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EVALUATION OF BACTERIAL AND FERTILITY STATUS OF RAWALPINDI CULTIVATED SOIL WITH SPECIAL EMPHASIS ON FLUORIDE CONTENT

HAROON ILAHI[1], FAZLI WAHID[1], RAFIULLAH[1*], MUHAMMAD ADNAN[1], JEHANGIR AHMAD[1], MUHAMMAD AZEEM[1], IJAZ AMIN[1], HAZRAT AMIN[2]

[1]Department of Agriculture (Soil Sciences) University of Swabi, KP, Pakistan
[2]Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar, Pakistan
Corresponding author Email: rafiullah@uoswabi.edu.pk

Abstract

Soil contains variety of the microscopic organism (bacteria, virus and fungi) and chemical compounds (organic and inorganic). This research work focus to calculate the physical and chemical parameter of the agriculture soil of selected land of Rawalpindi with addition to prokaryotes. Different methods were followed to assess the physiochemical characteristics. Further isolated bacteria were identified by gram staining, microscopy and different biochemical tests. Findings of this research revealed that the pH was neutral which indicate the low availability of the nutrients for plants, apart from this all other physiochemical properties were in lower limits. Besides this fluorine ion were in range allotted by the WHO, but fluorine in the 0-21cm was more abundant than deeper layer (22-41). In targeted areas the site one has the higher concentration of the fluorine ion as compared to all other sites. In bacterial fauna Gram + bacteria were dominant in which predominant species was Arthrobacter. *Current study concluded that using of the fertilizer can enhance the soil fertility hence the production but with strong check and balance on the fluorine ion to prevent any serious future consequences.*

Keywords: Fluoride ion estimation, Rawalpindi agriculture soil, soil bacterial fauna, physical parameters, chemical parameters

INTRODUCTION

A soil is a fine part of the earth that consists of countless living organisms along with organic and inorganic compounds, making soil as a complex mixture. Formation of the soil is a result of natural weathering (Nortcliff, 2006). this is the part of the earth that support life but very limited research has carried out. Even research regarding the soil was initiated from the last 10 years. (Ezeaku, 2015; Singh et al., 2013). Soil fertility can be defined in terms of its physiochemical parameter and the presence of the microbes. Soil from the different places of the world will never be the same even sample from two different location of same backyard (Carter et all., 1998). Soil fertility can be change due to contamination and the main cause of the pollution for soil is mankind actions. Fluoride ion pollution is more common as compared to any other type of the pollution because of the pesticides contains fluoride ion, Industrializations and many more (Paul et al., 2011; Okibe et al., 2010; Mezghan et al., 2005). Severe fluoride pollution can cause serious health concerns like tooth decay, bones deterioration, skin infections, eye disease and can cause many heart diseases (Paul et al., 2011). Soil prokaryotes play a major role in the soil fertility; they directly affect the plant growth. Bacteria produce different hormones which promote the plant growth. They are the excellent immune stimulator for the plant and they aid in the nutrient uptakes (Dobbelaere et al., 2003; Hayat et al., 2010). Soil prokaryotes can be categorized into nodules promoting bacteria (NPR), growth promoting bacteria (PGPR) health promoter bacteria (PHPR) (Alvarez et all., 1996; Saleem et al., 2007; Khan et al., 2013). This research work intended to segregate, classify the soil bacteria and to evaluate the physical and chemical parameter of the Rawalpindi cultivation soil.

PROCEDURES

Soil Specimen locations & collection: 10 samples (total 40) were collected from the four different agriculture land of Rawalpindi. Selected areas are marked in the figure below (fig 1). Soil specimen were collected from two depths that is 0-21 cm and 22-41cm. Before sampling the upper portion of the soil was cleared from the grasses, litter, plant residues. Sampling was done with the help of the different tools like blades, spade and shovel. The collected samples were store in the clean sterile plastic bags which were labeled according to the location, time and amount of the sample. After that samples were carried to the Laboratory of University of Sargodha. The samples were air dried at normal room temperature. After homogenation the sample were filtered through 2mm of mesh strainer and store again.

Parameters & Methodology: By using the distilled water, suspension was prepared by following the process of syringe (Moshrefi, 1993). Special focus was specified to the negative F ion along with the parameters that has vital role in water retention and storing the nutrients. Parameters under study are given in the table 1 (other than negative fluorine ion).

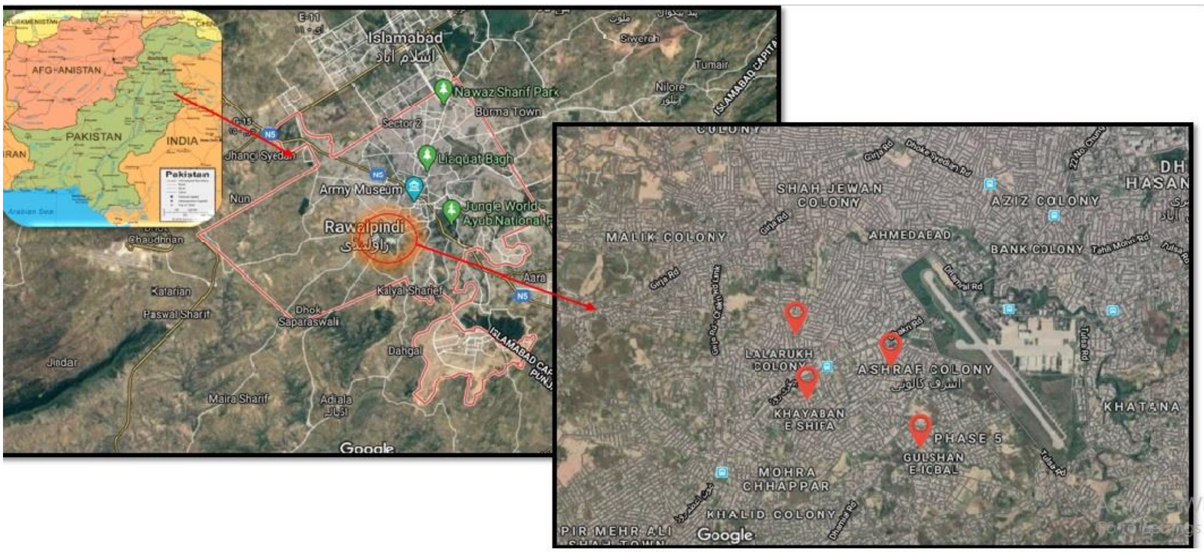


Figure 1: map of targeted areas (Gulshan E Iqbal, Khayaban E Shifa, Ashraf Colony and Lalarukh Colony)

Table: 1 Physico-chemical parameters

Concrete characteristics			**Synthetic characteristics**		
Soil Property	**Unit**	**Analysis Method**	**Soil Property**	**Unit**	**Analysis Method**
Humidity content	%	Drying in oven (Reeb and Milota, 1999)	chloride ion (cl)	Mg/100gm	Titration (International Standard ISO)
Consistency of the soil	-	Robinson's pipette (Jackson and Saeger, 1935)	Potassium (K)	Kg/ha	Flame photometer (Jankowski and Freiser, 1961)
Bulk	Gm/cm^3	Core sampling (Stone,1991)	Phosphorous (P)	Kg/ha	Spectrophotometer (Ganesh et al., 2012)
			Nitrogen (N)	Kg/ha	Micro kjeldhal (Bremner, 1960)
			pH/POH	-	Standard method

Fluoride (f) ion Approximation: The collected samples were air dried and chopped into fine particles by crushing. All large items like stones and other hard practical were removed. And the remaining samples were filtered for the small pebble. The fine homogenate, soil was disintegrated by the blending with NaOH a strong base in the crucible (temperature 6000C for 20 mints). Then using the deionized water samples was dissolved. After that Total Ionic Strength Adjustment Buffer TISAB was supplemented to prevent complex formations and maintain suitable ionic strength. Selective electrode procedure was adopted to evaluate the fluoride ion concentration (Zhang et al., 2010).

Separation of Bacterial Isolate: Serial dilution method was followed to isolate the bacterial fauna from the soil. One (1) gram of the fine clear soil sample suspended in the 10 ml of distilled water (diluted 10^{-1}) and then vortexed. And then further diluted to 10 $^{-6.}$ Now for final isolation, spread plate procedure was used and then for 24 hours the sample were incubate at 37 C. colonies that appear prominent was subjected to further analysis.

Classification and Description of prokaryotes: By using the 100 X microscope the size and shape of the bacteria were determined. Further following the procedures of the suggested by Rachel Watson the isolates were identified.

Statistical Analysis of the data: ANOVA was performed by using SPSS for all of the findings. Results are shown in the current research as mean and standard error.

FINDINGS

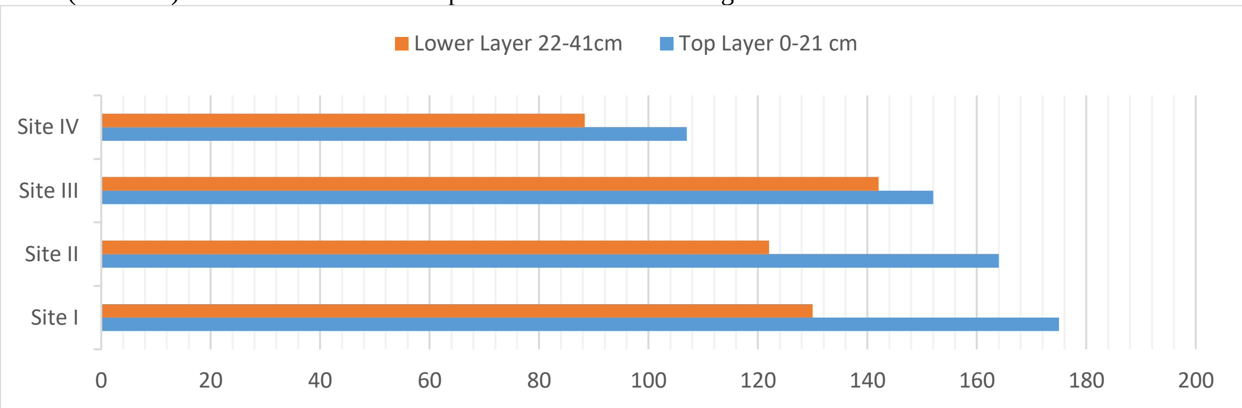
Physiochemical characteristics of the soil: pH range from 7.5 to 8.1. Humidity content was 3.07 to 13.20%. Soil texture is Deep brown loamy in all sites except site 4 which appears as rock red. Besides this carbon and organic compounds ranges in 0.311 to 0.948 and 0.517 to 1.63 respectively. Apart from this the most crucial parameter Nitrogen range from 95.2 to 140.7, Phosphorous ranges from 45.43 to 131.4 and in the last potassium 119.6 to 510. Findings detailed are summarized in the table 3.1 other than fluoride ion.

Table 2. Physiochemical findings of the collected soil samples of Rawalpindi agriculture land

	:Physical			Chemical						
Specimens locations	**Soil Consistency**	**Bulk Density**	**Moisture %**	**pH**	**Cl**	**% C**	**% OM**	**N**	**P**	**K**
I	Deep brown loamy	1.51±0 . 21	9.3	7.6± 0.01	5.39± 0.916	0.311 ± 0.029	0.517± 0.092	95.2± 12.08	131.4 ± 20.81	378.2± 9.35
II	Deep brown loamy	0.88± 0.08	13.20	7.5± 0.24	11.65± 0.492	0.842 ± 0.112	1.448± 0.261	100.0 ± 54.62	45.43 ± 34.66	510.8± 17.99
III	Medium brown loamy	1.21± 0.54	3.08	8.1± 0.14	15.91± 1.98	0.948 ± 0.154	1.634± 0.385	131.2 ± 38.29	122.3 ± 25.04	322.1± 12.58
IV	Rock red	1.64± 0.29	8.9	7.7± 0.23	4.01± 2.045	0.435 ± 0.023	0.749± 0.095	140.7 ± 47.24	120.4 ± 15.54	119.6± 29.02

2. Fluoride ion

Finding revealed that the upper layer 0-21cm is more concentrated with the fluoride ion as compared to the lower (21-41cm). Detail of the result is depicted in the table2 and figure 2.



Specimens locations	Soil fluoride ion (part per meter) (Mean ± SD)		p-value
	0-21 cm	22-41 cm	
I	175 ± 0.5	130 ± 1	0.001*
II	164 ± 0.5	122 ± 1	0.001*
III	152 ± 0.59	142 ± 0.5	0.001*
IV	107.57 ± 0.59	88.32 ± 0.57	0.001*

Bacterial Isolates and identification: Total eight colonies (2 from each site) were selected for the further projection of the experimental work. Result determined that total 6 samples were found gram positive while 2 samples were gram negative. Besides this *Arthrobacter* the gram positive bacteria were the most dominant species. Detail is given in the figure 2 and table 3.

	Sampling Location I		Sampling Location II		Sampling Location III		Sampling Location IV	
Prominent colony ID	**Pro-C I**	**Pro-C II**	**Pro-C III**	**Pro-C IV**	**Pro-C V**	**Pro-CVI**	**Pro-C VII**	**Pro-C VIII**
Strain identified	*Actinomyces israeli*	***Arthrobacter***	***Arthrobacter***	***Arthrobacter***	***Arthrobacter***	*Bacillus nealsoni*	*Azotobacter*	Bacillus stratosphericus
examination	Rod	Rod	Rod	Rod	Rod	circular	Spherical	Rod
appearance	smooth/dull	Rough	rough/ dull	rough/ dull	rough/dull	smooth/ shiny	Smooth	rough/dull
staining	+	+	+	+	+	+	-	+
Biochemical tests								
Oxidase	W	-	-	+	+	-	-	+
Catalase	-	+	+	+	+	+	-	-
2-nitrophenyl-ß Dgalactopyranoside	+	+	+	+	+	+	+	+
L-arginine	+	-	-	-	-	-	+	+
L-lysine	+	-	-	-	-	-	+	+
L-ornithine	+	-	-	-	-	-	+	+
Trisodium citrate	+	-	-	-	-	-	+	+
H_2S production	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-
Indole production	+	-	-	+	+	-	+	+
Acetoin production	+	-	-	_	_	-	+	+
Gelatinase	+	-	-	+	+	-	+	+
D-glucose	-	+	+	-	-	+	-	-
D-mannitol	-	+	+	+	+	+	-	-
Inositol	-	+	+	+	+	+	-	-
L-rhamnose	-	-	-	-	-	-	-	-
D-sucrose	+	-	-	-	-	-	+	+
D-melibiose	-	-	-	-	-	-	-	-
L-arabinose	+	+	+	-	-	+	+	+
NO_2 production	+	+	+	+	+	+	+	+
Reduction to N_2 gas	+	-	-	-	-	-	-	-

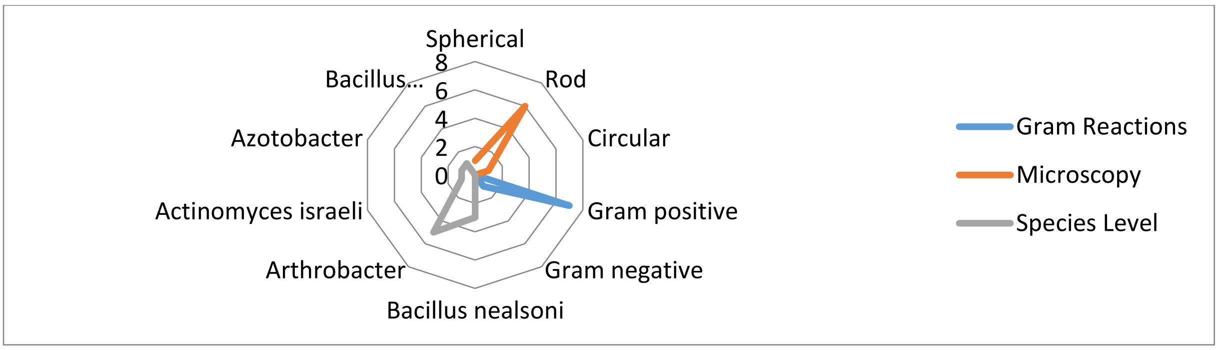
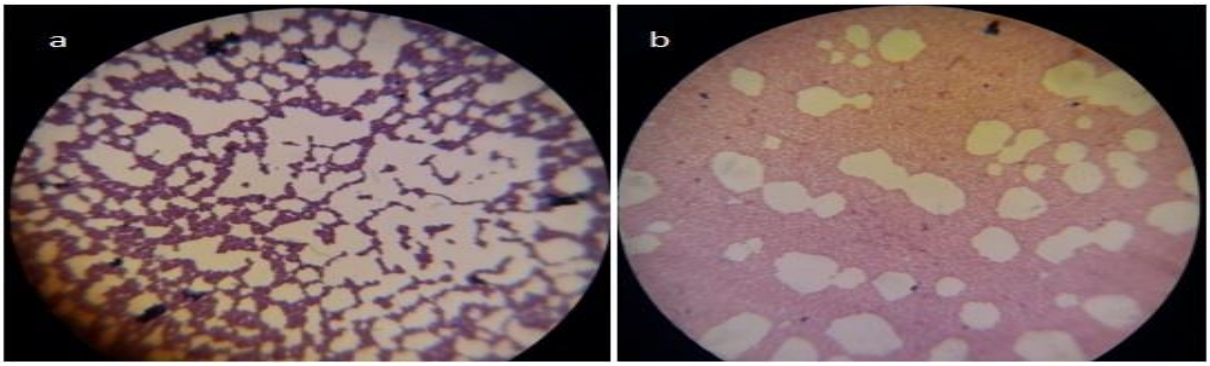


Figure 3: detail of the identified bacteria Figure Gram staining; Gram Positive (a), gram negative (b)



DISCUSSION

Soil is considered crucial factors for primary energy source in the ecosystem. And many researches have been carried out to improve the soil fertility and enhance the production. For the better production availability of the microbes and nutrient is very essential. pH at both extremes low or high can directly affect the nutrient availability for the plants, which in turn effect the plant growth. Lack of nutrient not only affect the plant growth but can also effect the activities of the beneficial microbes present in the soil ecosystem (Dora, 2019). Current result revealed that pH in all four sites range from 7.4 to 8.1 (moderately alkaline). Which is totally an unfavorable condition because slightly low pH can favors the availability of all essential nutrients thus promoting plant growth (Lončarić et al., 2008; Dora, 2019; Pushpa et al., 2015).

Soil texture can influence the water retention capacity, soil erosion and nutrient storage for example sandy texture cannot hold water for longer period even though it can recharge so easily and fast. Soil with heavy texture can hold water for longer due to availability of the small pores (Hewitt, 2004). Current study revealed that soil that has dark brown loamy texture has higher amount of the humidity while rock red soil has greater concentration of the N:P:K. potassium play a vital role in the plant growth and major activities of the plant body like cell division cell permeability, defense, and starch formation apart from this it also control many enzyme activity. It also aid in the closing and opening of the stomata with the help of chlorine ion (Sardans and Penuelas, 2015; Prajapati and Modi, 2012). Besides this Cl also helps in neutralizing the ions, current study concluded that location 3 has higher concentration of chlorine ion.

Fluoride ion was more abundant in the upper layer of the depth (0-21 cm) as compared to the deeper layer (22-41cm). The availability of the higher concentration of the fluoride ion in the upper layer might be possible because of Al $(OH)_3$, O_2_Atom and different silicate mixtures present in the top layer which retain the concentration of the fluoride ion in the top layer (Luther et al., 1996). Concentration of the fluoride ion in soil means the fertility of the soil is low, because it locks the entire available nutrient in the soil in the form of complexes which can lower the production of the crops (Zhu et all. 2007). Fluoride itself is unavailable for the plant due to its undissolved nature. But the availability of the clay and slight acidic environment can alter its solubility and make it available for the plant uptake. Fluoride ion concentration in current research work is below from the 200-300 ppm which is an optimal level suggested by WHO. But due to rapid industrialization and using pesticides containing F ion in the selected areas can increase the fluoride ions concentration which can prove as a toxic material for both plant and animals. Maintaining the range of fluoride in optimum is a challenging job because ability of the topsoil to retain the F ion always change with the progression of time. Apart from this highly acidic soil can promote fluorine ion leakage to the underground water, making it highly polluted which can in turn develop many serious health concerns. It can also cause toxicity in the plants (Mezghani et al., 2005).

Apart from the nutrient and minerals microorganism is also very important factor in the determining the soil fertility, they can influence the fertility directly or some time indirectly. Oftenly fertile soil comprises many type of microbes like fungi, bacteria etc. bacteria are the group of single cell microscopic prokaryotes that performed main part in ecosystems functions. Some of these bacteria are the decomposer while some of them change the nutrient from for livings organism (Walter, 1959).

In this experimental work we isolate 5 diverse species of bacteria which was dominates by Gram + ***Arthrobacter*** bacteria. One Gram – species of bacteria were also isolated that is *Azotobacter*. *Azotobacter* is able to survive in the soil without host plant body and can play a major role of nitrogen fixation. Besides this the other gram positive bacteria *Actinomyces israeli* belongs to the family phylum Actinobacteria are well-known for its enzymes which help in the degradation of the lignin, chitin and many organic compounds like plant material. Apart from this many other strains were also identified like Bacillus nelsoni, ***Arthrobacter*** and Bacillus stratsphericus. Genus Bacillus performed numerous dynamic roles in the environment functions like plant growth promoter as they produce phytohormones which act as immune stimulator for plants against many infections. Besides this they also help in nutrient cycling. Despites these important functions these species are exploited in agro-biotechnology sector (Krid et al., 2012; Radhakrishnan et al., 2017).

CONCLUSION

Upper layer of the depth 0-41 is more concentrated with Fluoride ions. In which location 1 is being more concentrated than any other location. pH should be maintained in slightly lower which can favor the availability of the nutrient. Though all other parameters were in their optimal level but still fertilizer can enhance the crop production. Special concentration should be given to the fluoride ion as increase level of fluoride ion can developed severe health concerns.

AUTHORS' CONTRIBUTIONS

Conceived, designed and performed the experiment; H Ilahi, Data analysis; Jehangir Ahmad, Ilahi and Fazli Wahid. Materials and Methods; M Azeem, Ijaz Amin, Muhammad Adnan & H Amin, Reviewed and wrote the paper; Rafiullah & H Ilahi.

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