PHYSIOCOCHEMICAL AND MICROBIAL ANALYSIS OF WATER AND SOIL SAMPLES IN PROPOSED COAL MINE AREA AT LATEHAR.

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CERTIFICATE

This is to certify that the thesis entitled "**PHYSICOCHEMICAL AND MICROBIAL ANALYSIS OF WATER AND SOIL SAMPLES IN PROPOSED COAL MINE AREA AT LATEHAR."** submitted by **Mr SHUBHANATH BEHERA** in partial fulfilment of the requirements for the degree of **Bachelor of Technology in BIOTECHNOLOGY** embodies the bonafide work done by him in the final semester of his degree under the supervision of the undersigned. The thesis or any part of it has not been submitted earlier to any other University / Institute for the award of any Degree or Diploma.

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ABSTRACT

The environmental quality is greatly focused on water and soil because of their importance in maintaining the human health and of the ecosystem. Soil and water are the two essential sources of life. The particulate organic matter, particulate mineral N and soil microbial biomass and their stocks over the soil profile are known to be indicators of soil. Water performs unique and indispensable activities in earth ecosystem, biosphere. Thus water is needed for recreation, transportation, and hydroelectric power, domestic uses etc. So for the quality testing physicochemical and biological parameters of soil and water are thoroughly examined. Soil and water quality are examined by taking physicochemical and biological parameters that has a major impact on agricultural productivity and human health. So for quality control physicochemical and biological parameters of soil and water are thoroughly examined. Soil and water quality can be determined by quantifying the physicochemical and biological parameters that has a major impact on agricultural productivity and human health.

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INTRODUCTION

Soil is comprised of minerals, soil organic matter (SOM), water, and air. The composition and proportion of these components greatly influence soil physical properties like including structure, and porosity. These properties influence air and water movements in soil, and thus the ability of soil to function. The organic fraction of a soil, although represents much less than 10% of the soil mass by weight, has a great effect on soil chemical and physical properties. Soil organic matter comprises of carbon, oxygen, hydrogen, nitrogen and smaller quantities of sulphur and other elements. Soil quality can be determined by quantifying the physical, chemical and biological parameters that has a major impact on agricultural productivity and sustainability. Soil is a mixture of mineral and organic constituents that are in solid, gaseous and aqueous states (Voroney et al., 2006). 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).

Water is the one of the essential source of life on earth. It also performs unique and indispensable activities in earth ecosystem, biosphere and biogeochemical cycles. Thus, high quality water is always a necessity for living organisms. Water is the most important component among the natural resources, and is crucial for the existing of all living organisms. Water is a resource that has many uses, including recreation, transportation, and hydroelectric power, domestic, industrial, and commercial uses. 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). 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).Fresh water is a finite resource, essential for agriculture, industry and even human existence, without fresh water of adequate quantity and quality, sustainable development will not be possible. Physicochemical analysis: The parameters like pH, dissolved oxygen (DO), Biological oxygen demand (BOD), chemical oxygen demand (COD), Total hardness (TH), calcium and magnesium are used to analyse using standard procedures.

Ideally, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Detection of faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment and it is not possible to examine water for every possible pathogen that might be present (WHO, 1993). Probably the most important pathogenic bacteria transmitted by the water route are Salmonella typhi, the organism causing typhoid fever, and Vibrio cholerae, the organism causing cholera (Madigan et al., 1997). Soil bacteria and fungi play pivotal roles in various biogeochemical cycles (BGC) (Molin and Molin, 1997; Trevors, 1998b; Wall and Virginia, 1999) and are responsible for the cycling of organic compounds. Soil microorganisms also

influence above-ground ecosystems by contributing to plant nutrition (George et al., 1995; Timonen et al., 1996), plant health (Srivastava et al., 1996; Filion et al., 1999; Smith and Goodman, 1999), soil structure (Wright and Upadhyaya, 1998; Dodd et al., 2000) and soil fertility (Yao et al., 2000; O'Donnell et al., 2001). Our knowledge of soil microbial diversity is limited in part by our inability to study soil microorganisms. Torsvik et al. (1990a,b) estimated that in 1 g of soil there are 4000 different bacterial ''genomic units'' based on DNA–DNA reassociation. It has also been estimated that about 5000 bacterial species have been described (Pace, 1997, 1999). Approximately 1% of the soil bacterial population can be cultured by standard laboratory practices. Microbial analysis: The microbial analysis like the numbers of bacterial and fungal colonies, Bacterial characterisation using biochemical tests according to Bergey's manual are used to be carried out.

OBJECTIVE AND OVERVIEW OF THESIS

OBJECTIVE

This project deals with the Physicochemical and Microbial Analysis of soil and water samples collected from Tubed , a village under the proposed coal mine area.

The research project was conducted with following aims and objective :

- ✓ To check the quality of water, whether it is safe for drinking purpose or not by the comparative physicochemical and microbial analysis of water samples using standard methods.
- ✓ To check the type of soil found there along with its physicochemical properties.
- ✓ To characterise the different bacteria found in soil.

OVERVIEW OF THESIS

In this study water and soil samples were collected from TUBED and physicochemical properties of soil and water were examined and analysed.

For Soil samples-pH,% of organic matter,% of $CaCO_3$ etc. and for Water samples-pH, TDS(total dissolved solids),TSS(total soluble solids),Sulphate concentration, Dissolved oxygen etc. like properties were analysed. Microbial analysis is carried out by isolation of bacteria followed by different biochemical tests.

LITERATURE REVIEW

LITERATURE REVIEW

The following is a brief review of scholarly work of different researchers in the field of water and soil quality studies along with microbial studies.

Fresh water is finite resource, essential for agriculture, industry and even human existence, without fresh water of adequate quantity, sustainable development will not possible [1]. Since water quality and human health are closely related, water analysis before usage is of prime importance. Certain physical, chemical and microbiological standards, which are designed to ensure that the water is palatable and safe for drinking before it can be described as potable [2]

Physicochemical property like pH for water should be in the range of 6.5 to 8.5 for drinking and domestic purposes[3]. As a momentous role of DO amount in water quality of ground water, the average concentration of DO was highest in post monsoon period and lowest in monsoon consequently increase in BOD and COD[4].The parameters like pH, dissolved oxygen(DO),biological oxygen demand(BOD),chemical oxygen demand(COD) total hardness(TH),calcium and magnesium were analysed using standard procedures [5]**.**

The fluctuations in optimum pH ranges may lead to an increase or decrease in the toxicity of poisons in water bodies [6]**.**The high level of total hardness is due to mixing of sewage effluents

into the rives. The permanent hardness is mainly caused by chlorides and sulphates [7]**.** Faecal coliforms counts/100 ml should be zero for water to be considered as no risk to human health. In general high levels of free CO_2 might be the reason for low pH values obtained in the river water samples, which may consequently affect the bacterial count [8].

Ground water contains high amount of various ions, salts etc. so if we were using such type of water as potable water then it leads to various water-borne diseases [9]. Unsafe drinking water contributed to numerous health problems in developing countries such as the one billion or more incidents of diarrhoea that occur annually [10]. The coliform bacterium is the primary bacterial indicator for faecal pollution in water [11-12]**.**

Concentration of DO is one of the most important parameters to indicate water purity and to determine the distribution and abundance of various algal groups [13]. High level of TDS in water used for drinking purposes leads to many diseases which are not water-borne but due to excess salts [14].

In one research paper the chemical oxygen demand (COD), total nitrogen, nitrate, nitrite, ammonium, orthophosphate and total phosphate concentrations of each sample were analysed using Aqualytic AL282 as described by the manufacturer. The concentrations of heavy metals (Pb+2, Cd+2, Cu+2, Al+3 and Hg+2) in water were measured using an atomic absorbance spectrometer. The temperature, pH, conductivity, dissolved oxygen (DO) concentration and turbidity were monitored on site using Corning Checkmate II with portable thermometer, pH,

turbidity and DO meters and Aqualytic turbidity meter respectively. Biological oxygen demand (BOD) values were obtained based on the instructional manual of the Aqualytic Sensomat System.[15]

PHYSICOCHEMICAL PROPERTIES

The physical properties of soils, in order of decreasing importance, are texture, structure, density, porosity, consistency, temperature, colour and resistivity along with concentration of different minerals and organic matter. Most of these determine the aeration of the soil and the ability of water to infiltrate and to be held in the soil. Soil texture is determined by the relative proportion of the three kinds of soil particles, called soil "separates": sand, silt, and clay. Soil porosity consists of the part of the soil volume occupied by gases and water. Soil consistency is the ability of soil to stick together. Soil temperature and colour are self-defining. Resistivity refers to the resistance to conduction of electric currents and affects the rate of corrosion of metal and concrete structures. The properties may vary through the depth of a soil profile. Soil is the system which supplies plant with available nutrients through the root. Physical and Chemical analysis of the soil are carried out to indicate the efficiency of soil for supplying plants with nutrients in available forms as well as identification of the factors affecting this efficiency in the soil. Therefore, besides perfect sampling in the field, soil samples must be properly prepared and analyzed in order to reach the correct evaluation of the soil nutritional status.

The functioning of an aquatic ecosystem and its stability to support life forms depend, to a great extent, on the physico-chemical characteristics of its water. The

key feature of an ecosystem is the interaction among the biotic and abiotic components. The external controls and internal interactions combine to produce a certain ecosystem structure and the species develop certain pattern of abundance seasonality, biomass and satisfaction. Any change in the abiotic components will be reflected in the biotic life. The physicochemical parameter of water are temperature, pH, electrical conductivity (EC), total dissolved solids

(TDS), turbidity, dissolved oxygen (DO), total alkalinity (TA), total hardness (TH), calcium (Ca^{++}) magnesium (Mg^{++}, potassium (K^{+}), chloride (Cl^{-}), fluoride (F^{-}), nitrate (NO_3^{-}), sodium (Na^{+})sulphate (SO_2^{-4}) and phosphate (PO_3^{-4}) etc

Porosity

Pore space is that part of the bulk volume that is not occupied by either mineral or organic matter but is open space occupied by either gases or water. Ideally, the total pore space should be 50% of the soil volume. The gas space is needed to supply oxygen to organisms decomposing organic matter, humus, and plant roots. Pore space also allows the movement and storage of water and dissolved nutrients.

There are four categories of pores:

- Very fine pores: < 2 microns
- Fine pores: 2-20 microns
- Medium pores: 20-200 microns
- Coarse pores: 200 microns-0.2 mm

Consistency

Consistency is known as the ability of soil to stick together and resist fragmentation. It is of rough use in prediction of cultivation problems and the engineering of foundations. Consistency can be measured at three moisture conditions: air-dry, moist and wet. A soil's resistance to fragmentation and crumbling is done in dry state by rubbing the sample. Its resistance to shearing forces is assessed in the moist state by applying pressure. The terms used to describe a soil in those three moisture states and a last state of no agricultural value are as follows:

 Consistency of Dry Soil: soft, loose, hard, extremely hard

 Consistency of Moist Soil: friable, firm, loose,extremely firm

 Consistency of Wet Soil: non-sticky, sticky or non-plastic, plastic

 Consistency of Cemented Soil: weakly cemented, indurated (cemented)

Temperature

Soil temperature regulates seed germination, root growth and the availability of nutrients to the plants. Soil temperatures range from permafrost at a few inches below the surface to 38°C in Hawaii on a warm day. The colours of the ground cover and its insulating ability have a strong influence on soil temperature. Snow cover will reflect light and heavy mulching will slow the warming of the soil, but at the same time they will reduce the fluctuations in the surface temperature. Below 50 cm (20 in), soil temperature seldom changes and can be approximated by adding 1.8°C (2°F) to the mean annual air temperature. Most often, soil temperatures must be accepted and agricultural activities adapted to them to: maximize germination and growth by timing of planting optimise use of anhydrous ammonia by applying to soil below 10°C (50°F) prevent heaving and thawing due to frosts from damaging shallow-rooted crops prevent damage to desirable soil structure by freezing of saturated soils improve uptake of phosphorus by plants.

Organic matter

Soil organic matter is composed chiefly of carbon, hydrogen, oxygen, nitrogen and smaller quantities of sulphur and other elements. The organic fraction of a soil, although usually representing much less than 10% of the soil mass by weight, has a great influence on soil chemical properties. The particulate organic matter, particulate mineral N and soil microbial

biomass and their stocks over the soil profile are known to be indicators of soil quality besides the total N content among which the soil organic carbon level is predominantly governed by the agro-climatic regime. The organic fraction serves as a reservoir for the plant essential nutrients, nitrogen, phosphorus, and sulphur, increases soil water holding and cation exchange capacities, and enhances soil aggregation and structure.

pH:

pH is affected not only by the reaction of carbon dioxide but also by organic as well as inorganic solutes those are present in water. Any change in water pH is accompanied by the change in other physic-chemical parameters18. pH maintenance (buffering capacity) is one of the most important attributes of any aquatic system since all the biochemical activities depend on pH of the surrounding water.

Hardness:

Hardness is an important parameter in decreasing the toxic effect of poisonous element. Water hardness is an aesthetic quality of water, and is caused mostly by the minerals calcium and magnesium.

Total Dissolved Solids (TDS):

It is the measure of the combined content of all inorganic and organic substances contained in a liquid in: molecular, ionized or micro-granular (colloidal sol) suspended form.

Dissolved oxygen (DO):

Dissolved oxygen in water plays a vital role for underwater life. It is what aquatic creatures need to breathe. Dissolved oxygen is often called DO. Dissolved oxygen (DO) is the amount of oxygen that is present in the water. It is measured in milligrams per litre (mg/L), or the number of milligrams of oxygen dissolved in a litre of water.

Biological oxygen demand:

Biochemical oxygen demand or B.O.D is the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period. The term also refers to a chemical procedure for determining this amount.

STUDY AREA:

The study areas are one randomly chosen villages at Latehar District in Jharkhand. The name of the village is Tubed. This village is under a proposed coal mining area of TUBED coal mines limited.

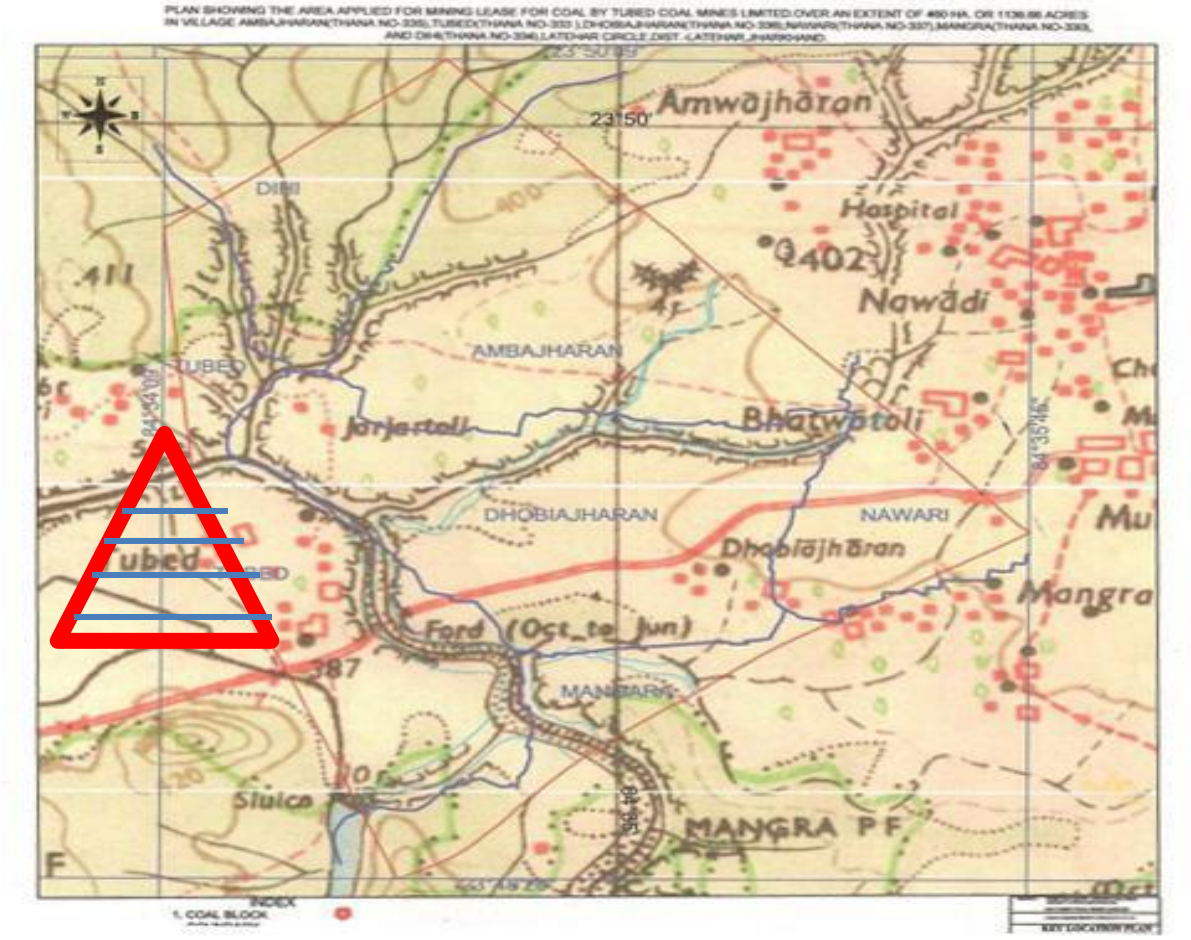


Fig.1 *Map of study Area*

Some part of this village is within the coal mining area. This village is the experimental sites for the analysis. Water samples were collected in two sections i.e Ground water sample and Surface water sample from that village. For soil samples soil from that village were collected from different places.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of sample:

Soil and water : Samples were collected from TUBED village from different location. For water sampling the method followed is as per the protocol described by Indian standard IS:3025 Part-I. For soil sampling the method followed is as per the protocol described by Indian standard IS:2720 Part-I . 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 and stored in refrigerator for further analysis.

Physiochemical analysis:

FOR WATER:

pH:

Principle: pH is defined as the logarithm to the base 10 of the inverse of the hydrogen ion concentration (or preferably H^+ ion). It can be the negative logarithm to the base 10 of H+ ion activity. pH maintenance is one of the most important attributes of any aquatic system since all the biochemical activities depend on pH of the surrounding water.

Procedure:

pH of the water was measured by the electrometric method according the Indian Standard IS 3025 : Part 12.For measuring the pH of water, the pH electrode is placed inside the beakers where the samples are collected and the constant values are recorded.3 readings are taken for the sample.

Dissolved Oxygen (DO):

DO is a very important parameter of water quality and an index of physical and biological process going on in water. Dissolved oxygen present in drinking water adds taste and it is highly fluctuating factor in water.

Principle:

Divalent manganese salt in solution is precipitated by strong alkali to divalent manganese hydroxide. It is rapidly oxidized by dissolved oxygen present in the sample to form trivalent or higher valency hydroxide. Iodide ions are added and acidified, which reduce tetravalent hydroxides back to their stable divalent state thereby liberating equivalent amount of iodine. This iodine is, equivalent to dissolved oxygen present in the sample.

The reactions are as follows:

$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K_2SO_4\&$

$2Mn(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2$

$MnO(OH)_2 + 2.KI + H_2O \longrightarrow Mn(OH)l + 4 + 2KOH$

$I_2 + 2S_2O_3^{-2} \longrightarrow S_4O_6^{-3} + 2I^-$

Procedure:

- To the water sample 2ml of $MnSO_4$ solution and 2ml of alkaline Potassium Iodide solution were added by one by one.
- The solution was shake thoroughly.

- 2ml if concn.H_2SO_4 was added slowly. Again the mixture was shake thoroughly to dissolve the precipitate.
- From the above solution 200ml was transferred to a conical flask.
- Few drops of starch indicator was added drop by drop.Then titrated against the sodium thiosulfate, till the blue colour turns into violet.

FOR SOIL SAMPLE

pH:

pH is the 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:

pH analysis of soil sample was carried out according to the Indian standards IS:2720(Part-26).This method is known as Electrometric method i.e. by pH meter.Befor the measurement the soil samples were prepared according to IS:2720(Part-1),1983.

- 30g of soil sample was sieved and mixed with 75ml of miliQ water in beaker and stirred.
- Then allowed to stand for 1h.After which pH meter was calibrated and pH was measured for 3times..

Organic Matter:

Organic matter is known as the reservoir of nutrients and water in the soil, plays an important role in surface crusting, reducing compaction and increases water infiltration into the soil.

Organic matter is stable in the soil. It has been decomposed until there is resistant to further decomposition. Usually, only about 5% of it mineralizes yearly. That rate will increase if temperature, oxygen, and moisture conditions become favourable for decomposition. In the soil testing only the stable organic matter was analysed.

Procedure:

- The soil was grounded and completely passed through 0.2 mm sieve (80mesh) and 1gm is placed at the bottom of a dry 500 ml conical flask.
- 10 ml of potassium dichromate (1N) was added in the 500 ml conical flask and conical flask was swirled gently to disperse the soil in the dichromate solution.
- Then 20 ml of sulphuric acid was run in and swirled again two or three times.
- The flask was allowed to stand for 30 minutes and there after 200 ml of distilled water along with 10 ml of ortho-phosphoric acid was added and also 1ml of diphenylamine indicator.
- The whole contents were titrated with ferrous ammonium sulphate solution till the colour flashes from blue – violet to green. For a final calculation, a blank was run without soil.

Calcium carbonate :

Percentage of calcium carbonate is defined as the total carbonates which is contained in 100 g of dry soil. Usually total carbonates is known as (CaCO3, MgCO3 etc) as CaCO3. Amount of calcium carbonate in soil indicates one of the most important soil properties Percentage of calcium carbonate, CaCO3 (%), is defined as the total carbonates which is contained in 100 g of dry soil.

5g of soil was weighed accurately and transferred into a 150 ml beaker.100 ml of HCl was added to it. It was kept at Room Temperature for 1 hour with vigorous stirring. After settling, 20 ml of supernatant liquid was taken and 6 to 8 drops of bromothymol blue indicator was added to it. Titration was performed with sodium hydroxide solution.

Water content of soil:

Soil water content is the physical parameter used to characterise the availability of water for plants in the soil. Water contained in soil is called soil moisture. The water is entrapped within the soil pores. Soil moisture is the major component of the soil in relation to plant growth. If the water content of a soil is optimum for plant growth, plants can easily uptake soil water but not all the water, held in soil, is available to plants. Soil water dissolves all the salts and makes up the soil sample solution, which acts as a medium for supply of nutrients to the plants.

Procedure :

The water content of soil sample can be calculate according to the Indian standard protocol IS:2720(Part II)-1973.This method covers the determination of the water content of soil expressed as a % of the oven dry weight.

Clean the container with lid, was dried and weighed (W1). The required quantity of the soil specimen was taken in the container crumbled and placed loosely, and weighed with lid (W2). Then it was kept in an oven with the lid removed, and the temperature of the oven was maintained at 110 ± 5°C (see Note). The specimen in the oven was dried for 24 h. Every time the container was taken out for weighing. The final mass (W3) of the container with lid was weighed with dried soil sample.

% of water content= $w=[(W_2-W_1)/(W_3-W_1)]*100$

MICROBIAL ANALYSIS

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 individually different colonies. In this technique 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} Sufficient amount of (approx. 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 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 Tubed village 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 petri plates containing nutrient agar medium. The petri plates were then incubated at 37oc for 24hrs.the colonies were taken observed and counted.

Bacterial Enumeration (Isolation of pure culture)

After the incubation different cultures were observed on the petri plates.Counting was done for each plate and different micorobes were identified on the basis of their colour and growth

pattern. Different isolated colonies were picked up and pure culture was obtained by streak plate method.

BIOCHEMICAL TESTS

GRAM STAIN:

The most important differential stain used in bacteriology is the Gram stain, named after Dr. Christian Gram. It is a differential stain which allows most bacteria to be divided into two groups, Gram +ve and Gram –ve bacteria. The Gram stain reaction is based on the difference in the chemical composition of bacterial cell walls. Gram +ve cells have a thick peptidoglycan layer, whereas in case of gram-ve it is much thinner and surrounded by outer lipid containing layers. The Gram+ve cell wall has a stronger attraction for crystal violet when gram's iodine is applied than the gran negative cell wall. Gram's iodine is a mordant which form a complex with the crystal violet that is attached more tightly to the Gram+ve cell wall than to the Gram negative cell wall.This complex can easily be washed from the Gram negative cell wall with ethyl alcohol. Gram+ve bacteria, however ,are able to retain the crystal violet and therefore will remain purple after decolorizing with alcohol. Since Gram-ve bacteria will be colourless after decolorizing with alcohol and counterstaining with counterstain safranin will make them appear pink.

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 treated with the Gram‘s iodine and left for 1 min.
- And gently washed with the tap water.
- Decolourized with 95% concentration ethyl alcohol reagent. It was added to the slide drop by drop until no further violet colour comes out.
- Gently washed with tap water.
- Counterstained with safranin for about 45 seconds.
- Gently washed with tap water.
- It was dried with filter paper and examined under oil immersion.

CATALASE TEST

Catalase test is used to identify organisms that produce the enzyme, catalase. Thisenzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.Catalase is produced by ceratain bacteria, which acts as a catalyst in break down of H_2O_2. . It is essential for differentiating Gram + ve coccus bacteria like Staphylococcus and Streptococcus.

$$2H_2O_2 \longrightarrow 2H_2O+O_2$$

This test is usually carried out by adding 3-4drops of H_2O_2 to an overnight growth on agar plate and formation of vigorous bubbling will signifies positive result.

METHYL RED TEST

Some bacteria perform mixed acid fermentation. The by products are mixture of large amounts of less stable acids. The methyl red test test for the ability to perform mixed acid fermentation.MR-VP broth contains glucose, peptone and a phosphate buffer. Organisms those perform mixed acid fermentation produce enough acid to overcome the buffering capacity of the broth, so a decrease in pH results. Organisms that perform other kinds of fermentation cannot overcome the buffering capacity of the broth.

Procedure

- The inoculated MR-VP broth was incubated for 48-72hours at 37°c after which, one millilitre (1ml) of the broth was transferred into a small tube.
- Few drops (2-3 drops) of methyl red were added to it.
- Colour change of the medium provides the result. If the colour is red then the bacteria is MR positive otherwise if colour is yellow, then the bacteria is MR negative.

UREASE TEST

The urease test identifies those organisms those are capable of hydrolysing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease positive Proteeae from other enterobacteriaceae. Uera broth is a differential medium that tests the ability of an organism to produce an exoenzyme, called urease, that hydrolyses urea to ammonia and carbon dioxide.

$(NH_2)_2CO+H_2O \longrightarrow CO_2+2NH_3$

Urease test media contain 2% urea and phenol red as a pH indicator. An increase in pH due to the production of ammonia results in a colour change from yellow to bright pink(pH 8.2).Urea broth is a highly buffered medium requiring large quantities of ammonia to raise the pH above 8.0 resulting in a colour change.

CITRATE UTILISATION TETS

The citrate utilisation test is used to determine the ability of a bacterium to utilize citrate as its only source of carbon. Bacteria can break the conjugate base salt of citrate into organic acids and CO_2.The CO_2 can combine with the sodium form the conjugate base salt to form a basic compound, sodium carbonate. A pH indicator in the medium detects the presence of the compound by turning blue which is a positive result.

Citrate = Oxaloacetate+ Acetate

Oxaloacetate=Pyruvate+CO_2

The inoculated medium was incubated for 48 to 72 hours .The colour of the medium signifies the result. If the colour change in media from green to blue then the bacteria is citratepositive. If the media retain the green colour after incubation period then the bacteria is citrate negative.

INDOLE TEST

The indole test is important in the grouping and identification of anaerobic bacteria. The indole test screens for the ability of an organisms to degrade the amino acid tryptophan and produce indole. Tryptophan is an amino acid that cam undergo deamination and hydrolysis by bacteria that express tryptophanse enzyme.

Tryptophan + Water=Indole + Pyruvic acid + Ammonia

One percent tryptophan broth was taken in a test tube and inoculated with bacteria colony. After 48 hours of incubation period at 37°C, one millilitre (1ml) of chloroform was added to the broth. The test tube was shaken gently. 5 drops of Kovács reagent was added directly to the tube. This was also shaken gently and allowed to stand twenty (20) minutes. The formation of red coloration at the top layer indicated positive and yellow coloration indicates negative.

OXIDASE TEST

The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colourless reagent becomes an oxidized coloured product.

A small piece of filter paper was soaked in 1% Kovács oxidase reagent and dried. (The composition of Kovács oxidase reagent is 1% tetra-methyl-p-phenylenediamine dihydrochloride in distilled water.)

Using a inoculation loop a well isolated colony was picked from a fresh bacterial plate (18 to 24 hours) and rubbed into the filter paper soaked with Kovács oxidase reagent.

Colour change indicates the result of this test. Microorganisms are oxidase positive when the colour changes to dark purple within 5 to 10 seconds.

Microorganisms are delayed oxidase positive when the colour changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the colour does not change or it takes longer than 2 minutes.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

SOIL CHARACTERISATION

Location	pH	% of Organic Matter	% of $CaCO_3$	Moisture content(%)
TUBED	6.336	1.3621	16.74	9.42
	6.51	0.475	18.135	7.35
	6.69	0.3162	20.925	8.78

Table 1: *Different physiochemical parameters of soil*

pH study

The soil pH is slightly acidic or slightly alkaline which preferable for the agricultural view.

WATER CHARACTERISATION

Location		pH	TDS(mg/ml)	TSS(mg/ml)	Dissolved Oxygen(mg/ml)
TUBED	SW	7.30	68	396	8.33
	GW	6.97	20	154	7.41

Table 2: Different physiochemical parameters of water.

pH study:

The pH of water is a very important property because it will decide whether the water is suitable for drinking purpose. The pH of the water samples collected from TUBED is in the range of 6.5 to 7.5 which indicates that it can be used as drinking water.

TDS and TSS study

The TDS value of both the samples are found to be under 100mg/ml and 500mg/ml.The lower TDS value of water indicates that presence of suspended particles are very low so it is suitable for drinking water as well as agricultural point of view.

Dissolved Oxygen (DO):

Dissolved oxygen of both the water samples are found to be 8.33mg/ml and 7.41mg/ml. The reason for the low dissolved oxygen content was due to decomposition of organic matter, which indicates a pollution load in the water. The deficiency of the oxygen in the water is shelter for bacteria and other pathogens, which are anaerobic [16].

MICROBIAL ANALYSIS OF SOIL SAMPLE

Sample Site	Dilution	No. of colonies	Inoculum size()	CFU/ml
TUBED	10^{-4}	70	0.1	$70x10^{-4}$
	10^{-5}	38	0.1	$38x10^{-5}$
	10^{-6}	28	0.1	$28x10^{-6}$

Table 3: Total Bacterial Count.

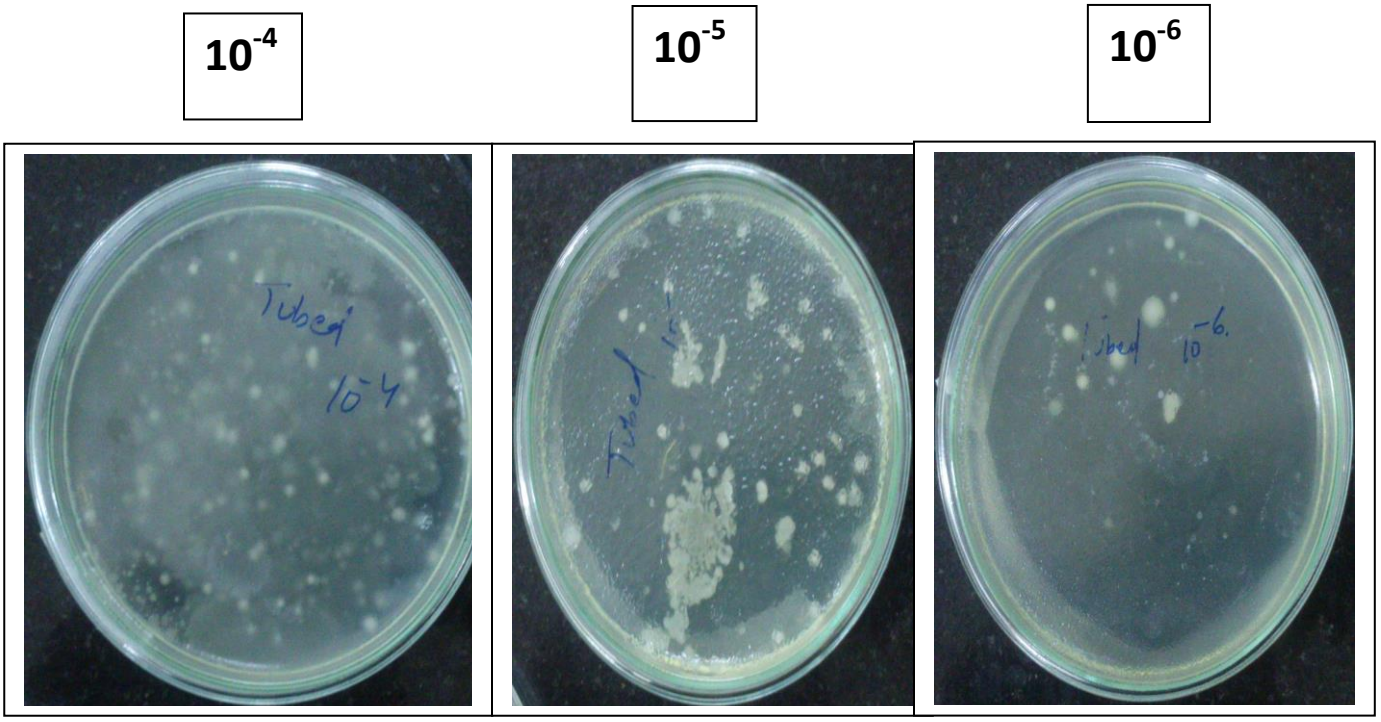
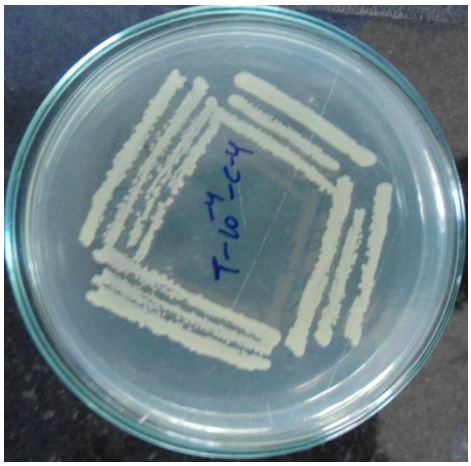
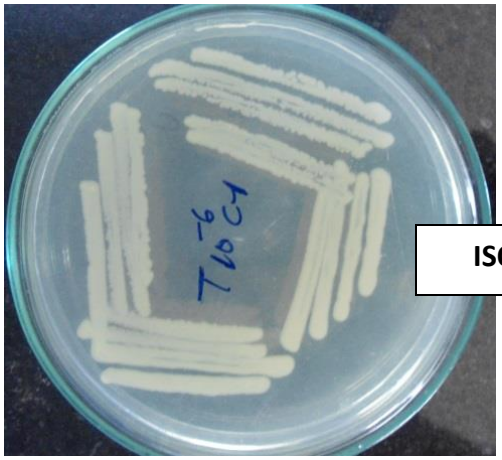


Fig.2-3 Bacterial enumeration Pure culture isolated by streak plate method.

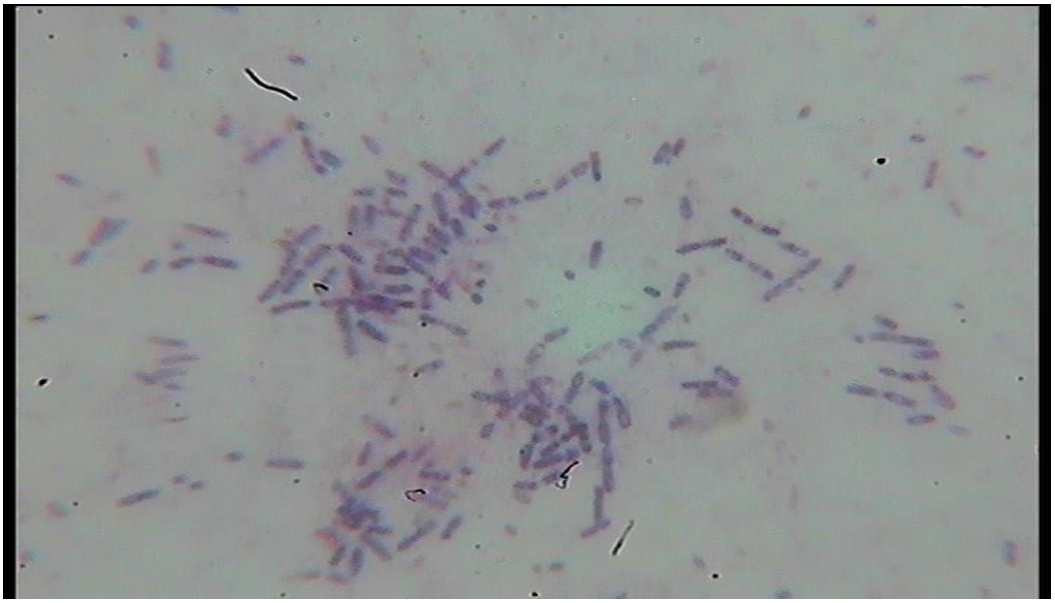




ISOLATION OF PURE CULTURE BY STREAK PLATE

GRAM STAIN

The strains were identified as gram negative.

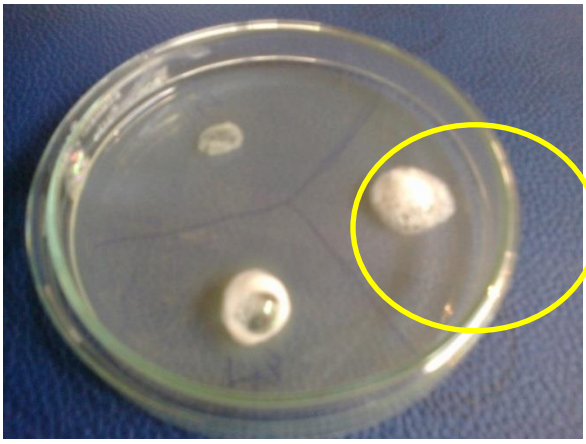


GRAM NEGATIVE
Shape-BACILLI

Fig 4:*Gram Staining*

From the figure it is clearly visible that that isolated bacteria is Gram negative bacteria and bacilli shape in nature because it gives a pink colouration of safranin counterstain.

CATALASE TEST

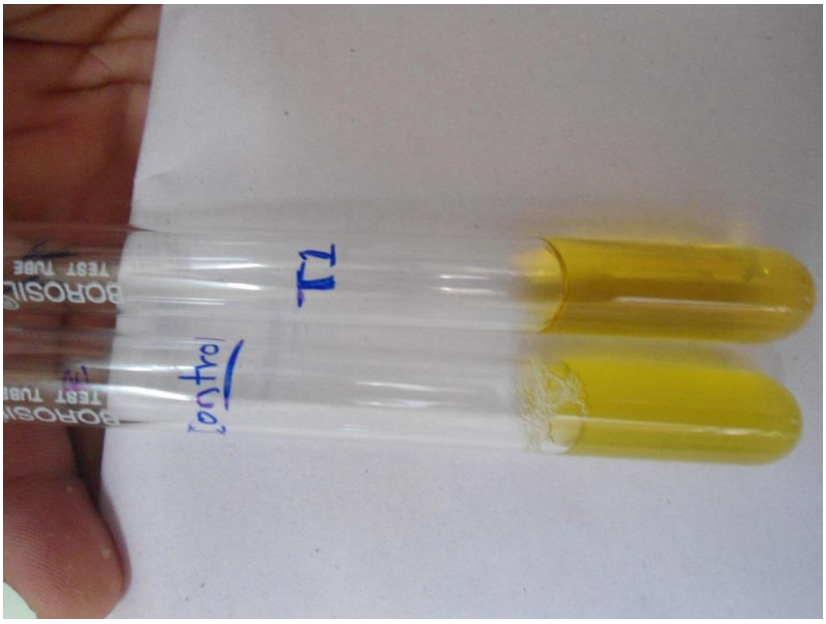


CATALASE TEST----POSITIVE(+)

Fig.5:*Catalase Test*

Formation of vigorous bubble signifies that the bacteria isolated is catalase positive.

METHYL RED TEST

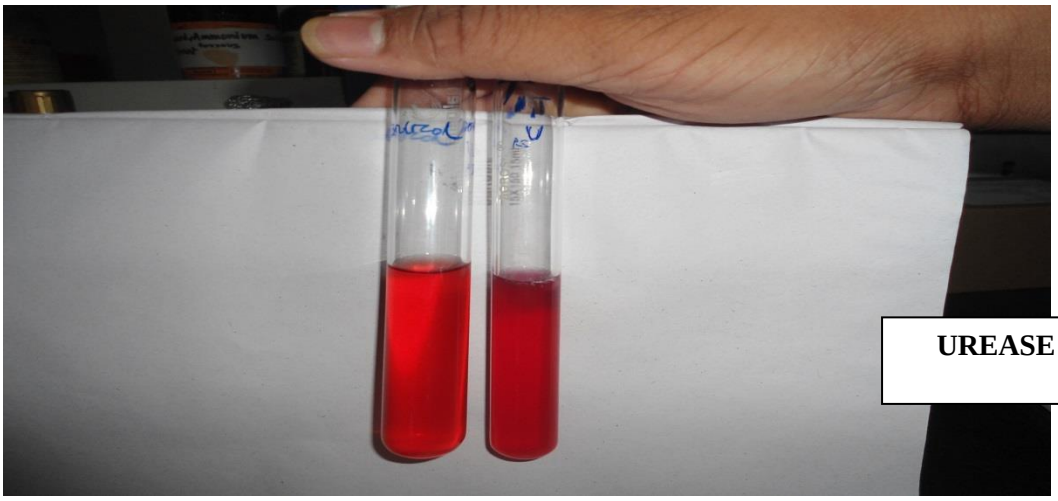


METHYL RED TEST—NEGATIVE(-)

Fig.6:*MR Test*

After the addition of methyl red the colour of the culture media change to yellow colour which concluded that the isolated bacteria is MR negative.

UREASE TEST



UREASE TEST-POSITIVE(+)

Fig.7:*Urease Test*

After 72hr of grow there is slightly change in the colouration of the culture media i.e red colour to slightly pink colour. So the isolated bacteria is **Urease positive**.

CITRATE UTILISATION TEST

CITRATE TEST---POSITIVE(+)

Fig.8:*Citrate Utilisation Test*

Change in the colour of the agar media i.e forest green colour to blue colour states that tha bacteria used is **Citrate positive.**

INDOLE TEST

INDOLE TEST—NEGATIVE(-)

Fig.9:*Indole Test*

In this indole test there is no sign of formation of any pink or red colour ring on the top of the media, so from which it is concluded that the isolated bacteria is **Indole negative.**

From the above biochemical tests it is found that:

LIST OF BIOCHEMICAL TEST	RESULT
GRAM STAINING	NEGATIVE
SHAPE	UNIFORMLY BACILLI
CATALASE TEST	POSITIVE
MR TEST	NEGATIVE
UREASE TEST	POSITIVE
CITRATE TEST	POSITIVE
INDOLE TEST	NEGATIVE
OXIDASE TEST	NEGATIVE

Table 4:*Results of Different Biochemical Test*

By comparing these biochemical tests results with BERGY'S MANUAL it is assumed that the isolated bacteria from the soil sample may be *Enterobacter spp*.

LIST OF BIOCHEMICAL TEST	***Enterobacter spp***	**RESULT**
GRAM STAINING	NEGATIVE	NEGATIVE
SHAPE	UNIFORMLY BACILLI	UNIFORMLY BACILLI
CATALASE TEST	POSITIVE	POSITIVE
MR TEST	NEGATIVE	NEGATIVE
UREASE TEST	POSITIVE	POSITIVE
CITRATE TEST	POSITIVE	POSITIVE
INDOLE TEST	NEGATIVE	NEGATIVE
OXIDASE TEST	NEGATIVE	NEGATIVE

Table 5:*Comparision Between* ***Enterobacter spp. And Unknown Bacteria isolated.***

Other biochemical tests like Sucrose fermentation and Glucose fermentation can be carried out for the isolated bacteria for further confirmation. The results will also be positive for both these tests.

CONCLUSION

CONCLUSION:

Physicochemical properties of water and soil samples collected in the two villages in Latehar region have been presented. All the parameters for each soil sample and water samples are within the permissible range. The soil pH is slightly acidic or slightly alkaline which preferable for the agricultural view. The water pH is also within the range of 6.5 to 7.5 which indicates that it can be used as drinking water. The soil was found to be sand type and black in colour .The microbial studies can be utilized for the prevention of any pathogenic diseases caused by the microbes found in soil and water. Regular check up of the aquatic life can help in maintaining ecological balance. The analysis of soil texture can be helpful for the farmers in their irrigation and vegetation purpose.

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