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STUDY THE PHYSICOCHEMICAL AND MICROBIOLOGICAL PARAMETERS OF DIFFERENT SOIL SAMPLES

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ABSTRACT
In this study, physico-chemical and microbiological properties of different soil samples was studied. The total of four soil samples (S1, S2, S3 and S4) was analyzed for bulk density, pH, electrical conductivity, total organic carbon, moisture percentage, total nitrogen percentage, phosphorus, potassium, total plate count by following the standard protocols. It was observed that pH ranged from 6.6 to 7.8, bulk density ranged from 1.1-1.6 mg/m, conductivity ranged from 229.7 – 434.3 µs/cm, amount of organic carbon lies between ranges of 1.42 - 2.78%, nitrogen lies between ranges of 0.014 – 0.018 %, phosphorus content ranged from 2.81 – 29.61 mg/kg, potassium content ranged from 210 – 567 mg/kg respectively in all four soil samples. While microbiological analysis revealed highest total viable count of bacteria in S3 soil sample and lowest in S2 soil sample.

KEYWORDS: Physico-chemical, Microbiological, Soil Analysis.

INTRODUCTION
Various physicochemical properties of soil such as moisture content, specific gravity Nitrogen as a fertilizer needed for the growth of plants. Potassium is used for flowering purpose, as well as crucial for fruit quality photosynthesis, formation of protein and reduction of diseases.

The roots of plants well developed in the presence of potassium. In plant cell wall, which provides normal transport and retention of other elements was carried out in the presence of calcium.[1] Naturally soil includes organic and mineral components comprehend into horizons, which differ from narrow to wide range among themselves as well as from underlying components in their morphology, physical make-up, biological characteristics and chemical composition.[2]

Soil can develop from accumulated plant residues, weathered rocks or volcanic ash deposits. Thus soil forms a medium for plant in which they can grow and perform varieties of functions crucial to life and generally all plants grow by absorbing nutrients from soil.

Now a day's, in India instead of manures wide range of fertilizers in large amount are being used. As a result of which there is a huge increment in productivity of crop, while the quality of soil support declines regularly. Therefore, there is need to evaluate the soil parameters in urgency through which we can recommend the best suited fertilizer for the soils.[3]

It is becoming challenging day by day to control the negative effects of chemical fertilizers to the soil, plants, animals and human being due to their increasing applications in the fields. Therefore the physicochemical analysis of soil has become necessary.[4] Soil properties which are conscious to variation in the management can be used as indicators. Indian agriculture keeps an eminent position in the globe for the cultivation of rice, sugarcane, wheat, pulses and vegetables.

Microbiological analysis of soil Soil is the portion of the earth's crust where biology and geology meet together, the land surface which provides a habitat to the microorganism, plants, animals.[5] Soil lip with macroscopic life such as nematodes, earthworms, insects and mites, as well as microscopic life (fungi, bacteria, protozoa, algae and viruses), and also the root systems of plants.

Generally soil is the supportive habitat for the propagation of microorganisms, with micro colonies, developing around soil particles.[6] The soil microorganisms are sensitive to environment and have been shown various microbial population and fertility potential according to environment.[7]

Fertilizers supply nutrition for plant and microorganisms. The application of these fertilizers affects the population and growth of microbes found in soil as individual or in colonies

MATERIAL AND METHODS

Sample collection: The aim of this study was to determine the physicochemical and microbiological status from soil samples and isolation of plant growth hormones producing microbes and their fertility potential. Before sampling 15 mm topsoil was removed. Soil samples were collected from different plant rhizosphere at the depth of 15cm. A composite sample of about 500 g was taken through mixing of representative soil sample. These soils were first sieved by scientific sieve shaker with approximately 2 mm spacing to remove the coarser particles. The sieved out fine particles then dry at room temperature for several hours in order to completely remove any trace of moisture. The samples were carefully labeled and packaged.

Physico-chemical analysis of soil: Samples were analyzed for physicochemical parameters such as pH, EC, Nitrogen, organic carbon, bulk density, colour, moisture, phosphorous content, and organic matter, by employing standard methods.[8]

pH analysis: The pH meter was calibrated by using 4.01, 6.86, 9.18 buffer solution. The pH of 1:5 Soil: deionised water suspension was determined by calibrated pH meter at room temperature.

Bulk density: Bulk density was determined by ratio of oven dry mass of the soil to the bulk volume of the soil using bulk density apparatus.

Moisture content

The moisture content was determined by the ratio of the weight of water in the soil to weight of the dry soil. The ratio was expressed as percentage. The percentage moisture content was corrected by using moisture correction factor (MCF).6

$$MCF = \frac{100 + \% \text{ Moisture}}{100}$$

Organic carbon

Soil organic carbon was estimated, following titration method of Walkley and Black (1934) [9]. To 5 g of oven dried mine spoil, 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 were added in a 500 ml flask, thoroughly shaken for 5 min, and was allowed to stand for 30 min. The suspension was diluted with 200 ml of distilled water, followed by 1 ml of 85% H_3PO_4, and 1 ml of diphenylamine indicator. The mixture was titrated against 1N $(NH_4)_2Fe(SO_4)2.6H_2O$, until the colour of the mixture flashed to green. Then, 0.5 ml of 1N $K_2Cr_2O_7$ was added, and the titration was completed by adding 1N $(NH_4)_2$ $Fe(SO_4)2.6H_2O$, till the last traces of blue color disappeared.

Organic carbon (%) was calculated as

$[(V1-V2)/W]\times0.003\times100$

Where V1=volume of 1 N $K_2Cr_2O_7$

V2=volume of 1 N $(NH_4)_2Fe(SO_4)2.6H_2O$

W=wt of soil sample.

Total nitrogen

Total soil nitrogen was determined by Kjeldahl method. Spoil sample of 10 g was transferred to a 300 ml Kjeldahl flask and was moistened with 25 ml of distilled water; allowed to stand for 30 min. To it, 20 g of sodium sulfate and the catalyst mixture (20 g copper sulfate, 3 g of mercuric oxide, 1 g selenium power were ground) was added. To one part of this mixture, 20 parts of anhydrous sodium sulfate was added, and a pinch of granulated zinc was added to the suspension, followed by 35 ml of conc. H_2SO_4. The resulting mixture was subjected to low heat treatment for 30 min for digestion, till the digest become yellow and colorless. The digest was then cooled and 100 ml of water was added, and allowed to stand for 5 min. The supernatant was then transferred into a flask. 25 ml of 4% boric acid was pipetted into a 500 ml conical flask, and 5 drops of mixed indicator (0.5 g bromo-cresol green and 0.1 g methyl red dissolved in 100 ml of 95% ethyl alcohol) was added. The glass tube attached to the lower end of the condenser as dipped into the boric acid solution. The condenser was connected to the flask and 100 ml of 40% NaOH was added slowly through the separating funnel. By heating the mixture, 150 ml of distillate was collected in the conical flask and was titrated against N/14 H_2SO_4, till the faint pink coloration was reached. A blank was run instead of soil.

Total soil nitrogen (%) was calculated as

$[(T-B)\times N\times14.007\times100]/W;$

where T and B are the volume of titrant used against sample and blank;

N=normality of titrant and W=weight of sample.

Soil texture: Soil texture analysis included the estimation of clay (<0.002 mm), silt (0.06 mm-0.002 mm) and sand (2 mm-0.06 mm) percentage. Soil sample (50 g) was taken in a 500 ml heat resistant bottle, calibrated up to 250 ml. To this, 125 ml of water was added and the mixture was swirled to wet the spoil thoroughly. 20 ml of 30% hydrogen peroxide was added to it and the bottle was gently rotated. Few drops of amyl alcohol were added to the mixture and kept in a boiling water bath, till the reaction was complete. Then, 2 g of sodium hexametaphosphate was added, followed by water, to make it up to 250 ml, and was shaken for 28 hr in a mechanical shaker. Then, the contents were transferred to a 1 L sedimentation cylinder and the volume was made up to 1 L. A blank cylinder was maintained by dissolving 2 g of sodium hexametaphosphate in water and made up to the mark with water. Both experimental and blank samples were placed in a water bath to maintain a constant temperature (25 ± 2°C). After 30 min, the sample cylinder was mixed vigorously with a plunger. The Bouyoucos hydrometer

readings were taken exactly at 40 sec and 5 hr for the samples, and at 5 hr for the blank. Temperature of the water bath was recorded. The percentage of sand, silt and clay were determined as per the following calculation.

$$\text{Sand\%} = \frac{\text{Oven dry sand mass}}{\text{Original sample mass}} \times 100\%$$

$$\text{Slit \%} = \frac{\text{Oven dry slit mass}}{\text{Original sample mass}} \times 100\%$$

$$\text{Clay\%} = 100-(\text{Sand\%}+\text{Slit \%})$$

Phosphorus analysis: Water extracted P from soils were analyzed by ascorbic acid colorimetry (Murphy Riley) method. To prepare samples, 4.0 mL Reagent B and 19.0 mL Distilled water was added to 2.0 mL of each extract. Standards consisting of 5.0 mL of each standard P solution (0.1 ppm to 1.0 ppm P), 4.0 mL Reagent B, and 16.0 mL DI water and a 0.0 ppm P standard consisting of 4.0 mL Reagent B and 21.0 mL DI water were also prepared. Samples were allowed 30 minutes for color development.

The absorbance of the samples and standard solutions at 882 nm was measured with a UV/VIS spectrometer.

Microbiological Analysis of Soil

Isolation of micro-organisms

From the soil samples serial dilutions were prepared and a volume of 0.1 ml was spread on pikovskaya medium for the isolation of P solubilizing microbes. The inoculated Petri-dishes were incubated 48 h on 37°C. The isolates were maintained on pikovskayas broth for further analysis.[10]

Morphological Characterization: The microbial identification was done on the observation of colony morphology and their microscopic observation.

RESULTS AND DISCUSSION

Physicochemical analysis of Soil

The physico-chemical values of the soil samples collected at different sampling sites were calculated (**figure 1**). The results (**Table 1**) indicate that the quality of soil considerably varies from location to location and sample to sample. Soil collected from different rhizosphere show the different colour that is important for the soil diversity on behalf of chemical variation and responsible for the diversity of vegetation. The analysis of chemical properties indicates that the black soil samples of plant rhizosphere were generally slightly acidic and other show alkaline pH. The ranges of pH of soil were 6.7-7.9. The pH values of the impacted soils lie within the acidic range and may not support the growth of most crops that thrive in alkaline soil, this may lead to loss of macro minerals needed for plant growth. Thus acidification of soil depletes important nutrient elements such as potassium, calcium and magnesium.[11] pH is a measure of the hydrogen ion concentration i.e. acidity or alkalinity of the soil. pH can affect the availability of nutrients and activity of many essential micro-organisms. The pH of a soil may influence crops grown in the field and the types of soil microbiota.[12] pH is an important parameter as it helps in ensuring availability of plant nutrients e.g. fe, Mn, Zn and cu are more available in acidic than alkaline soils. It also helps in maintaining to soil health.[13] An examination of soil samples (Table 1) shows that the values for pH range from 6.7 to 7.9 indicating that the soils are alkaline and under such conditions the solubility of minerals decreases creating nutrient deficiencies in the soils.[14] Black soils have high pH of when may be due to presence of high exchangeable cations on the exchange complex and may be due to presence of high exchange complex and may be due to calcareousness.[15] From this we can conclude that pH is mainly dependent on exchangeable cations and calcium carbonate[16], which in turn are controlled by topography and physiographic position. The results indicate that the soil in the region is acidic as the pH of all soil samples lie within a range from 5.2 to 6.2, which is consistent with the range (4.5 to 9) specified by Costerton[17] for microbial activity to take place, though it is argued by Harris[18] that pH within a range from 5 to 9 has no effect on corrosion.[19] The low pH may have affected fungal growth in the contaminated soil, which was observed to be low. It has been shown that optimal activity for microbial degradation occurs at pH 7.4 while considerable inhibition can be seen both at pH 4.5 and 8.5.[20] Soil pH levels that are too high or too low (Figure 1.) lead to deficiency of many nutrients, decline in microbial activity, decrease in crop yield, and deterioration of soil health. For example, soil pH values below 5.5 and between 7.5 and 8.5 limit availability of phosphate to plants. The pH values of the impacted soils lies within the acidic range and may not support the growth of most crops that thrive on alkaline soil, this may lead to loss of macro minerals needed for plant growth. Thus acidification of soil depletes important nutrient elements such as potassium, calcium and magnesium.[21]

Electrical conductivity showed variation between 255 µs/cm to .49 ds/m for red soil and .16ds/m to .33ds/m for black and yellow. Generally it is believed that higher the concentration of ions in the soil solution more is its electrical conductance. Table 1 shows the electrical conductivity (EC) values of soil sample at different site. Electrical conductivity can serve as a measure of soluble nutrients for both cations and anions. Soil EC indicates the mineralization of organic matter in soil and serves as a measure of soluble nutrients.[22] Eigenberg et al., 2002[23] revealed that nitrogen content of soils may be monitored using EC measurements mentioning significant positive relationship. Azeez and Van Averbeke, 2012[24] found that electrical conductivity of soil significantly increases with the application of poultry, cattle and goat manures and the potential of

manure-induced soil salinization was very high in poultry manure and goat manure compared with cattle manure. Dikinya and Mufwanzala, 2010[25] revealed increased electrical conductivity with increasing rates of chicken manures. Soil electrical conductivity values were moderate but could peak higher locally, indicating potential soil salinity problems; this was likely due to insufficient leaching of salts in some profiles. Factors contributing to high salt concentration included insufficient rainfall or water application, the presence of soil layers impeding drainage, and high initial amounts of salts in certain profiles. Cations in the soil solution indicated a high proportion of sodium with respect to calcium, magnesium and potassium.

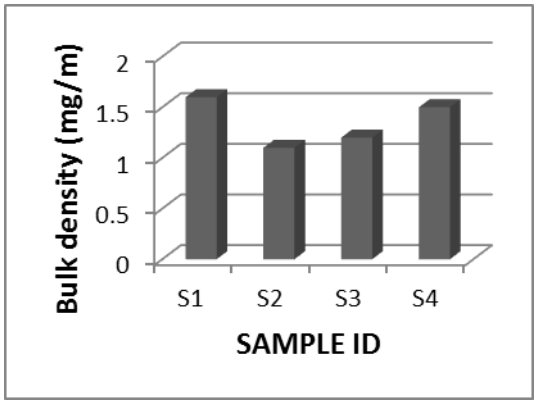
The amount of organic carbon was found between the range of 1.42-2.78% respectively. The maximum organic content 2.78% observed in sample S4. Sample S1, S2 and S3 obtained 2.15, 2.08 and 1.42% (Table 1) respectively. Boyd et al., 2002[26] recommended that range of organic carbon 1.0-3.0 % is the best range of agriculture. Boyd (1994) also reported that organic carbon value 0.60-1.50 % are highly suitable for aquaculture.[27] Ahmed (2004) reported that organic carbon range 0.95 to 1.50 % is the suitable range for aquaculture of Bangladesh.[28] It is apparent from the present study that comparatively high amount of organic carbon was determined black soil of Rajgarj and Hoshangabad district. The increased organic carbon of the soil might be attributed to unused supplemented feeds were used and stocking density was precisely low. It showed noted that the quantity of supplemented feed was increasing day by day, which may be the reason of the comparatively higher value of organic carbon in soil.

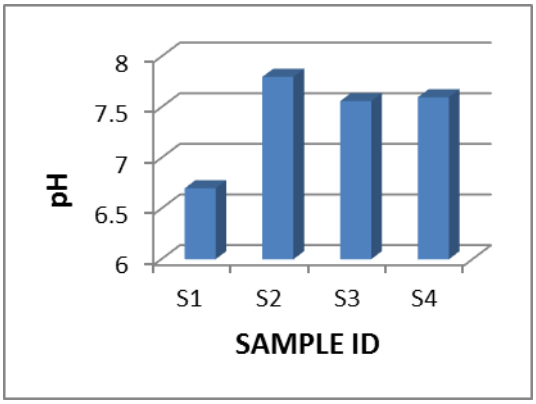
The S2 soil sample has low bulk density (1.1mg/m), while sample S1, S3 and S4 have high bulk density. High bulk density may be due to high clay and that too of well shrink type overburden leading to compaction. It was observed that the bulk density is dependent on calcareous and saline nature of soils, but independent on whether the soil is acidic or alkaline. Jones (1983)[29] reported that the impact of the texture on bulk density is much, because of the organic carbon. Wagner et al., 1994 estimated soil bulk density using soil texture parameters along with organic carbon content values.[30] Bernoux, et al., 1998[31] found a correlation between texture and bulk density. Kumar et. al., 2009[32] indicated that soil texture specific tests would be required to determine the correct organic matter level to achieve a target bulk density to avoid the problem of compaction.[1]

Soil moisture is the amount of water in a given amount of soil present in the form of capillary water, which is used by plants during the photosynthesis. Soil moisture of all samples ranged between 6% to 64.7% The increase level of moisture in the surface and sub-surface soils in not unconnected with intense rainfall and flooding which proceeded the period of sampling. The high moisture may lead to the problem of wet ability and soil aeration, which may affect the nutrient status of the soil.[14]

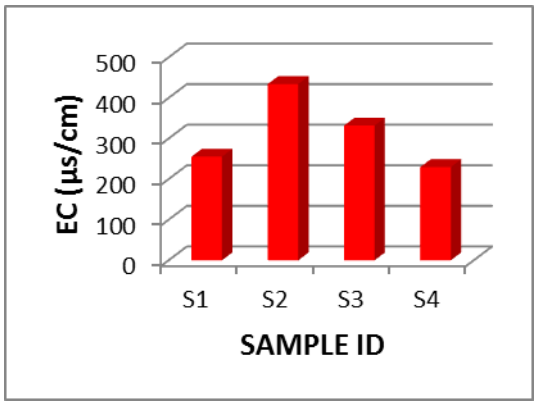
Phosphorous in the present investigation vary from 2.81 mg/Kg to 29.10 mg/Kg (Figure 1). The highest value of P was found in a S1soil sample 29.10mg/kg may be due to use of excessive phosphorous fertilizers. Application of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop. Previous researches have already reported the importance of phosphorus.[33] Phosphorus is one of the key macronutrient required for plant growth and metabolism. Inorganic phosphate supplied to the soil as a fertilizer is rapidly converted into unavailable form. Soluble P converted into insoluble phosphate involves microorganisms. The available nitrogen was measured for all samples, its value about 0.017 for S1, 0.016 for S2, 0.014 for S3and 0.018% nitrogen investigate in S4. Soil nitrogen content is an important environmental factor that affects the rate of nutrient uptake by plants. For that matter, the higher concentration of nitrogen in the upper layer of soils may be due to the presence of immobilized nitrogen in the detritus on the soil surface[34], which is prone to microbial decomposition in soils in the subsequent layers. Total Nitrogen (TN) levels in soils depend on the organic matter build up in different systems, supported by the significant positive correlation between TN and TOC in soils.[35]

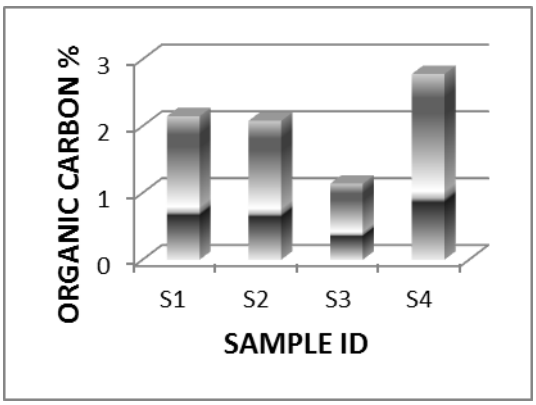
Figure 1 represent the range of Low, Medium and High potassium content as per standard of soil analysis. This values are used to determine the category of soil whether the soil sample have Low, Medium or High content of Potassium Experimental values of quality characteristics especially for available Potassium of soil with their fertility index are presented in the Table . Data presented in Table 2 shows that soils of sample S3 contain lower available Potassium (210 mg/kg) and sample S2 have high range of available potassium (567 mg/kg) that might be due to poor or excessive use of fertilizer. Potassium is absorbed by the plant in larger amounts the than any other mineral element except nitrogen and in protein photosynthesis, fruit quality and reduction of diseases.[36] Available potassium of cultivated soils and barren land soils was between medium to high and some cases it is the entry of only that nutrient which is not toxic to the found in medium, whereas in the garden soils it was medium.[37] P. G. Gajghane et al., 2015[38] reported the effect of potassium and sulphur levels on soil fertility status after harvest of mustard. They observed significantly increases in organic carbon, calcium carbonate and improvement in the available nitrogen (317.23 kg ha-1), phosphorus (18.87 kg ha-1), potassium (407.03 kg ha-1) and sulphur (9.72 kg ha-1) status after harvest of mustard by the addition of doses fertilizers, respectively.



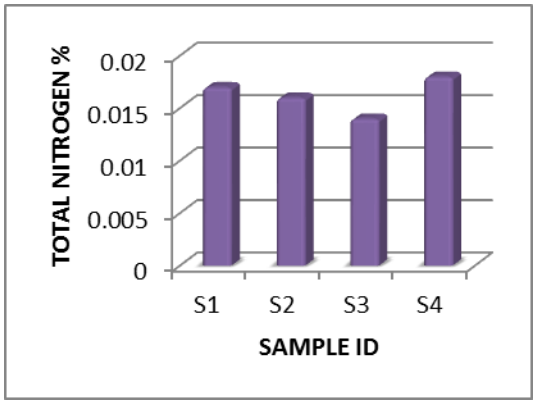


Bulk Density of Soil Samples Ph Of Soil Samples.

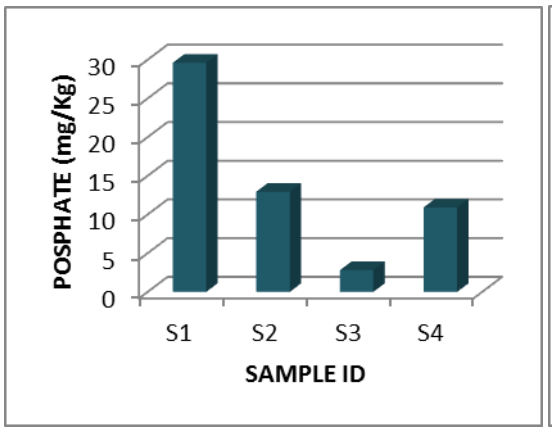


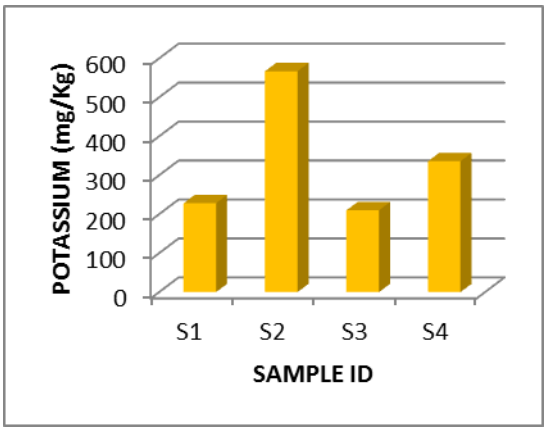


Conductivity of Soil Samples Organic Carbon of Soil Samples.



Total Nitrogen of Soil Samples.





Phosporus of Soil Samples Potassium of Soil Samples.

Figure. 1: Physicochemical analysis of soil samples collected from various agrofields.

Table. 1: Physicochemical Analysis of soil samples collected from different Rhizosphere.

S. No.	Parameters / Study Area	S1	S2	S3	S4
1	Bulk density (mg/m)	1.6	1.1	1.2	1.5
2	pH	6.7	7.8	7.56	7.6
3	EC (µs/cm)	255.3	434.3	331.8	229.7
4	OC%	2.15	2.08	1.41	2.78
5	N (%)	0.017	0.016	0.014	0.018
6	P (mg/Kg)	29.61	12.95	2.81	10.92
7	Potassium (mg/Kg)	228	567	210	336

MICROBIOLOGICAL ANALYSIS

Total plate count: The isolation process is a procedure of isolation the mixture of colonies to a single colony. This process was done by using the streaking method to obtain pure cultures. The soil's samples were added with 1 Liter pure water to obtain solution sample before transferred onto nutrient agar plate. It is important that the numbers of colonies developing on the plates are not being too large. On crowded plates some cells may not form colonies, and some colonies may fuse, leading to erroneous measurements. So, to obtain the appropriate colony number, the samples need to be diluted. This solution samples were diluted up to 10^{-3}. By using spread plate method, the diluted samples were transferred into nutrient agar plate and the bacteria were grown on it. From the observation, these samples take about three until four days to growth on the plate. The maximum total of 42 microbial isolates were obtained from the analyses of S3 soil sample, whereas sample S1, S2 and S4 showed 32, 9 and 24 colonies through soil dilution agar plating (**Figure 2**). The results of the total viable count are summarized in Table 2. Different samples showed different cfu/ml. After the bacteria were grown on a culture plate, colonies with distinctive morphology were selected (Figure 2). Four colonies were selected for further study. The successful recovery of viable cells from permafrost depends on a number of factors. The occurrence of viable microorganisms was independent of the depth of permafrost sampling and sometimes even increased with depth. Rich media favor morphological diversity, while diluted media (with low nutrient contents) enhance the quantitative recovery of viable microorganisms.[39] Microorganisms work incognito to maintain the ecological balance by active participation in carbon, nitrogen, sulphur and phosphorous cycles in nature.[40]

Table. 2: Total viable count of oral bacteria.

S. No.	No. of colony	Dilution factor
S1	32	10^3
S2	9	10^3
S3	42	10^3
S4	24	10^3

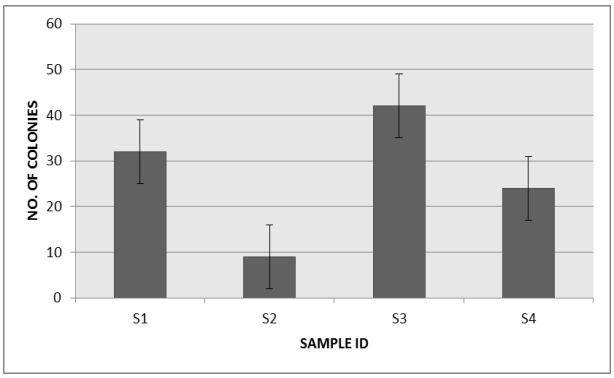


Figure. 2 Total plate count of soil samples collected from different rhizosphere

CONCLUSION

Study of physicochemical parameters of soil is very important with respect to the proper management of soil. S3 soil sample was recorded with the highest cell count while S2 having lowest cell count. Due to the overuse of chemical fertilizers in the need of quick response the quality of soil has reduced to very poor grade and also have adversely affected the microbial flora of the soil which is helpful in plants growth and development. So, in order to improvise the soil fertility status it has become necessity to cross check the physicochemical and microbiological parameters of soil.

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