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IMPACT OF SEWAGE ON RHIZOSPHERE AFFECTING THE MORPHOLOGY AND GRAIN PRODUCTION IN *PISUM SATIVUM*

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Abstract

The study aimed to determine the impact of sewage on rhizosphere affecting the morphology and grain production in *Pisum sativum*. For this purpose different types of sewage were collected from different part of the Varanasi city.

The sewage of industrial effluent used for mixing in the field of pulse crop had an adverse effect on plant growth and productivity due to their toxic effect. But the sewage of domestic effluent brings done their toxic effect on the pulse crop. this indicated that the sewage of domestic effluent is suitable for the mixing in field of pulse crop.

Key words : Pisum sativum, morphology, domestic effluent, pulse crop.

Introduction

Soil is a mosaic of microbial population, which is governed by many factors. Growing plant roots are a major factor influencing the microbial population by secreting organic substance. Sloughed of tissues serves directly as nutrient, source for micro organism. The rhizosphere is quite distinct from the soil which is not influenced by the growing root and characterized by increased microbial population.

The plant roots affect the microbial population and the microorganism influences the plant growth. This mutual effect is changed by many factors, such as age of the plants, plant species, light, availability of oxygen, CO_2, moisture, pH organic matter etc.

Right from the earlier ages the relationship that exist between plant roots and the soil where the roots are anchored had been a matter of great importance. The geo-chemical or physico-chemical interaction between the roots and soil has been a topic of wide study in the early and middle of nineteenth century. It took almost a century for soil scientists to realize the fact that a part from the purely physico-chemical relationship, a very strong biological phenomenon also exist in the soil as a whole and also in the soil which is very close to the root system soil microbes adhere to the surface of the plant root so as to form a mantle and many types of interactions exist amidst them and with the plant roots. Rhizosphere soil can be defined as that part of the soil in which microbial activity is modified by the presence of root but in which the microbial environment is mostly at soil origin.

The higher amount of organic substances present in sewage improves the physico chemical qualities of soil. Numerous scientists proved sewage irrigation as a very successful irrigational facility and studied its impact on various physico-chemical properties of soil. Wichelns *et al*. (1987), Jadheo *et al*. (1992), Cakmack & Marschner (1993), Namje (1995), Cheng (1996), Wang *et al*. (1997), Gulati and Lenka (1999), Pandey *et al*. (2002), Cherki *et al*. (2002) and Chen *et al*. (2005) have studied the impact of sewage irrigation on properties of soil. Although, the soil component of agro ecosystem has the ability to assimilate certain amount of waste materials, the successive addition of domestic waste in large quantities or over long period above its absorption capacity results into a series of edaphic problems and ultimately make soil unproductive by drastic change in its physico-chemical and biological properties (Dotson, 1973; Terman *et al*., 1973).

The disposal of domestic waste as sewage is a problem being faced by Municipalities throughout the world. As steps are taken to maintain and improve the quality of surface water, the quantities of sewage continue to increase and Municipalities are confronted with an urgent need to develop safe and feasible alternative practice for sewage management. Sewage treatment itself which presumably could accommodate increasing quantities of sewage is agricultural utilization. Its organic matter content, which constitutes 40 to 50 per cent of the solid fraction, is known to improve the physico-chemical properties of soil. Nitrogen and phosphorus ranging from 1 to 8 and 0.5 to 4 per cent, respectively in sludge's are nutrients, essential for growth (Mays *et al*., 1973). However, Singh (1982), Page *et al*. (1987), Gulbahar *et al*. (2005) have reported that their disposal over prolonged period may contaminate the soil environment by their enrichment with heavy metals which are potentially harmful to plants and their consumers.

Many workers have established that rhizosphere effect is due to the secretion of nutrients by roots. Roots secrete many organic and inorganic substances, which directly serve as food for the micro-organisms. These root secretions also remove the wide spread fungistasis in soil and induce spore germination and development of mycelium. Microbial community assembly in the rhizosphere is governed by abiotic and biotic factors (de Ridder-Duine *et al*., 2005; Santos Gonzalez *et al*., 2011). Many studies have demonstrated that soil has a profound influence on the assembly of bacterial and micorrhizal fungal communities in the rhizosphere (Andrew *et al*., 2012; Inceoglu *et al*., 2012).

Rhizosphere microflora have so far been studied with three viewpoints *viz*. (i) Effect of plants on micro-organisms, (ii) Effect of micro-organisms on plant growth and (iii) Interaction amongst microbes in the rhizosphere. The aspects (ii) and (iii) are very little investigated. Bowen and Rovira (1961) reported that micro-organisms affected the seed germination and seedling growth.

The age as a factor influencing the rhizosphere microflora is known from many earlier workers, but the real cause is not clearly understood. Many workers established the correlation between the changes in bacterial population and root excretions but this does not seem to apply with rhizosphere fungi. The foliar application of different chemicals and the study of their effect on the rhizosphre and rhizosphere microflora is little investigated. The foliar application of different chemicals is a good device to change the microbial population in the rhizosphere, which may serve to control root diseases.

Rhizobial cells are stimulated more around the roots of legumes than those of non legumes (Brown, 1961; Rovira, 1961; Banerjee *et al*., 1997). The individual strains of rhizobia are more vigorously stimulated by such hosts which they are able to infect than the rest of the legumes (Wilson, 1930).

Rhizospheric micro-organism depends not only on the level of plant uptake of water and nutrients, but also on the nutrient supply and distance from the root surface. In many studies, it was showed that the ions of essential elements either accumulate or decrease across the rhizosphere.

Materials and Methods

The study was carried out during first week of the month from July 2016 to June 2017 between 8 A.M. to 10 A.M. Samples of different sites of sewage water were collected at monthly intervals. Sewage samples were collected in ten replicates from each of the sewage site in clean plastic containers using standard methods of collection.

The temperature, transparency and pH were analysed at the site. Samples were brought to the laboratory and kept in preservator at 40ºC for further analysis of various physico-chemical parameters in alkalinity, nitrate, phosphate, dissolved oxygen (DO) biochemical oxygen demand (BOD), Chemical oxygen demand (COD) Calcium and Potassium water samples collected in different sites were analysed for various physico-chemical parameters.

The analysis of the collected samples of different sewage sites were studied by the methods described in standard methods for the analysis of water and waste water, APHA (2005), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Temperature was measured at the site using a Celsius thermometer. An electronic pH meter (CK-701) to the accuracy of 0.05 was used for the measurement of pH. The pH meter was standardized with stock buffers before each reading.

Heavy metals analysis in sewage samples

Only three heavy metals have been taken into account *viz*. Chromium, Nickel and Magnesium in present investigation atomic absorption spectroscopy method was used. However few problems are encountered in the determination of trace concentrations of element in dilute aqueous solutions by atomic absorption spectroscopy.

Soil sampling and analysis

Soil samples were collected from the surface upto 30 cm depth.

Table 1 : Physico-chemical characteristics of collected sewage.

Parameter	Domestic Sewage I	Sewage of Carpet Indu. Site II	Sewage of Dairy Indu. Site III	Sewage of Paper Indu. Site IV	Sewage of Refinery Indu. Site V
Temperature (ºC)	28.28±1.31	26.32±1.05	26.20±1.62	28.32±1.34	27.38±1.78
pH	7.5±0.34	8.2±0.13	6.90±0.10	8.2±0.16	7.8±0.18
TDS	2875±21.36	2060±5.81	1700±11.78	3324±45.41	3052±36.08
TSS	945±7.34	762±5.81	285±2.62	907±6.78	1132±7.50
BOD (mg/L)	240±2.32	2158.34±11.41	1046.48±5.47	1200±7.06	125.31±2.34
COD (mg/L)	408.72±2.41	1.01±0.09	1952.3±9.05	2720±10.47	2031±8.42
DO (mg/L)	1.7±0.13	1.02±0.13	0.80±0.10	0.51±0.15	0.76±0.12
Phosphate (mg/L)	3.42±0.80	46.36±1.81	6.84±0.24	1.20±0.13	1.78±0.15
Nitrogen (mg/L)	36.74±2.24	9.03±0.26	62.30±2.63	3.42±0.89	10.47±0.77
Potassium (mg/L)	2.59±0.41	102.0±2.10	1.38±0.34	1.34±0.61	1.21±0.45
Chloride (mg/L)	29.90±1.42	91.41±2.00	70.34±1.03	10.45±1.00	7.84±0.81
Sulphate (mg/L)	32.65±1.08	91.41±2.00	86.52±1.86	12.34±1.31	27.31±1.89
Calcium (mg/L)	42.83±1.16	47.62±1.20	12.41±0.84	9.41±0.29	15.38±0.75
Magnesium (mg/L)	7.36±0.48	15.27±0.31	2.52±0.14	1.25±0.16	2.01±0.41
Chromium (mg/L)	2.36±0.81	15.82±1.02	-	0.21±0.02	0.35±0.01
Nickel (mg/L)	3.27±0.12	0.405±0.10	-	0.27±0.01	0.18±0.01

± Standard Error.

Table 2 : Biological characteristics in Isolated Rhizospheric soil of *Pisum sativum* under different pollution stress.

Biological Parameter	Number of species per ml. in different type sewage				
	Domestic Sewage I	Sewage of Carpet Indu. Site II	Sewage of Dairy Indu. Site III	Sewage of Paper Indu. Site IV	Sewage of Refinery Indu. Site V
Bacterial (per ml. of sewage)					
Escherichia coli	2258±61	1562±52	3805±101	2781±71	1027±25
Streptococcus faecolis	1807±41	1131±28	2485±68	1223±18	729±11
Clostridium perfringens	976±10	432±12	806±14	657±9	320±04
Pseudomonas	589±12	420±8	1528±27	431±10	965±12
Micrococci	982±13	515±9	1072±15	785±13	407±11
Fungi (per ml. of sewage)					
Achlya sp.	78±1.34	-	25±1.0	29±1.08	12±1.06
Saprolegnia sp.	39±1.31	5±0.81	41±1.31	15±0.91	-
Yeast	107±1.31	-	46±1.31	36±1.45	96±2.01
Pythium sp.	52±2.03	16±1.49	25±1.34	21±1.36	8±0.89
Allomyces sp.	33±1.34	-	20±1.0	14±1.36	-
Algae (per ml. of sewage)					
Chlorella	10±0.81	2±0.13	5±0.23	8±0.41	-
Eudorina	12±0.48	10±0.48	4±0.21	10±0.41	4±0.41
Volvox	17±1.31	10±1.01	11±0.98	15±1.10	8±0.9
Anabaena	05±0.34	2±0.42	-	-	-
Cosmarium	15±1.31	-	10±1.32	11±1.34	14±1.05
Scenedesmus	18±1.34	-	7±1.0	9±1.21	10±0.98
Microsystis	10±1.31	6±1.01	11±1.31	3±0.76	5±0.51
Pinnularia	13±1.02	4±0.08	10±0.87	11±0.97	6±1.0
Cyclotella	8±0.97	4±0.81	-	13±1.31	-
Navicula	7±1.31	5±0.72	6±0.41	2±0.36	-

The method of separating the soil particles into groups in known as mechanical analysis *i.e.* percentage ratio of different size particles was done by Robinson Pipette Method (Piper, 1966).

Analysis of rhizosphere

Operationally, the rhizosphere can be defined as the region, extending a few millimetres from the surface of each root, where the microbial population of the soil is influenced by the chemical activities of the plant.

The no. of bacteria in the rhizosphere usually exceeds the numbers in the neighbouring soil by a factor of 10 and often by a factor of several hundred. The rhizosphere to soil ratio (R : S) collect rhizospheres effect can be calculated by dividing the number of micro-organisms in the rhizosphere soil by the number in the soil free from plant growth greater rhisophere effects is seen with bacteria (R : S values from 10 to 100 or sometimes more) than with Actinomycetes and fungi white regard to protozoa and algae there are negligible change. The rhizosphere effect increase with the age of the plant and normally reached its maximum at the stage of greater vegetative growth following the death of the plant, the microbial population reverted gradually to the level of that in the surrounding soil.

Growth characteristics

The individuals of pulse crops were picked out at regular interval of 30 days and different morphological characteristics were analysed such as plant height weight of shoot, amount of leaves length of roots, amount of flowers, and amount of pods, mean fruit weight, production of fruit etc.

Results and Discussion

In the present study different type of sewage were collected from different part of the Varanasi city *i.e.* domestic sewage (site-I), sewage of carpet industry (site-II), sewage of dairy industries (site-III), sewage of paper industries (site-IV) and sewage of Refinery industry (site-V).

Several authors have studied the physical chemical properties of sewage water with reference to irrigation (Devi, 1991; Garg, 1998).

In the study, it was observed that there were variations in different type of sewage at different level.

The physico-chemical characteristics of natural field soil of *Pisum sativum* growing field at different pollution stress were recorded and found with increasing concentration of all the analysed parameters with increasing the pollution load. The mechanical composition

Table 3 : Physico-chemical characteristics of experimental soil before mixing of collected sewage.

Physico-chemical parameters	**Experimental soil**
Mechanical composition (%)	
Sand	58.73±2.34
Clay	20.58±1.64
Silt	27.36±1.02
Colour (Dry)	Light grey (dry), Olive brown (wet)
Texture class	Sandy Loom
Soil moisture (%)	19.35±0.97
Water holding capacity (%)	42.64±1.36
pH	7.3±0.80
Potassium (mg/g)	0.69±0.02
Nitrogen (mg/g)	0.05±0.001
Phosphorus (mg/g)	0.06±0.005
Sodium (mg/g)	0.57±0.03
Calcium (mg/g)	2.13±0.16

± Standard Error.

(sand, clay and silt particles) showed the sand particles found in higher concentration that of silt and clay particles. The colour of natural field soil was found light grey at site I and brownish black at site IV. The texture of all the sites found similar as sandy loams. The soil moisture content was reported higher at site IV and minimum at site I. The nutrient concentrations were reported maximum at site III and minimum at site I.

The physico-chemical analysis of the collected sewage effluent revealed that the temperature was reported to be maximum in the sewage collected from site IV and run parallel to the atmospheric temperature. The maximum concentration of dissolved oxygen was reported at site I and it decreases at site II, III, V and IV, respectively. This occurs due to increasing amount of microbial activity for the decomposition of organic matter present in the domestic sewage effluents. In decomposition process most of the oxygen, which is dissolved in the effluent water, was consumed by the microbial activity. Due to this region the biochemical oxygen demand and chemical oxygen demand gradually increase in collected sewage effluent from site I to site IV, II and III respectively. The other chemical constituents such as nitrate, phosphate, potassium, chloride, calcium, sodium and alkalinity were reported to be maximum in the sewage effluent collected from site II followed by site III and site I respectively. This occurs due to high

Table 4 : Physico-chemical properties of experimental soil after mixing the collected sewage.

Parameter	**Domestic Sewage Pot set I**	**Sewage of Carpet Indu. Pot set II**	**Sewage of Dairy Indu. Pot set III**	**Sewage of Paper Indu. Pot set IV**	**Sewage of Refinery Indu. Pot set V**
Mechanical composition (%)					
Sand	57.32±1.34	57.58±1.20	58.34±1.62	58.62±1.37	57.51±1.05
Clay	21.25±1.52	21.10±1.58	21.72±1.06	21.41±1.38	21.0±1.52
Silt	28.37±1.71	27.89±0.34	28.41±1.26	27.62±1.09	27.58±1.06
Colour (Dry)	Olive Brown	Olive Brown	Olive Brown	Olive Brown	Olive Brown
Texture class	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Soil moisture (%)	24.34±2.31	22.34±1.62	25.38±1.05	24.31±1.36	23.13±1.23
Water holding capacity (%)	42.06±1.06	41.67±1.58	43.73±2.34	41.98±	42.01±1.05
pH	7.4±0.86	7.8±0.25	7.51±0.62	7.9±0.51	7.5±0.38
Potassium (mg/g)	1.65±0.16	1.87±0.08	1.20±0.12	0.97±0.02	0.83±0.03
Nitrogen (mg/g)	0.85±0.01	1.01±0.05	1.10±0.06	0.72±0.02	0.58±0.01
Phosphorus (mg/g)	1.01±0.02	0.48±0.01	1.32±0.03	0.54±0.02	0.65±0.01
Sodium (mg/g)	0.59±0.01	0.49±0.03	0.60±0.04	0.51±0.01	0.46±0.06
Calcium (mg/g)	3.51±0.02	3.67±0.11	2.23±0.12	3.08±0.08	2.87±0.10

discharge of these elements from household effluent and industrial wastes. All the analysed heavy metals i.e. magnesium, chromium and nickel were reported as maximum in the sewage effluent collected from site II. This indicated a high discharge of these metals from carpet industries of that site.

In the biological analysis, it is clear all the analysed microbes in the rhizospheric soils of *Pisum sativum* was reported maximum were a medium concentration of toxic metal was found and they are also found in increasing trend with the plant age and at the time of harvesting. The number of microbes in the rhizospheric soil of *Pisum sativum* the pulse crops was found in decreasing trend.

Similar results had also been reported in the analysis of experimental soil. This is due to the mixing of collected sewage in the experimental soil. After mixing of sewage sample, the all physico-chemical parameters and heavy metals of experimental soil changed with the physico-chemical parameters and heavy metals of the sewage effluents due to sedimentation of the chemical elements, which are dissolved in the sewage effluent.

The culture experiments reveals that, almost all growth characters *i.e.* shoot length, root length, fresh weight and dry weight of shoot and root, number of leaves, amount of fruits, diameter of fruit, biochemical content *i.e.* sugar, protein and nutrient uptake the pulse crop *i.e. Pisum sativum*. Further it decreases with decrease in the nutrient concentration of experimental soil. Thus the pulse crops *Pisum sativum* may show a good performance of growth under domestic sewage mixed soil, which also enhanced the maximum growth of rhizospheric microorganisms and the soil in which industrial sewage were mixed shows decreasing trend or adverse effect on the growth parameters.

The sewage of industrial effluent used for mixing in the field of pulse crop had an adverse effect on plant growth and productivity due to their toxic effect. But the sewage of domestic effluent brings down their toxic effect on the pulse crop. This indicated that the sewage of domestic effluent is suitable for the mixing in field of pulse crop. The present investigation approves the beneficial effects of domestic sewage which is free from toxic chemical, which ultimately enhance the growth of rhizospheric microorganism.

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