Characterization of rhizospheric soil of *Abelmoschus esculentus* (L.) Moench grown in paper mill effluent infested area

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ABSTRACT

Soil pollution can be defined as the phenomenon in which the harmful chemicals and wastes mix directly or indirectly into the soil and it starts affecting the soil-plant microbes' interactions. This results into soil contamination and if the situation remains continued the soil gets degraded and ultimately becomes sterile. Among different soil pollutants, industrial wastes and effluents generated from the industrial processing contain severe toxins which are disposed off in most of the cases in very precarious ways. Generally, the liquid toxic effluents are disposed into the environment without proper treatment and they get easily fallen into the agricultural land through drains. Among different industrial effluents, paper mill effluents are dangerous as it contains heavy metal elements and several disease-causing chemicals which have drastic negative impact on human as well as on plant health. So, it is necessary to analyse the properties of soil samples collected from different industrial zone and then it can help us to identify the amount of toxic materials present in that zone. The current work focuses on the characterization of rhizospheric soil samples collected from Abelmoschus esculentus (L.) Moench plants grown in papermill effluent infested area of Nadia, W.B.

Key Words: *industrial waste, heavy metal, rhizosphere, soil pollution, effluent*

Introduction:

Soil is the uppermost layer of earth's crust and it is made up of minerals, organic material and different organisms which all together support the total living system (Asema et al, 2015). Soil is the mother bed of beneficial as well as harmful microorganisms and the ecological balance is maintained by their active participation in the biogeochemical cycling in environment (Talwar and Chatli, 2018). The physicochemical analysis of soil samples denotes the properties and qualities of soils. The rhizospheric microbes play an important role in the availability of different mineral nutrients in the soil and they obviously help in maintaining the balance of soil ecosystem. The rhizospheric microorganisms particularly PGPR i.e. Plant Growth promoting Rhizobacteria stimulate plant growth by direct as well as indirect mechanisms.
PGPR stimulates the secretion of IAA, production of HCN, solubilization of phosphate, production of siderophore and controlling of seed-borne pathogens etc. (Roy Chowdhury, 2020). So, from this point of view it is clear that the rhizospheric microorganisms play a vital role in plant's growth and hence the determination of rhizospheric soil's quality is necessary.

Materials and Methods:

- ***Collection of soil:***

Rhizospheric soil samples of *Abelmoschus esculentus* (L.) Moench plants were collected from different paper mill effluent infested areas of W.B. During collection the upper 10 cm. soil layers were discarded and then the rhizospheric bulk soil was collected by following the shaking procedure and subsequent brushing of rhizospheric zone of plants. Total 6 soil samples were collected from the rhizospheric zone of *Abelmoschus esculentus* plants grown in different field in that area. After that those six soil samples were mixed together properly and were stored aseptically in sterile polythene bags. Then those packets were kept in aseptic condition as far as possible for further use.

- ***Characterization of soil samples:***

The characterization of the collected rhizospheric soil sample was done on the basis of three parameters- p^H, temperature, and total moisture content.

- ***Determination of total moisture content of soil:***

The total moisture content determination was done following Rowell's method (Rowell, 1995). It was measured as % moisture on weight basis. 10 gm rhizospheric soil sample was taken in a clean 100 ml. glass beaker and was properly dried on an oven at 60 °C temperature for consecutive 2 days. The data regarding dried soil sample's weight was recorded and the total moisture content of soil sample was determined by following equation,

$$\text{Moisture content (M \%)} = W_1\text{-}W_2/W_1*100$$

Where,

W_1= Initial weight of soil sample

W_2= Final weight of soil sample

- ***Determination of p^H of soil:***

Determination of p^H of collected soil sample was crucially done by using p^H meter (Systronics, model no.361). In a 100 ml. glass beaker, 10g rhizospheric soil sample was collected and then about 50 ml. distilled water was added. After that the beaker was kept on a magnetic stirrer for 30 minutes. After that period the total content was allowed to settle down and then the p^H was recorded.

- ***Determination of temperature of soil:***

Determination of p^H of collected soil samples was done by using the Soil Probe Thermometer (Model: Luster Leaf 1618 Rapitest Soil thermometer). The thermometer was dipped into the soil sample for about 15 cm depth and the data was recorded when the mercury level got stabilized.

- ***Chemical analysis of soil sample:***

1. ***Organic Carbon (C):***

1 gm sieved soil sample was collected in a clean, dry conical flask and 10 ml. of potassium di chromate and 20 ml. sulphuric acid (conc.) were added followed by continuous shaking. After that the whole solution was kept in stable condition for 30 mins for occurring of the complete reaction. After certain period, 200 ml. distilled water was poured to the conical flask for dilution. Thereafter 10 ml. orthophosphoric acid and 1 ml. o-phenanthroline indicator was mixed. A dark blue colouration came. Then this solution was titrated against freshly prepared 0.5 N ferrous ammonium sulphate till the colour turns into bright green. Organic matter present in the sample gets oxidized by the reaction with mixture of potassium dichromate and concentrated sulphuric acid. The extra potassium dichromate which doesn't get reduced by the organic material of the sample, is analysed by the titration method applying standard ferrous ammonium sulphate solution along with orthophosphoric acid using o-phenanthroline indicator. A blank (without soil) set was also continued following the similar steps. The % of organic carbon in soil sample was determined by using the following formula:

$$\text{Organic carbon (\%)} = \frac{N \times (X-Y) \times 0.003 \times 100}{W}$$

Where, N = normality of ferrous ammonium sulphate

W = gm of soil sample collected

X = the volume (ml) of 0.5 N ferrous ammonium sulphate necessary for reduction of 10 ml potassium di chromate solution (blank reading)

Y = volume (ml) of 0.5 N ferrous ammonium sulphate necessary for reduction of the extra volume of chromate (sample reading).

2. ***Phosphorus (P):***

It was measured by following $NaHCO_3$ extraction method proposed by Olsen et al. (1982). At first 5 gm soil sample was taken in a conical flask and 100 ml 0.5 N $NaHCO_3$ (pH=8.5) was added into it. After that one teaspoon of carbon black was added. Then the whole solution was shaken vigorously for 45 min and then allowed to be stable for overnight. Then the solution was filtered and the filtrate was used for phosphorus estimation. At first 5 ml filtrate was taken in a 25 ml. volumetric flask, 5 ml. ammonium molybdate and 1 ml

stannous chloride were added into it. The volume was maintained up to 25 ml with addition of distilled water. The optical density (O.D.) of the solution was recorded at 660 nm using UV-VIS spectrophotometer (Model-119, Systronics, India). A blank set was also run following the above-mentioned procedure but it was without soil sample and the O.D. value was recorded at similar wavelength. The total content of phosphorus was calculated by the following formula:

$$\text{Available Phosphorus (mg /kg)} = \frac{C \times V}{v \times w}$$

Where, C= concentration of Phosphorus as calculated from the standard curve
V= final volume of extractant (ml)
v = volume of aliquot (ml)
w = weight (g) of soil sample analysed.

3. Potassium (K):

Exchangeable potassium ion (K^+) in soil was extracted in ammonium acetate following the repeated leaching methodology and after that the total concentrations were measured with the aid of Atomic Absorption Spectrophotometer (Model 2380, Perkin Elmer, USA).

4. Zinc (Zn) and Sulphur (S):

The amount of Zinc (Zn) was measured by using Atomic Absorption Spectrophotometer (AAS) (Ghaedi *et al*., 2013).

5. Boron (B):

Hot water extraction method is most popular method to estimate the total content of boron (B) in soil samples. The availability of boron in the test soil sample (1:2 soil: extractant, 0.02M CaCl2, 5 minutes boiling) was done by following the azomethine-H method, proposed by Gaines and Mitchell's (1979).

6. Electric Conductivity:

Conductivity meter (Model 303, Systronics, India) was used for the determination of the electric conductivity of soil sample.

7. Isolation of microorganisms from soil sample:

Isolation of microbial population from rhizospheric soil samples was done by conventional serial dilution method by pouring technique on culture media (Cappuccino and Sherman, 2007). For calculation of total microbial (bacterial) load in the samples, 1 ml. of each dilution was poured on previously sterilized Nutrient Agar plates. Then the plates were incubated at 28± 2°C for consecutive 2 days. For calculation of total microbial (fungal) load in the samples, 1 ml. of each dilution was poured on previously sterilized Rose Bengal Agar plates and were incubated following the same procedure. After the period, appearance of bacterial and fungal colonies were observed.

Results and discussion:

The data regarding the physical characterization of rhizospheric soil of *Abelmoschus esculentus* reveals that the soil colour was blackish and its pH was 8 (lower pH favours the growth of fungi and higher pH allows the growth of bacteria) and the moisture content of the sample was 51%.

Table 1: Physical characterization of collected rhizospheric soil samples:

Code of sample	Soil colour	Soil type	Soil smell	Soil p^H	Soil temperature (^{0} C)	Soil moisture (%)
A1	Blackish	Loamy	Normal	8	33	51

Table 2: Rhizospheric soil analysis report:

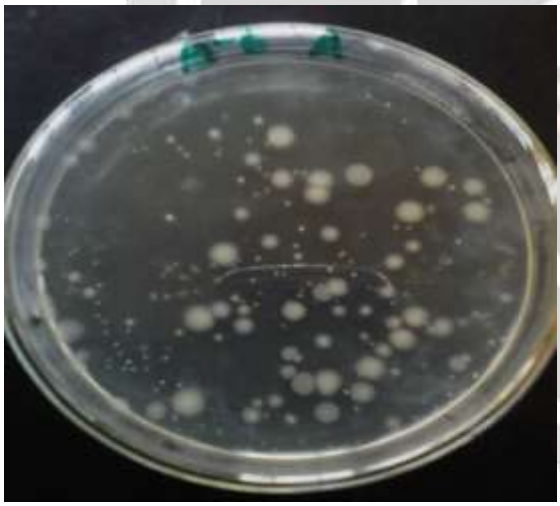
The Organic Carbon content was 0.70%, Phosphorus content was 24kg/ha and others are depicted below.

Sample Code	EC (ds/m)	Organic Carbon (C) (%)	Phosphorus (P) (kg/ha)	Potassium (K) (kg/ha)	Zinc (Zn) (ppm)	Boron (B) (ppm)
A1	0.35	0.70	24	146	0.80	0.18

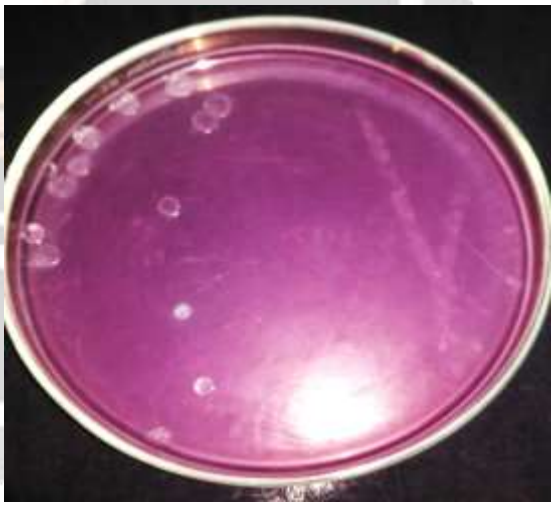
Table 3: Microbial community of soil samples:

Soil Sample	Medium used	CFU (x 10^6)		Colony characters
		Bacterial	Fungal	
A_1	Nutrient Agar medium for bacterial culture and Rose Bengal Agar medium for fungal culture.	47	05	Bacterial colonies are round, whitish, smooth. Fungal colonies are white, fluffy mycelial growth.

There was a diverse pattern of bacterial and fungal growth on NA media and Rose Bengal Agar media. Bacterial colonies were more in number as compared to fungal colonies. The bacterial colonies were more or less round, whitish in colour and of smooth margin. The fungal colonies were white and fluffy mycelial growth was formed.



Bacterial colonies grown on NA media



Fungal colonies grown on Rose Bengal Agar Media

Conclusion:

It is always necessary to determine and analyze the chemical characters of agricultural soil particularly in industrial area. The crops and vegetables grown in those agricultural land are consumed by human as well as by other animals. So, through food chain the chemical elements are entering into the living system. If the soils are properly analyzed and if the data regarding the chemical constituents (those constituents may come from industry or from pesticide/agrochemical's residue) are checked regularly then it becomes easy to control the soil pollution rate. In this study, the chemical constituents present in the soil were measured and the microbial load is also determined. From the result it is clear that the bacterial load is higher than the fungal growth and as the bacteria are isolated from the rhizospheric soil, it can be assumed that most of these bacteria will belong to the

PGPR group. PGPRs help in growth promotion and yield improvement of crops and vegetables. So, the PGPRs isolated from those soil will be quite tolerant against the chemical effluents mixed in the soil. Hence, if those PGPRs are isolated and characterized and applied under that specific pollutant stress, they can enhance plant growth widely.

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