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Research Paper

STUDY OF IN-SITU AND CULTURABLE BACTERIAL COUNTS AND ITS RELATION WITH VARIOUS PROPERTIES OF SOILS NEAR JUNAGADH

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Abstract

Soil is an important part of an ecosystem and various biogeochemical cycles play an important role in the soil with process of nitrification, nitrogen fixation, phosphate solubilization etc. Soil microbes are one of the important biota of the soil. In the present study attempt has been made to study culturable bacteria in soil and its relation with soil parameters such as electrical conductivity, pH, and percentage of organic compound and total nitrogen, phosphorus and potassium. Also study of in situ analysis with buried slide technique was carried out. Soil samples were found to be of neutral pH, low salinity, high nitrogen, low organic carbon, range of phosphorus and potassium from medium to high. Site GB showed maximum number of colonies with phosphate solubilizers and nitrogen fixers. *In situ* analysis showed different bacteria as well as fungi.

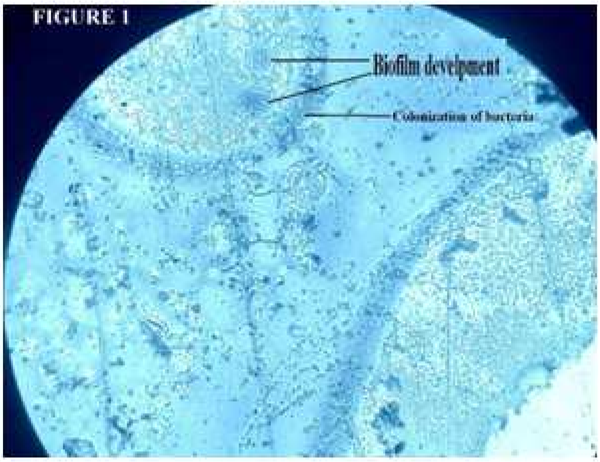
Key words: Phosphate solubilizers, organic carbon, total nitrogen, phosphorus, potassium.

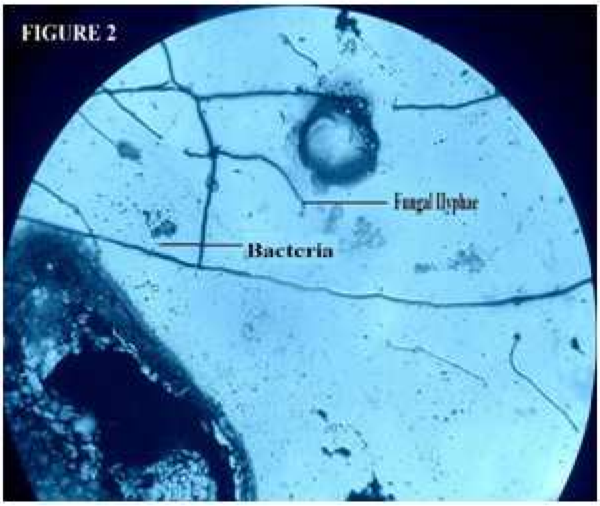
INTRODUCTION

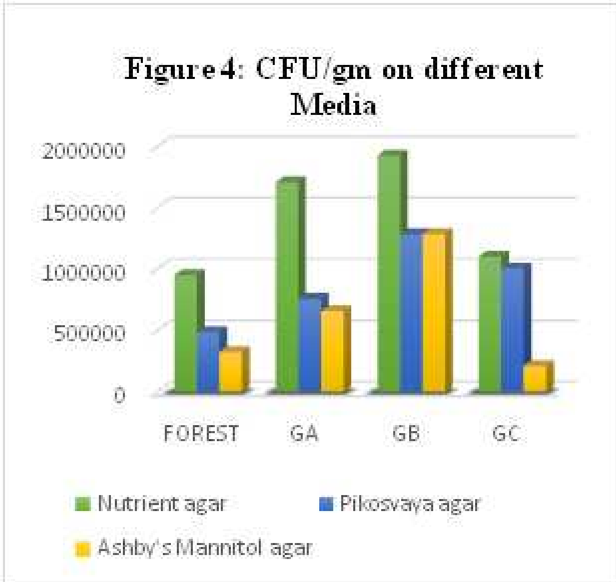
The “roots” of human understanding of soil biology and ecology can betraced into antiquity and probably even beyond the written word. Wecan only imagine hunter–gatherer societies attuned to life cycles ofplant roots, fungi, and soil animals important to their diets, their welfareor their cultures, and particularly to environmental conditionsfavourable to such organisms. Indeed, early agriculture must certainlyhave developed, at least in part, from a practical knowledge of soils andtheir physical and biological characteristics.

Formation of soil is a pedogenic and biogeochemical process that is dependent of the translocation, bio cycling and transformation of materials deposited and degraded by microorganisms on the uppers soil layer. These processes are developed a long time and space, and have as final product the formation of soils with different physical and chemical characteristics.Various soil microorganisms, control ecosystem functioning through decomposition and nutrient cycling and may serve as indicators of land-use change and ecosystem health[1]. However, the study of soil microorganisms is difficult and our current understandinglimited. The sheer number, astonishing diversity and small size of these communities become more apparent as our technologies to explore them have improvedin recent years[2]. What we do know is that soil microbial communitiesare dynamic and diverse [3]almost beyond measure[4], and that some patterns seem to hold on a global scale.

The type of vegetation cover and dominant tree species of forest ecosystems affects the chemical, biological and biochemical properties of soils[5]. Due to the accumulation of organic residue, a significant proportion of the nutrients extracted from soil are retained by trees, and the decomposition of plant residue releases nutrients, which can be reused by vegetation and microorganisms[6]. In the present study attempt has been made to study in situ characteristics of bacteria and counts of bacteria and its relationship with different physiochemical parameters of soil.







MATERIALS AND METHODS

Soil samples

Soil samples from planted area near Junagadh (GA, GB &GC)and forest (FO) near Junagadh. From each place three samples were collected. The rhizosphere soil around the root was carefully dug up to 30 cm depth collected and transferred into polythene bags. The soil samples were stored in refrigerator till further use. The representative was collected from forest randomly up to 30 cm depth.

In situ microscopic Observation(Buried slide technique)
Glass slide were washed and treated with alcohol. Slide were buried at different site in soil at a depth of up to 30 cm for 15 days for study soil microbial flora structure.[7]

Enumeration

Samples of soil were plated on nutrient agar, Pikovskaya agar[8] and Ashby's[9] agar with different dilution and using spread plated technique. Colonies were counted at 24 hours interval until 72 hours and colony forming unit per gram was calculated for each sample.

pH and electrical conductivity (EC, dS/m) were determined in the supernatant solution of 1:5 soil/water ratio (w/v) using a pH meter and conductivity bridge / meter [10] respectively.

Organic carbon was determined by the wet digestion method of Walkley and Black (1934a)[11].

Nitrogen , Potassium and Phosphorus were determined by Jones [12].

Interpretation of all the physical and chemical properties of soilwere done according to Methods ManualSoil Testing in India[13].

All the statistical calculation were done using Microsoft Office Excel (2013) and XLSTAT- PRO 7.5.

RESULTS AND DISCUSSION

In situ microscopic Observation

Buried slides were taken out of the soil and observed for microbial flora. Various types of bacteria and fungi were observed in the soil. Formation of biofilm was observed (FIGURE 1). Fungi (FIGURE 2) and Bacterial colony (FIGURE 3) were observed. Bacteria has ability to colonize and form special growth characteristics under soil environment. Researchers have shown that biofilms are not simply organism-containing slime layers on surfaces but also represent biological systems with a high level of organization where bacteria form structured, coordinated, functional communities[14].

Enumeration

Colony forming unit per gram was calculated by counting bacterial colonies on Nutrient agar plates, bacterial colonies which were able to solubilize tri-calcium phosphate on Pikovskaya agar plates and nitrogen fixers which showed growth on Ashby's Mannitol agar.Site GB colony forming unit per gram as compared to other sites.

Table 1: Colony forming unit per gram of soil on various media.

Sample	Nutrient agar	Pikovskaya agar (For Phosphate Solubilizers)	Ashby's Mannitol agar (For nitrogen Fixers)
FOREST	950000	489000	325175
GA	1708833.333	758000	659878.1667
GB	1926666.667	1285000	1285000
GC	1099956.667	1005000	210612.5

pH and Electrical Conductivity

The measure of soil pH is an important parameter which helps in identification of chemical nature of the soil[15] as it measures hydrogen ion concentration in the soil to indicate it's acidic and alkaline nature of the soil.pH of the samples was found to be between 7 and 8 indicating the existence of a variety of soils that are neutral to slightly alkaline nature.

Conductivity(dS/m), as the measure of current carrying capacity, gives a clear idea of the soluble salts present in the soil. It plays a major role in the salinity of soils. Lesser the EC value, low will be the salinity value of soil and vice versa.Conductivity(dS/m) was found to be low at all the sites indicating low salinity. Even though, soil conductivity is influenced by many factors, high conductivities are usually associated with clay-rich soil and low conductivities are associated with sandy and gravelly soils. This is a result of the shape and physical properties of the particles which make up the soil.There are various factors that affect the soil electrical conductivity such as pore continuity, water content, salinity level, cation exchangecapacity, depth and temperature[16].

Table 2 : Selected Physical and Chemical Properties of soil

Parameters	FO			GA			GB			GC		
E.C.	0.21	±	0.02	0.19	±	0.02	0.28	±	0.01	0.11	±	0.02
pH	7.69	±	0.06	7.93	±	0.05	7.42	±	0.07	7.81	±	0.05
P	19.00	±	0.58	25.33	±	1.45	32.67	±	1.76	21.7	±	1.2
K	190	±	5.5	350.7	±	5.8	342.7	±	4.1	370.7	±	5.8
% OC	1.05	±	0.01	0.93	±	0.04	0.88	±	0.04	0.89	±	0.02
%TN	1.32	±	0.05	1.24	±	0.03	1.12	±	0.02	1.44	±	0.02
%OM	1.81	±	0.02	1.61	±	0.06	1.51	±	0.07	1.54	±	0.03

EC – Electrical Conductivity, P- Phosphorus (kg/ha) , K- available potassium,
OC- % Organic Carbon; OM- % Organic Matter, TN- % Total Nitrogen

Percentage Organic carbon (OC) and percent organic Matter (OM)

The importance of organic matter in the soil is implied in the definition of soil, which recognizes fertility status of the soil, as a unique feature distinguishing soil from the parent rock/ other non-fertile soils. It increases the soil fertility / nutrient status and controls erosion and runoff of the soil and water, besides it is a major determinant of improved soil structure, moisture content and general nutrient status of the soil. The percentage of organic carbon ranged from 0.89 to 1.05and percentage of organic matter was found to be in range of 1.51 to 1.81 in the study area. Depending upon theorganic carbon content (%), the quality of soil may be graded as low, medium and high. Majority of the soilsamples appear to possess low percentage of organic carbon content (Table 2).Soils with low carbon are due to good aeration which increase the oxidation of organic matter present in the soil[17].

Phosphorus

Phosphorus is the second most important macronutrient available in the biological systems, which constitutesmore than 1% of the dry organic weight. It is also a second most limiting factor often affecting plant growth,which exists in the soil in both organic and inorganic forms.The phosphorus (kg/ha) was found in range of 19 to 25.33kg/ha. Phosphorus content was found to be medium to high.

Nitrogen

Plants take up nitrogen generally as nitrates under aerobic conditions and as ammonium ions during anaerobicconditions. Nitrogen is most often the limiting nutrient for the plant growth. The percentage of total nitrogen was found in range of 1.12 to 1.44.Total nitrogen content was found to be high.

Potassium (K)

Next to nitrogen and phosphorous, potassium is the most critical essential element in influencing plant growth and production throughout the world. Potassium plays essential role in plants. It is an activator for dozens of enzymes responsible for plant process. The Potassium (K) (kg/ha) values varied from 190 to 370.7 kg/ha (Table 2). Potassium in soil samples ranged from moderate to very high.

Relation among soil parameters.

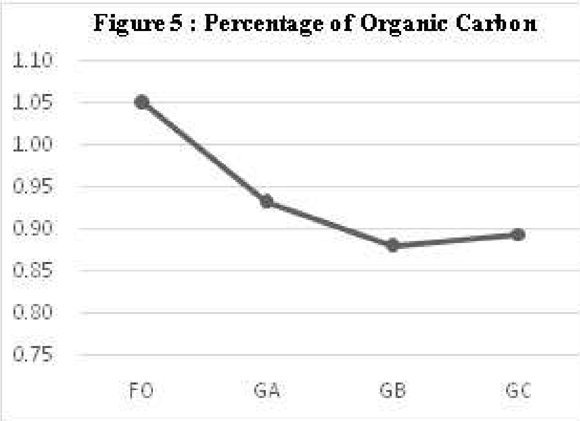
Correlation matrix is given in Table 3. Correlation studies of electrical conductivity with pH, Potassium, percent organic carbon, percent organic matter and percent total nitrogen was found to be negative and with that of phosphorus was found to be positive. pH was negatively correlated with phosphorus and electrical conductivity, and positively correlated with Potassium, percent organic carbon, percent organic matter and percent total nitrogen. Phosphorus and potassium show strong relationship with each other (r=0.631). Organic carbon showed strong relationship with pH and total nitrogen. Nitrogen showed positive correlation withpH.

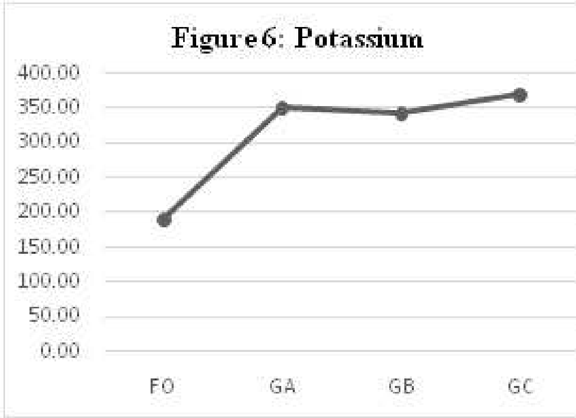
Table 3: Matrix correlations among different soil properties in tabular form.

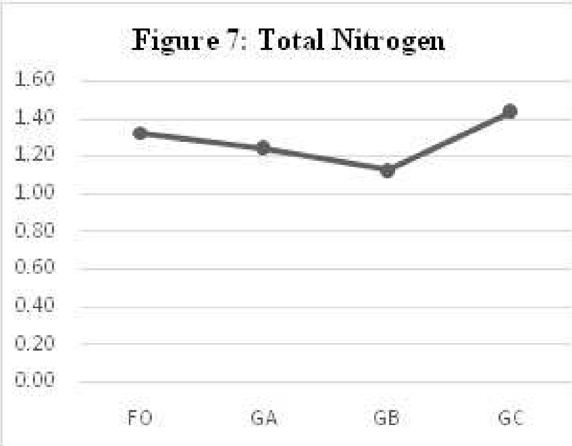
Soil Parameters	E.C.	pH	P	K	% OC	%TN	%OM
E.C.	1.000						
pH	-0.764	1.000					
P	0.564	-0.615	1.000				
K	-0.295	0.051	0.621	1.000			
% OC	-0.167	0.491	-0.882	-0.845	1.000		
%TN	-0.893	0.488	-0.677	0.038	0.250	1.000	
%OM	-0.167	0.491	-0.882	-0.845	1.000	0.250	1.000

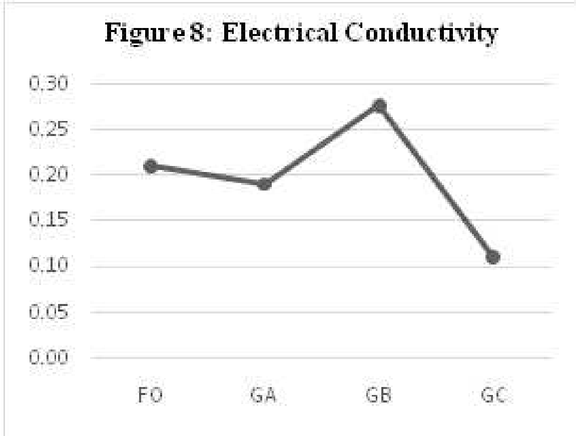
Relation of Bacteria with soil parameters.

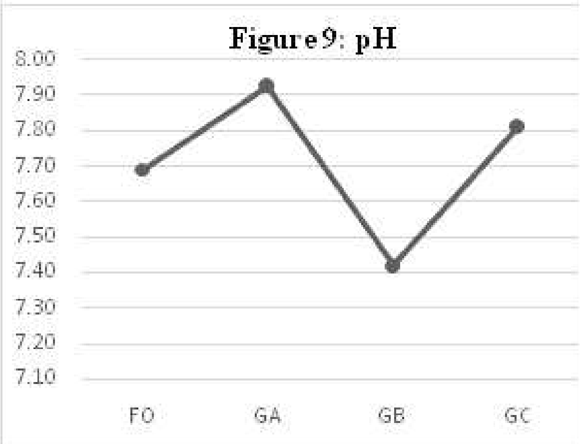
In the present study pH was found to be neutral to slightly alkaline which is an indication that population of bacteria were high as compared to acidic pH soils[18]. In the given study pH at site GB was neutral and maximum bacteria population was found at the same site. Electrical conductivity was found to be less than 2 dS/m indicating low saline and supporting growth of bacteria, increase in >2dS/m will have negative impact on respiration, decomposition, nitrification and denitrification[19].Soil organic carbon is the major source of energy for the soil microorganisms and Organic Carbon reaches its optimum value when the soil pH is in between 5.00 to 6.00 and decreases when lower than 5.00[20]. Nitrogen addition in temperate forests typically increases microbial biomass at a short term basis [21] but over the longer term, biomass generally decreases [22]. Total Nitrogen was found to be high and has negative impact over nitrification process.The organisms involved in phosphorus (P) cycling in soils are highly varied, and microorganisms probably play the most important role. However, more than 99% of soil microorganisms have not been cultured successfully[23]. Microorganisms play an important role in all three major components of the soil P cycle i.e. dissolution, precipitation, adsorption, desorption, mineralization and immobilization. Excess potassium leads to decrease in count of bacteria in soil.

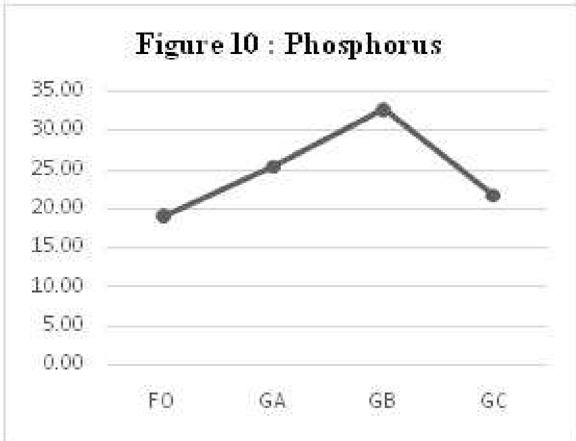












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