification system utilizing seven conventional biochemical test and five carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated by a colour change in the media that can be either interpreted visually or after addition of the reagent.

INTERPRETATION OF RESULT:

Phenylalanine deamination test:

2-3 drops of TDA reagent was added. Development of dark green colour within one minute indicates a positive reaction.

Nitrate reduction test:

1-2 drops of sulphanilic acid and 1-2 drops of N,N-Dimethyl-1-Napthylanine reagent. Immediate development of pinkish red colour on addition of reagent indicates positive reaction.

Preparation of inoculum:

- The organisms to be identified were first isolated and purified. Only pure culture was used.
- Kit was opened aseptically. Inoculate each well with 50µl of the prepared inoculm by surface inoculation method.
- The kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

INCUBATION:

- Temperature of incubation 35±37^{o}c. Duration of incubation 18-24 hours.

Table-4: Result interpretation chart for HI ASSORTED BIOCHEMICAL TEST KIT

Sl. no	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Citrate utilization	-	Detects capability of organism to utilize citrate as a sole carbon source	Yellowish green	Blue	**Yellowish green**
2	Lysine decarboxylase	-	Detects lysine decarboxylation	Olive green	Purple	**yellow**
3	Ornithin decarboxylase	-	Detects ornithin decarboxylation	Olive green	purple	**Yellow**
4	Urease	-	Detects urease activity	Orangish yellow	Pink	**Orangish yellow**
5	Phenylalanine deamination	2-3 drops of TDA reagent	Detects pH enylalanine deamination activity	Colourless	Green	**Colourless**
6	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2 drops of N,N-Dimethyl-1-Napthylamine	Detects Nitrate reduction	Colourless	Pinkish red	**Colourless**
7	H_2S production	-	Detects H_2S production	Orangish yellow	Black	**Orangish yellow**
8	Glucose	-	Glucose utilization	Red	Yellow	**Red\pink**
9	Adonitol	-	Adonitol utilization	Red	Yellow	**Red\pink**
10	Lactose	-	Lactose utilization	Red	Yellow	**Red\pink**
11	Arabinose	-	Arabinose	Red	Yellow	**Red\pink**
12	Sorbitol	-	utilization Sorbitolutilization	Red	Yellow	**Red\pink**

EXTRACELLULAR ENZYMATIC ACTIVITIES OF MICROORGANISM

Starch hydrolysis

Starch is a high molecular-weight, branching polymer composed of glucose molecule linked together by glycosidic bonds. The degradation of this macromolecule first requires the presence of the extracellular enzyme amylase for its hydrolysis into shorter polysaccharides, namely dextrins,and ultimately into maltose molecules. The final hydrolysis of this disaccharide, which is catalyzed by maltase, yields low molecular weight, soluble glucose molecules that can be transported into the cell and used for energy production through the process of gycolysis. In this experimental procedure starch agar is used to demonstrate the hydrolytic activities of these exoenzymes. The medium is composed of nutrient agar supplemented with starch, which serves as the polysaccharide substrate. The detection of the hydrolytic activity following the growth period is made by performing the starch test to determine the presence or absence of starch in the medium. Starch in the presence of iodine will impart a blue black colour to the medium, indicating the absence of starch splitting enzymes and representing a negative result. If the starch has been hydrolyzed, a clear zone of hydrolysis will surround the growth of the organism. This is a positive result.

Casein hydrolysis

Casein, the major milk protein, is a macromolecule composed of amino acid subunits linked together by peptide bonds (CO-NH). Before there assimilation into the cell, proteins must undergo step-by-step degradation into peptones, polypeptides, dipeptides, and ultimately in to their building blocks, amino acids. This process is called peptonisation, or proteolysis, and it is mediated by extracellular enzymes called proteases. The function of these proteases is to cleave the peptide bond CO-NH by introducing water in to the molecule. The reaction than liberates the amino acids. The low molecular weight soluble amino acids can now be transported through the cell membrane into the intracellular amino acid pool for use in the synthesis of structural and functional cellular proteins. In this experimental procedure, milk agar is used to demonstrate the hydrolytic activity of these exoenzymes. The medium is composed of nutrient agar supplemented with milk that contains the protein substrate casein. Similar to other proteins, milk protein is a colloidal suspension that gives the medium its colour and opacity, because it deflects light rays

rather than transmitting them. Following inoculation and incubation of the agar plate cultures, organisms secreting proteases will exhibit a zone of proteolysis, which is demonstrated by a clear area surrounding the bacterial growth. This loss of opacity is the result of a hydrolytic reaction yielding soluble, no colloidal amino acids, and it represents a positive reaction. In the absence of protease activity, the medium surrounding the growth of the organism remains opaque, which is a negative reaction.

Gelatin hydrolysis

Gelatin is an incomplete protein because it lacks essential amino acid tryptophan; its value in identifying bacterial species is well established. Gelatin is a protein produced by hydrolysis of collagen, a major component of connective tissue and tendons in humans and other animals. Below temperature of 25^{o}C, gelatin will maintain its gel properties and exist as a solid at temperatures above 25^{o}C, gelatin is liquid. Liquefaction is accomplished by some microorganisms capable of producing a proteolytic extracellular enzyme called gelatinase, which acts to hydrolyze this protein to amino acids. Once this degradation occurs, even very low temperatures of 4^{0}C will not restore the gel characteristic. Gelatin deep tubes were used to demonstrate the hydrolytic activity of gelatinase. The medium consists of nutrient supplemented with 12% gelatin this high gelatin concentration results in a stiff medium and also serves as the substrate for the activity of gelatinase. Following inoculation and incubation for 48hours, the cultures were placed in a refrigerator at 4^{0}C for 30 minutes. Cultures that remain liquefied produce gelatinase and demonstrate rapid gelatin hydrolysis. All culture was re-incubate for a additional 5 days. Again they were refrigerated for 30 minutes and observed for liquefaction. Cultures that remain liquefied were indicative of slow gelatin hydrolysis.

Carbohydrate Fermentation

Many organism use carbohydrates differently to obtain energy depending on their enzyme complement. Some organisms are capable of fermenting sugars such as glucose an aerobically while others use the aerobic pathway.

The purpose of this test is to determine whether an organism can ferment a specific carbohydrate with production of acid. In this test lactose is used. The indicator is bromothymol blue which turns yellow when pH is low.

Citrate Utilization:

This is the test for the ability of bacteria to convert citrate (an intermediate of the Krebs cycle) into oxaloacetate (another intermediate of the Kerbs cycle). In this media, citrate is the only carbon source available to the bacteria. If it cannot use citrate then it will not grow. If it can use citrate, then the bacteria will grow and the media will turn a bright blue as a result of an increase in the pH of the media. To inoculate this slant, transfer loop is used.

Indole production:

This test is done to determine if bacteria can breakdown the amino acid tryptophan into indole. SIM media or TSB (Tryptic Soy Broth) is inoculated using a transfer needle. After incubating the bacteria for at least 48 hours, Kovac's reagent is added to the media to detect if indole has been made by the bacteria. The development of a red/pink layer on top of the media is a positive result (the bacteria can breakdown tryptophan to form indole). Failure to see a red layer is a negative result (indole was not formed from tryptophan).

MRVP (methyl red-Vogues Proskauer):

This test is used to determine two things. The MR portion (methyl red) is used to determine if glucose can be converted to acidic products like lactate, acetate, and formate. The VP portion is used to determine if glucose can be converted to acetone.

These tests are performed by inoculating a single tube of MRVP media with a transfer loop and then allowing the culture to grow for 3-5 days. After the culture is grown, about half of the culture is transferred to a clean tube. One tube of culture will be used to conduct the MR test, the second tube serves as the VP test.

A. **MR (methyl red) test**: Methyl red is added to the MR tube. A red colour indicates a positive result (glucose can be converted into acidic end products such as lactate, acetate, and formate). A yellow colour indicates a negative result; glucose is converted into neutral end products.

B. **VP (Vogues Proskauer) test:** First alpha-napthol (also called Barritt's reagent A) and then potassium hydroxide (also called Barritt's reagent B) are added to the VP tube. The culture should be allowed to sit for about 15 minutes for color development to occur. If acetone was produced then the culture turns a red color (positive result); if acetone was not produced then the culture appears yellowish to copper in color (a negative result).

Triple sugar Iron (TSI) and Hydrogen sulphide production (H_2S):

Looks at fermentation of glucose, lactose, and sucrose and checks if hydrogen sulphide is produced in the process. Basically a pH indicator will change the colour of the media in response to fermentation. Where that colour change occurs in the tube will indicate what sugar or sugars were fermented. The presence of a black colour indicates that H_2S was produced. In this media, H_2S reacts with the ferrous sulphate in the media to make ferrous sulphide, which is black. To inoculate, use a needle to stab agar and then uses a loop to streak the top slated region. In addition to TSI media, SIM media can be used to determine if H_2S is produced. A black colour in the SIM medium following inoculation and incubation indicates that H_2S is made by the bacteria.

Urease:

This test is used to detect the enzyme urease, which breaks down urea into ammonia. Ammonia is a base and thus will raise the pH of the media if it is present. This change in pH is indicated by a pH indicator called phenol red which is present in the media. A colour change from yellow to bright pinkish-red is positive; lack of colour change is a negative result. Inoculation in liquid media is done by transfer loop.

Catalase:

This test is can be used to detect the enzyme catalase. This enzyme is responsible for protecting bacteria from hydrogen peroxide (H_2O_2) accumulation, which can occur during aerobic metabolism. If hydrogen peroxide accumulates, it becomes toxic to the organism. Catalase breaks H_2O_2 down into water and O_2. To perform the catalase test simply smear a small amount of the test organism onto the lid of a petri plate/culture dish. Then add a drop of hydrogen peroxide to the smear. If bubbles become visible (these would be the O_2 bubbling up)

then the test is positive and you can conclude that the organism makes catalase. A lack of bubbles indicates the absence of catalase. **Most aerobic organisms make catalase.**

Oxidase test:

To perform this test simply swab some of your test culture into one of the boxes on an oxidase dry slide. If a colour change to purple or blue is evident at 30 seconds-1 minute then the result is positive. It is important that the test is read by one minute to ensure accurate results (avoid false negatives and false positives). This laboratory test is based on detecting the production of the enzyme cytochrome oxidase by Gram-negative bacteria. It is a hallmark test for the *Neiserria*. It is also used to discriminate between aerobic Gram-negative organisms like *Pseudomonas aeruginosa* and other *Enterobacteriaciae*.

Motility test:

The motility test is not a biochemical test since we are not looking at metabolic properties of the bacteria. Rather, this test can be used to check for the ability of bacteria to migrate away from a line of inoculation thanks to physical features like flagella. To perform this test, the bacterial sample is inoculated into SIM or motility media using a needle. Simply stab the media in as straight a line as possible and withdraw the needle very carefully to avoid destroying the straight line. After incubating the sample for 24-48 hours observations can be made. Check to see if the bacteria have migrated away from the original line of inoculation. If migration away from the line of inoculation is evident then you can conclude that the test organism is motile (positive test). Lack of migration away from the line of inoculation indicates a lack of motility (negative test result).

4.6 Identification of phytoplankton:

Water was collected from the surface with minimal disturbance with the help of phytoplankton net (20 μm) (fig: 17) and immediately fixed with 5% formalin. The preserved samples were kept in refrigerator until analysis. Before identification water samples were allowed to settle for 24 hours and the supernatant was decanted until a concentrate 10 ml was achieved. Few drops of concentrated sample were taken in a common glass slide and observed

under sedverg rafter chamber under light microscope For identification of common diatoms examination of raw (without acid cleaned) material in a water mount was done as stated earlier. Several keys and illustration were consulted to confirm identification.

5. RESULTS AND DISCUSSIONS

pH Study:

The pH of water is a very important property because it will decide whether the water is suitable for drinking purpose. The overall pH of Koel River is 7.6 which indicate that the pH of water is slightly alkaline i.e., it is suitable for drinking and other purposes.

Hydro biological studies of Lake Mirik in Darjeeling, Himalayas was done by Jha and Barat (2003).The pH of lake was reported to be acidic in nature. The present study states that the nature of water is alkaline.

The pH of water sample from 3 different sites is-8.2, 8.10, and 7.01

Table: pH of water from three different sites

site	pH
Site-I	8.2
Site-II	8.10
Site-III	7.01

Soil texture analysis:

For site I

Weight of total soil sample: 200g

Table9: soil texture for site -I

Soil texture	wt	%
Gravel	2.2g	1.1
Sand	30.4g	15.2
Silt	17.4g	8.7
Clay	152.2g	76.1

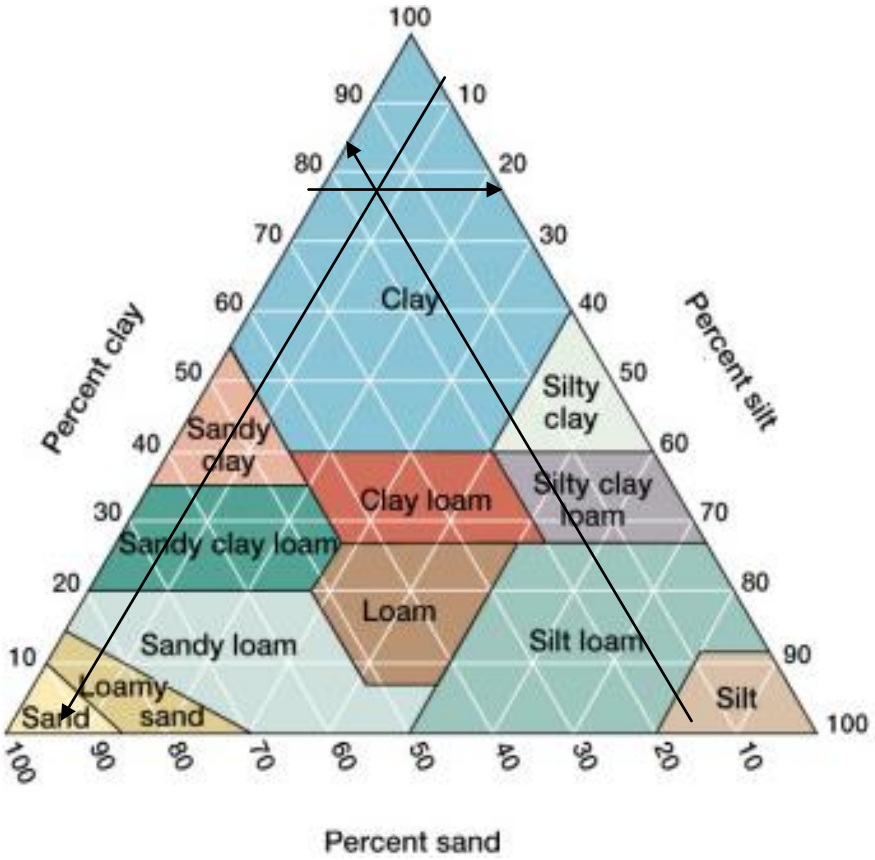


Fig6: Trigon plot for site -I

For site II

Wt of total soil sample: 100g

Table10: soil texture for site-II

Soil texture	wt	%
Gravel	5.3g	5.3
Sand	25g	25
Silt	11g	11
Clay	61.5g	61.5

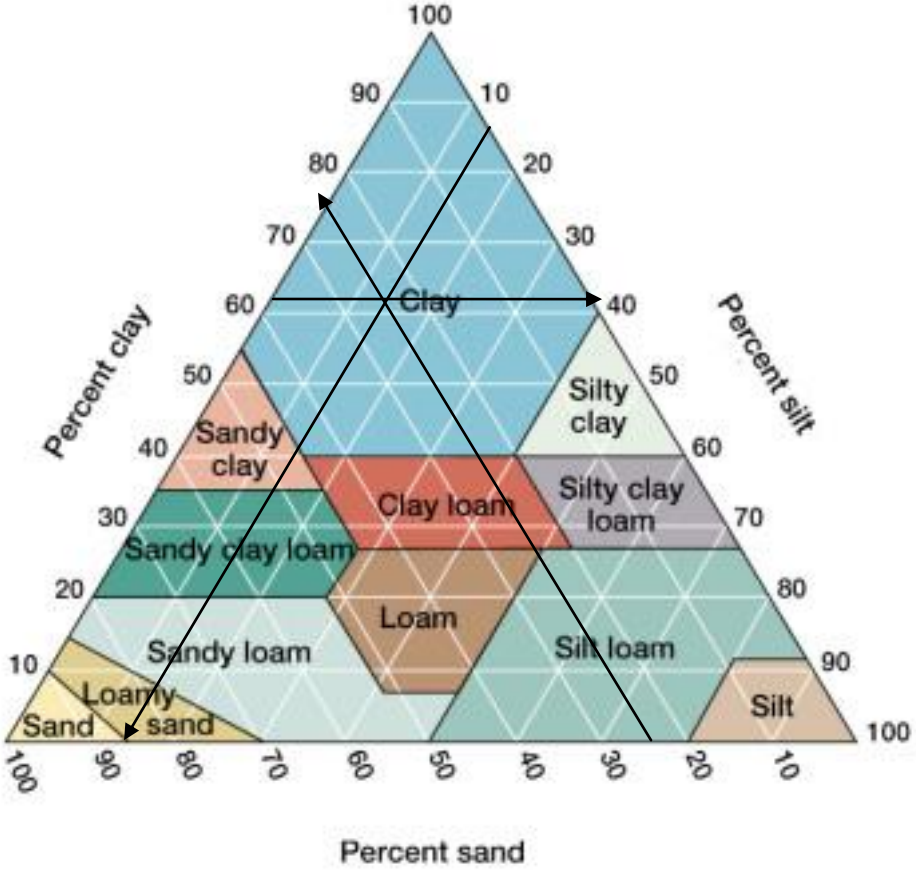


Fig7: Trigon plot for site-II

The texture of soil reported in table and table indicate that the percentage of clay soil is more and from the above two Trigon graphs it is clear that the soil is of clayey type.

Bacterial count

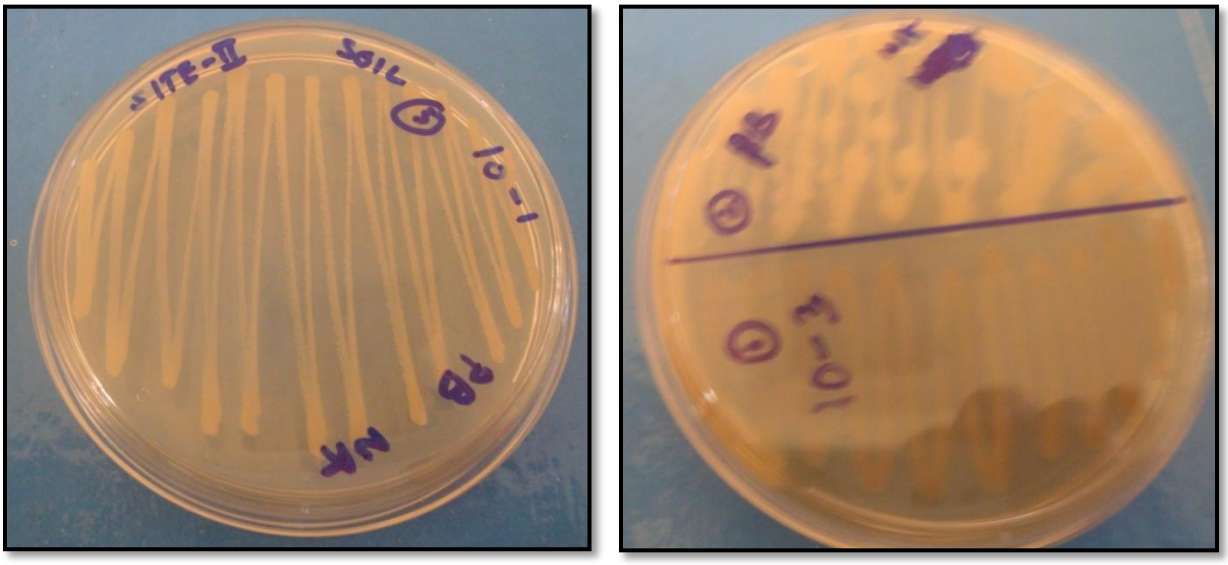
Table11: Bacterial count for water sample;

Site	Dilution	No. of colonies	Inoculum size	CFU/ml
I	10^{-1}	TNTC	0.1	
	10^{-2}	80x4	0.1	$0.32x10^{6}$
	10^{-3}	79x4	0.1	$0.316x10^{7}$
II	10^{-1}	110x4	0.1	$0.4x10^{5}$
	10^{-2}	80x4	0.1	$0.32x10^{6}$
	10^{-3}	70x4	0.1	$0.28x10^{7}$

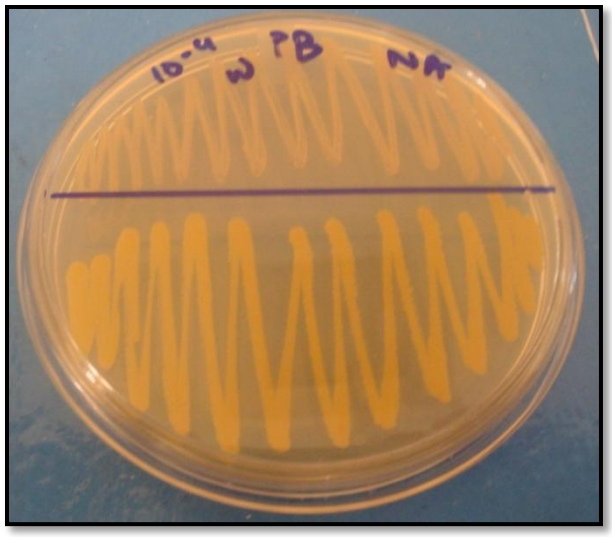
Table12: Bacterial count for soil sample:

Site	Dilution	No. of colonies	Inoculation size	CFU/g
I	10^{-1}	TNTC	0.1	
	10^{-2}	232	0.1	$2.32x10^{5}$

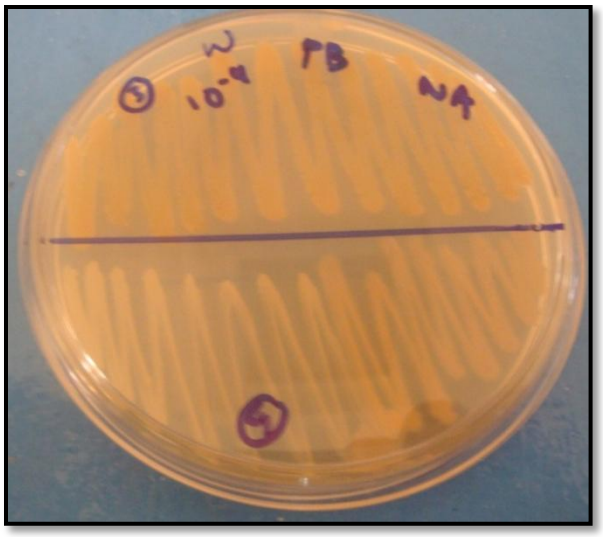
	10^{-3}	150	0.1	$1.5x10^{6}$
II	10^{-1}	110	0.1	$1.1x10^{4}$
	10^{-2}	106	0.1	$1.06x10^{5}$
	10^{-3}	98	0.1	$9.8x10^{5}$



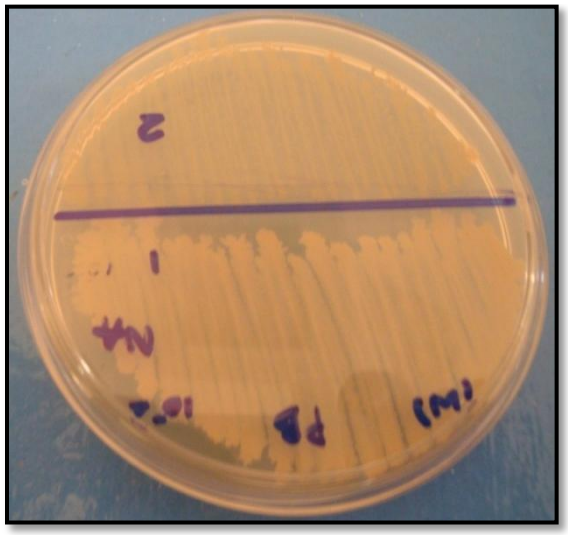
(a) (b)



(c)



(d)



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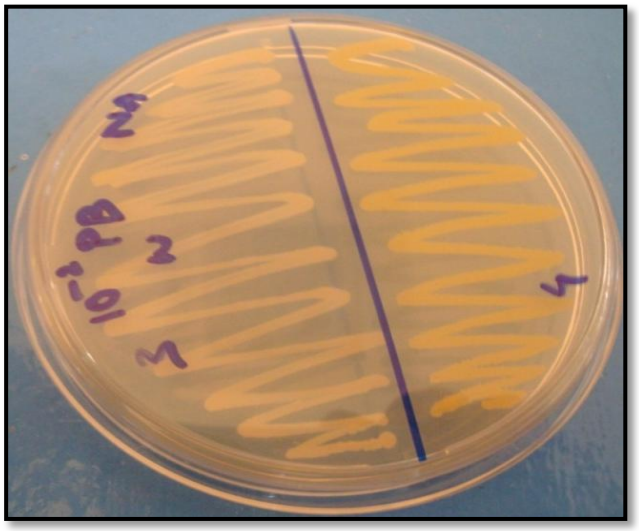


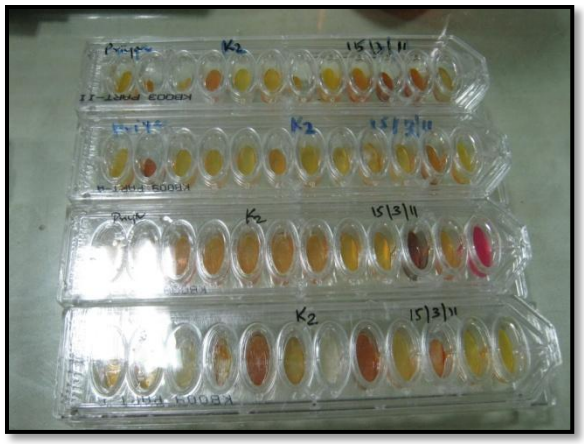
(f)

Fig8 (a-f): Plates showing isolation of pure culture by streak plate method

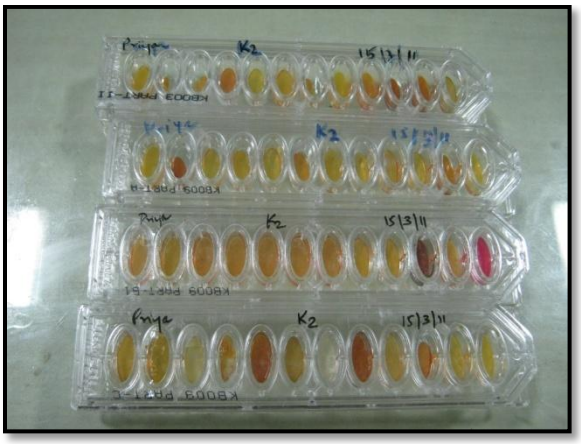
Bacterial colony counts and MPN counts of soil (moist) and water samples collected in September 1957 and June 1958 have been consolidated by sampling period. In general, the soil is a much more productive source of microorganisms than is water. The coliforms were an exception: they were present in all 16 water samples, and in only 5 of the 16 soil samples. The upper limit of the coliform count in soil (1,400 per gm.), occurring in soil sample number 4 (September 1957) ,was very high, whereas the other 5 soil samples taken at that time were negative for these organisms(Fourvelle,1958). The present study shows high bacterial count in 10^{-1} being too numerous to count, the number decreasing further with dilution factor 10^{-2} to 10^{-3}.

Biochemical test:

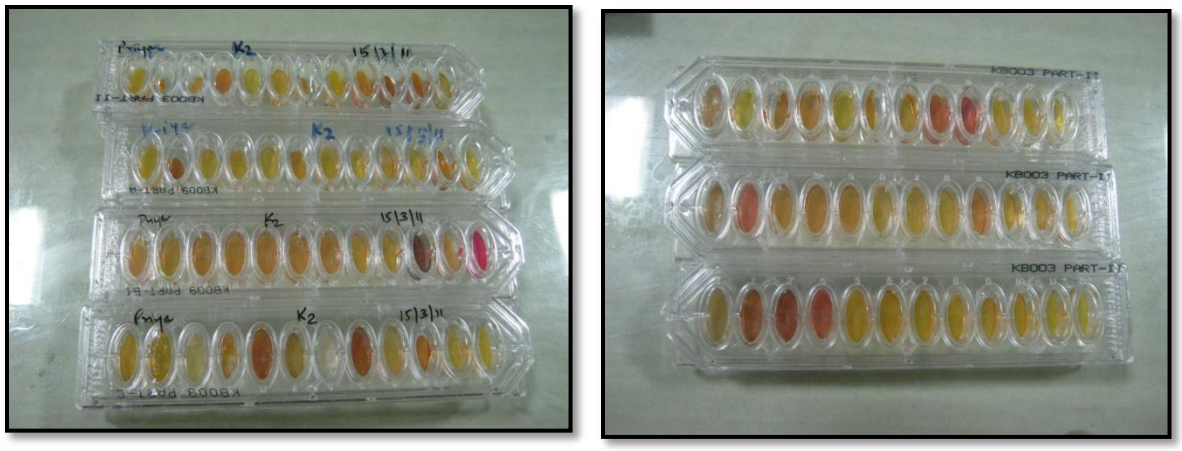
After Gram's staining, the two isolates K1, K2 and k3 are identified as gram positive staphylococcus type of bacterium. Biochemical test results of two pure culture strains isolated from fertilizer berth namely K1, K2 and K3 are tabulated below and figures were presented in figure9.



(a)



(b)



(c) (d)

Fig9 (a-b): Biochemical test kit result

Table-13: Biochemical test results of K1, K2 and K3 isolates (part A)

Sl.no	TEST	K1	K2	K3
1	Lactose	+	-	+
2	Xylose	-	-	-
3	Maltose	-	+	+
4	Fructose	+	-	+
5	Dextrose	+	+	+
6	Galactose	+	-	-
7	Raffinose	-	-	-
8	Trihalose	-	+	+
9	Melibiose	-	-	-
10	Sucrose	-	+	+
11	L-Arabinose	-	-	-
12	Mannose	-		-
13	Inulin	-	-	-
14	Sodium gluconate	-	-	-
15	Glycerol	+	-	+
16	Salicin	-	-	+
17	Dulcitol	-	-	-
18	Arabitol	-	-	-
19	Inositol	-	-	-
20	Sorbitol	-	-	-
21	Mannitol	+	-	+
22	Adonitol	-	-	-
23	α –Methyl-D-glucoside	-	-	-

24	Rhammose	-	-	-
25	Cellobiose	-	-	-
26	Melezitose	-	-	+
27	α –Methyl-D-Mannoside	-	-	-
28	Xylitol	-	-	-
29	ONPG	+	-	+
30	Esculin	+	-	+
31	D-Arabinose	+	-	-
32	Citrate utilization	+	+	+
33	Malonate	+	+	+
34	Sorbose	-	-	-
35	control	-	-	-

Table-14: Biochemical test results of K1, K2and K3 isolates (part-B)

Sl no	TEST	K1	K2	K3
1	ONPG	+	+	+
2	Lysine utilisation	-	-	-
3	Ornithine utilisation	+	+	+
4	Urease	+	+	+
5	Phenylalaninedeamination	-	+	+
6	Nitrate reduction	-	-	+
7	H_2S production	+	-	-
8	Citrate production	+	+	+
9	Voges proskauer's	+	-	+
10	Methyl red	+	-	+
11	Indole	-	-	-
12	Malonate	+	+	+

+: Positive

- : Negative

Gram staining:

The strains were identified as gram positive

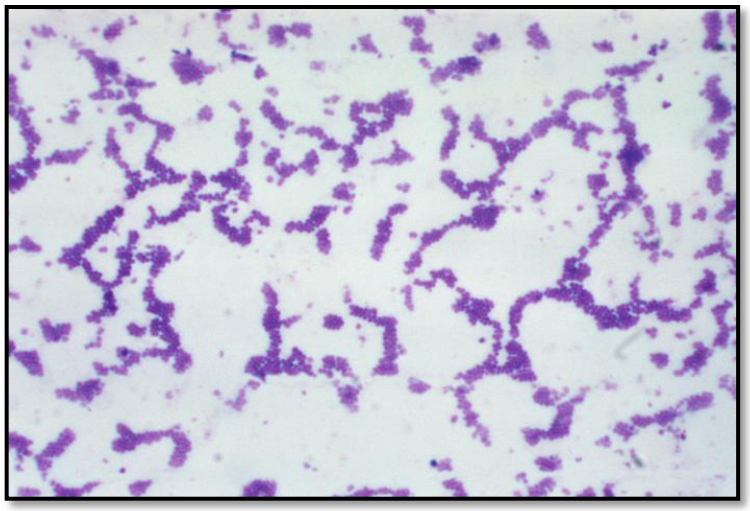


Fig10: Stained picture of Staphylococcus

The number of Gram-negative rods isolated from the soil approximated the number of spore formers taken (120/102). However, considerably more of the Gram-negative forms were found in water (240/70) and the difference appears to be significant. Boyd and Boyd (1963) found that Gram negative bacteria were predominant in arctic lake water. The present study reports the gram positive bacteria were predominant in Koel River.

Identification of bacteria:

STRAIN1

1. *Staphylococcus cohnii subsp. urealyticus* ~ 81% (acc: 40%)

2. *Staphylococcus felis* ~ 81% (acc: 40%)

3. *Staphylococcus pseudointermedius* ~ 76% (acc: 40%)

4. *Staphylococcus carnosus subsp. carnosus* ~ 76% (acc: 40%)

STRAIN2

1. *Staphylococcus saprophyticus subsp. saprophyticus* ~ 83% (acc: 40%)

2. Staphylococcus auricularis ~ 80% (acc: 40%)

3. *Staphylococcus aureus subsp. anaerobius* ~ 78% (acc: 40%)

4. *Staphylococcus warneri* ~ 78% (acc: 40%)

STRAIN3

1. *Staphylococcus simiae* ~ 74% (acc: 36%)

2. *Staphylococcus nepalensis* ~ 73% (acc: 36%)

3. *Staphylococcus piscifermentans* ~ 72% (acc: 36%)

4. *Staphylococcus aureus subsp. aureus* ~ 66% (acc: 38%)

The microorganisms play a vital role in determining the quality of soil. The microorganisms identified from a small amount of sample are of staphylococcus sp.

Staphylococcus **(family: *Staphylococcaceae*, Genus: *Staphylococcus*)**

Staphylococcus (from the Greek: *staphyle*, "bunch of grapes" and kókkos, "granule") is a genus of Gram-positive bacteria. Under the microscope they appear round (cocci), and form in grape-like clusters.

The *Staphylococcus* (fig10) genus includes at least forty species. Of these, nine have two subspecies and one has three subspecies. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are a small component of soil microbial flora.

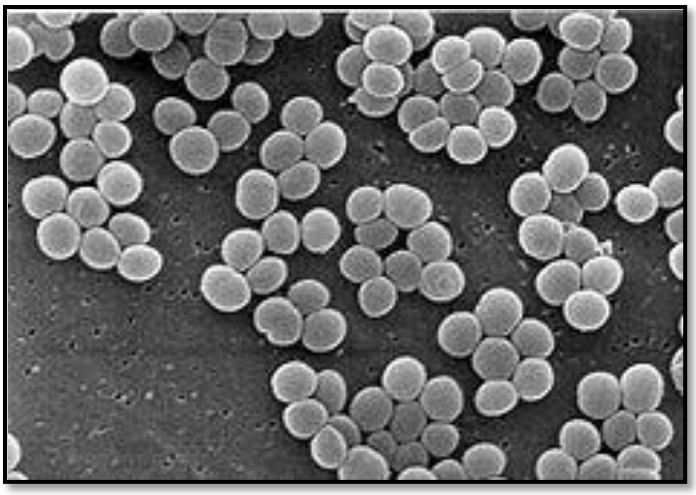


Fig11: *Staphylococcus* colony

Collection of phytoplankton:

Fig12: Collection of phytoplankton with the help of phytoplankton net

Fig13: Phytoplankton net

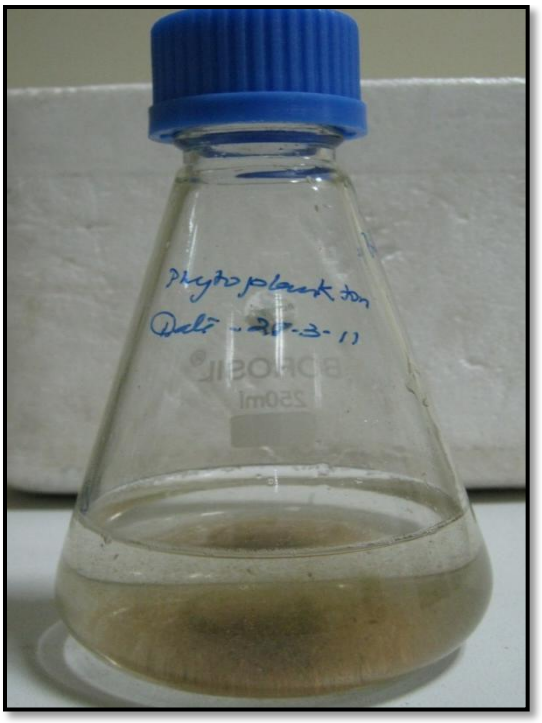


Fig14: Collected Phytoplankton

Identification of phytoplankton

Table14: Name of organism and their abundance

Sl no	Name of organism	abundance
1	*Spirogyra*	D
2	*oscillatoria*	D
3	*Chromolina ovalis*	D
4	*Diatoms*	SD
5	*Scendusmus dimorphus*	SD
6	*Nostoc*	R
7	*Chrysococcus cornuta*	R
8	*Penium sylvanigra*	R

D: Dominant

SD: Sub Dominant

R: Rare

Spirogyra (Family: *Zygnemataceae*, Genus: *spirogyra*)

Spirogyra is a genus of filamentous green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in freshwater areas, and there are more than 400 species of *Spirogyra* in the world. *Spirogyra* measures approximately 10 to 100μm in width and may stretch centimeters long. This particular algal species, commonly found in polluted water, is often referred to as "pond scum"(fig15).

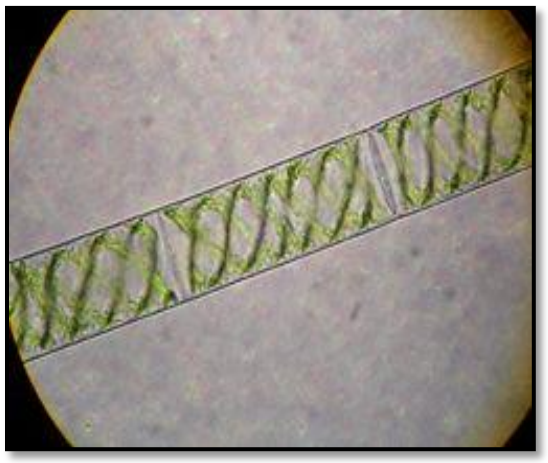


Fig15: *Spirogyra*

2. ***Oscillatoria*** (Family: *Oscillatoriaceae*, Genus: *Oscillatoria*)

Oscillatoria is a genus of filamentous cyanobacterium which is named for the oscillation in its movement. Filaments in the colonies can slide back and forth against each other until the whole mass is reoriented to its light source. It is commonly found in watering-troughs waters, and is mainly blue-green or brown-green. *Oscillatoria* is an organism that reproduces by fragmentation. *Oscillatoria*forms long filaments of cells which can break into fragments called hormogonia. The hormogonia can grow into a new, longer filament. Breaks in the filament usually occur where dead cells (necridia) are present. *Oscillatoria* uses

photosynthesis to survive and reproduce. Each filament of oscillatoria consists of trichome which is made up of rows of cells. The tip of the trichome oscillates like a pendulum. (fig16)

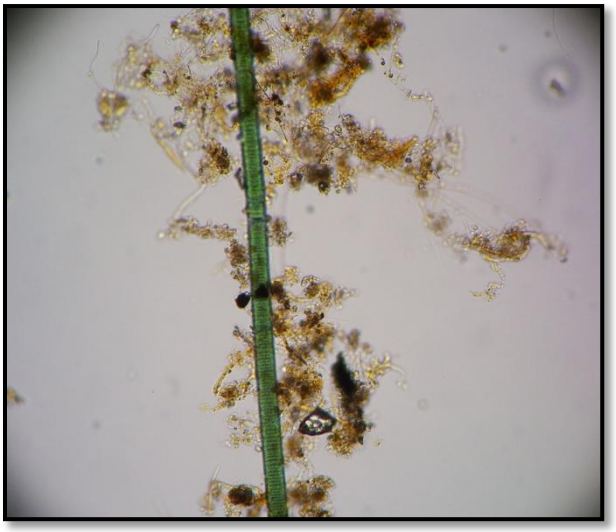


Fig16: *Oscillatoria*

3**. *Nostoc*** (Family: *Nostocacae*, Genus: *Nostoc*) (Fig17)

Nostoc is a genus of cyanobacteria found in a variety of environmental niches that forms colonies composed of filaments of monili form cells in a gelatinous sheath. *Nostoc* can be found in soil, on moist rocks, at the bottom of lakes and springs (both fresh- and saltwater), and rarely in marine habitats. It may also grow symbiotically within the tissues of plants, such as the evolutionarily ancient (Gunnera) or hornworts, providing nitrogen to its host through the action of terminally differentiated cells known as heterocysts. These bacteria contain photosynthetic pigments in their cytoplasm to perform photosynthesis.

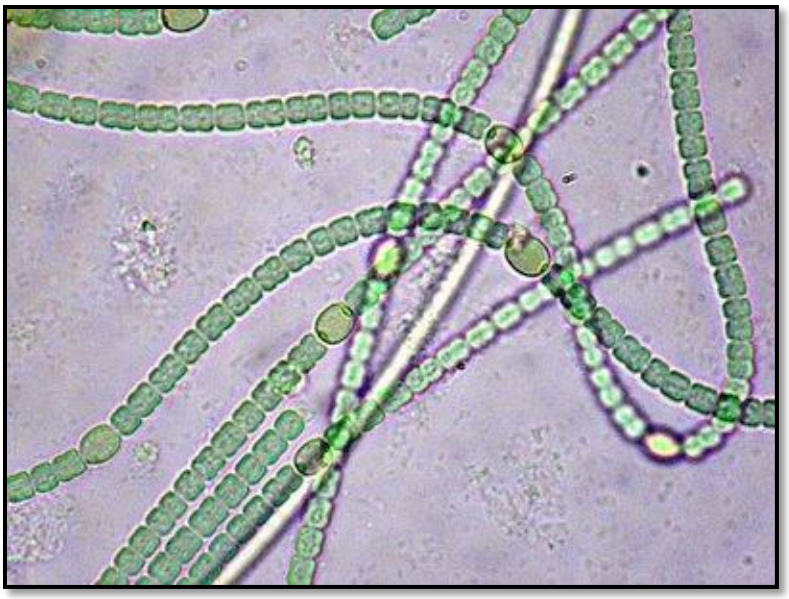


Fig17: *Nostoc*

4. ***Diatoms*** (class: *Bacillariophyceae*)

Diatoms are a major group of algae, and are one of the most common types of phytoplankton. Most diatoms are unicellular, although they can exist as colonies in the shape of filaments or ribbons (e.g. *Fragillaria*), fans (e.g. *Meridion*), zigzags (e.g. *Tabellaria*), or stellate colonies (e.g.*Asterionella*). Diatoms are producers within the food chain. A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silica (hydrated silicon dioxide) called a frustule. These frustules show a wide diversity in form, but usually consist of two asymmetrical sides with a split between them, hence the group name. Fossil evidence suggests that they originated during, or before, the early Jurassic Period. Diatom communities are a popular tool for monitoring environmental conditions, past and present, and are commonly used in studies of water quality.

5. ***Scenedesmus dimorphus*** (class: *chlorophyceae*)

Scenedesmus dimorphus is a freshwater unicellular algae in the class *Chlorophyceae*. The name means "having two forms. (fig18)

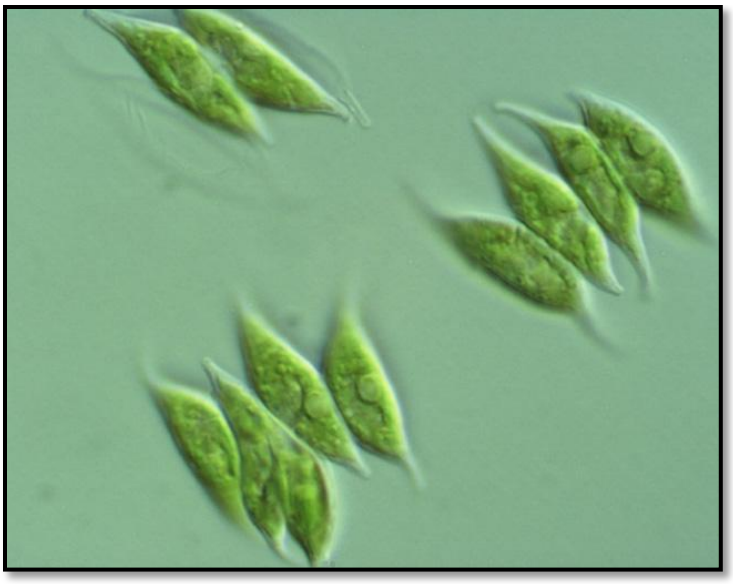


Fig18: *Scenedesmus dimorphus*

Hydro biological studies of lake Mirik in Darjeeling ,Himalayas was done by Jha and Bharat (2003).The phytoplankton analysis confirmed that the lake was polluted due to contaminates let into the river. The present study does not show that the water is polluted.

Huddar (1995) also reported the dominance of *Cyanophyceae* in the fresh water bodies of Dharwad district of Karnataka. The present study also states the dominance of *Cyanophyceae*, the other dominant phytoplankton being *Oscillatoria.*

It is well established fact that the members of *Cyanophyceae* dominate the phytoplankton of ponds and lakes in the tropical areas. Sewage contaminated water bodies harbor more of *Cyanophyceae*(Pande and Tripathi,1988).In the present study *Cyanophyceae* is dominant and the nature of river is slightly eutrophic.

A total of 79 phytoplankton species were recorded in the international section of River Minho without taking into account other phytoplankton organisms which could not be identified to species level. The groups more represented were Chlorophyta and Bacillariophyta, although Cyanobacteria were also present in significant numbers. (Vasconcelos and Cerqueira (2001).The present study reports 8 groups of phytoplankton in which spirogyra were dominant.

CONCLUSION

The water of Koel River is alkaline as observed from the experiments. The soil was found out to be of clay type. Isolated bacteria were identified to be *Staphylococcus cohnii subsp. urealyticus, Staphylococcus saprophyticus subsp. saprophyticus, Staphylococcus simiae*. The abundant phytoplankton is *Spirogyra* followed by *Oscillatoria*. These organisms can be used to maintain the quality of water supply from Koel River. The microbial studies can be utilized for the prevention of any pathogenic diseases caused by the microbes of this river. Regular check up of the aquatic life can help in maintaining ecological balance of Koel River. The analysis of soil texture can be helpful for the farmers in their irrigation and vegetation purpose.

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