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EFFECT OF INDUSTRIAL WASTE ON SOIL MICROBIAL COMMUNITY AND SEED GERMINATION OF DIFFEENT PLANT SPECIES

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ABSTRACT

The study was undertaken in greenhouse to investigate the effects of industrial waste on seed germination, speed of germination and fungal activities. Three types of industrial waste (i.e. fiber industry sludge, chemical industry sludge and marble waste powder) were used for this experiment. Six treatments were made using 20g quantities of industrial waste in each treatments. The treatments were made, as control, T1= fiber industry sludge, T2= fiber industry sludge-ash, T3= chemical industry sludge, T4= chemical industry sludge-ash and T5= marble waste powder. Two series of plants species were selected, including five crops species *Vigna radiata (L.) Wilczek, Pisum sativum L., Vigna mungo (L.)Hepper, Phaseolus lunatus L.* and *Lens culineris* Medic, while five wild (or naturalized) plant species *Cynodon dactylon (*L)*Pers., Azadirachta indica (L.) A. Juss., Parkinsonia aculeata L., Prosopis Juliflora (Swartz)* DC. and *Lactuca remotiflora* DC. The germination was recorded on alternate days. The soil samples from each treatment were collected to check the fungal activities. The results revealed that all plant species were suppressed in all the treatments compared with controls. The percentage of seed germination and speed of germination of *Phaseolus lunatus* and *Lactuca remotiflora* were suppressed in all treatments. Two fungal species *Aspergillus niger* and *Aspergillus flavus* were recorded from all treatments. No significant differences were found between these two species and among all treatments.

Keywords: Industrial Waste, Soil Microbial Community, Seed Germination

INTRODUCTION

Pakistan is one of the countries, which are being affected by various types of land, water and aerial pollution. According to (Saleem *et al*., 1993) about 9000 million gallons of untreated waste water is being discharged regularly in the water bodies of industrial areas. However, due to the unavailability of clean water many farmers use the polluted river water or even the combined industrial and domestic effluents to irrigate their crop fields. The leachate of the industrial effluent is absorbed in the soil or agricultural field while the remaining solid part (sludge) or other material stays over the soil surface in the form of a thick layer of waste. Most of the seeds and microbial activity is disturbed due to the layer of waste material. Industrial effluents cause adverse effects on plant growth. Metal toxicity may affect all forms of life including microorganisms, plants and animals but the toxicity varies with the organism (Ashraf and Tasneem 2007). Heavy metals exert toxic effect on soil microorganisms (Pawlowsk and Charval 2004). They alter the diversity, population size and overall activity of the soil microbial communities (Smejkalova et al 2003, Gupta 1992, Hattori 1996, Kelly *et al* 2003). The soil microorganisms like bacteria and fungi play an important role in soil nutrition by decaying plants and other organic matter in soil as nitrifiers (Mishra *et al* 2008).

Various studies have been conducted on chemical, bacteriological and fungal activities of the industrial effluents. Some of these industrial wastes are potential source of essential nutrients as well as contain toxic heavy metals in their composition (Timsina 1988, EISP 1987, Miyoshi 1987). These heavy metals accumulate in the soil for a long time and interrupt the overall growth of plants as well as the microbial activities of soil. The microbial community is linked to soil health and subsequently to plant health throughout the nutrient cycle. The majority of the microbial populations degrade matter but some times they are also affected by industrial waste.

The aim of this study is to ascertain the adverse effects of increasing industrial pollution on wild plants, ornamental plants and food crops. Present study is designed to observe the seed germination and speed of germination of various plant species under the influence of industrial waste and also check fungal activities under the same treatments. The quantitative and qualitative fungal activity was examined to understand the relationship among fungal group, plants species and industrial waste treatments, so as to determine what type of industrial waste becomes more problematic for plants and mycoflora when contained in the growth medium.

MATERIALS AND METHODS

This experiment consist of two parts: the first part deals with the effect of industrial waste on seed germination of some food crops while in second part wild (or naturalized) species were observed in same treatments. The industrial wastes were collected from the waste site of different industries: chemical industry, fiber industry and marble industry. The analyses of heavy metals of all industrial wastes were done by Atomic absorption spectrophotometer (model PG 990). Dry sludge of chemical and fiber industry were crushed in small pieces by an ordinary electrical grinder and incinerated up to 1100^0C until they were converted into ash. Sandy loam soil (500g) was filled in each pot having 10cm in diameter. Six treatments were made with three replicates including controls. Five healthy seeds of each of five species were sown in each pot at 1.5cm depth. A thick layer of industrial waste, fiber industry sludge, sludge-ash, chemical industry sludge, sludge-ash and marble waste powder (20g per pot) were spread. Percentages of seed germination was assessed every 2 days interval for sixteen days and speed of germination index "S" was calculated in both experiments as directed by Khandakar and Bradbeer (1983),

$$S = \left[\frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} \cdots \frac{N_n}{n} \right] x \frac{100}{1}$$

Where: N_1, N_2, N_3…N*n* = Number of seeds which germinate on Day 1,2,3…n following set-up of the experiment; S varies from 100 (if all seeds germinate on the first day following set-up) to 0 (if no seed have germinated by the end of the experiment).

Czepeek media was used to check the fungal activity under the influence of waste leachate and discharge of heavy metals in soil. The soil samples were collected and Rajendran & Kathiresan (2007) methods were followed for estimate the fungi. Triplicates of Petri plates were kept at 32 ºC for five to seven days in an incubator. Colonies were counted by colony counter. The fungal slides were prepared and observed under microscope. The colonies growing on plates were identified on the basis of micro and macro morphological features following various manuals (Thom and Raper, 1948, Ellis, 1971). Soil analyses pertaining to heavy metals were done by Atomic absorption spectrophotometer (model PG 990). The statistical analysis was performed using Friedman test for percentage of seed germination by SPSS ver.10 while analysis of variance (ANOVA) (Steel & Torrie, 1984) and Duncan's Multiple Range Tests (Duncan's 1955) on two fungal species *Aspergillus niger* and *Aspergillus flavus* were performed using Costat version. 3.

RESULTS AND DISCUSSION

The results showed that the percentage of seed germination and speed of germination were both adversely affected by five different types of industrial wastes (p at the most 0.05). The results clearly indicated that the germination was suppressed in all treatments compared to controls. The final percentage of germination was significantly abated in all test species except *Vigna mungo* by the treatments ($P<0.05$) (Table 2a). The greatest inhibition of germination occurred in *Phaseolus lunatus* followed by *Pisum sativum, Vigna mungo, Lens culineris,* and *Vigna radiata* in that order (fig1). *Parkinsonia aculeata* did not show any significant difference among treatments, while other species were significantly adversely affected by all treatments ($P<0.05$) (Table 2b). The highest inhabitation of germination was found in *Lactuca remotiflora* followed by *Parkinsonia aculeata, Azadirachta indica, Prosopis juliflora* and *Cynodon dactylon* (Fig2).

The results showed that the rate of seed germination was affected by five types of industrial waste. The results clearly indicated that germination was retrograded in all the treatments compared with controls. Chemical industrial sludge showed better result for all the crop and wild (or naturalized) species while fiber industry sludge-ash showed adverse effect on germination of both crop and wild (or naturalized) species compared with other treatments (Table 2a). This may be due to high amount of heavy metals present in fiber industrial sludge-ash because according to Shaukat *et al*., (1999) and Uzair *et al* (2009) heavy metals cause a significant adverse effect on seedling growth. Reduction of seed germination by heavy metals has also been recorded by various researchers (Morzek and Funiceli 1982, Brown and Wilkins 1986, Shaukat *et al*, 1999 and Burhan *et al* 2001).

Table 3a describes the speed of germination 'S' which showed marked differences compared to controls. The speed of germination was almost strongly retarded in all treatments. The crop species showed better results in chemical industry sludge treatment while adverse effects were observed in the sludge ash of the same industry (Table 3a). *Phaseolus lunatus* was adversely affected in all treatments compared to other species. The speed or germination of wild (or naturalized) species was recorded in Table 3b. Better results were observed in chemical industry sludge treatment, while adverse effects were found in sludge-ash of the same industry. Species showed

varied response over treatments *Lactuca remotiflora* responded adversely against all treatments compared to other species.

Table 1. Chemical properties of industrial sludge and marble waste powder.

Parameters	Units	Fiber industry (sludge)	Chemical industry (sludge)	Marble (slurry) waste powder
Cu	mg/kg	3.22	42.00	<0.5
Mn	mg/kg	534.35	120.0	<0.5
Pb	mg/kg	N.D	210.0	<0.5
Ni	mg/kg	3.22	50.00	<0.5
Zn	mg/kg	8.12	150.0	0.80
Cd	mg/kg	N.D	N.D	-
Co	mg/kg	422.22	-	-
Fe	mg/kg	1298.81	-	6.50
Cr	mg/kg	N.D	160	<0.5

Table 2a. Percentage of seed germination of crop species by Friedman Test.

Species	Mean Rank						Chi-square	Sig.
	control	T1	T2	T3	T4	T5		
Vigna radiata	5.67	3.67	2.67	4.67	2.33	3.00	11.444	0.039
Pisum sativum	6.00	3.83	2.00	4.83	2.17	2.17	12.806	0.025
Vigna mungo	4.67	4.33	2.00	4.50	2.67	2.83	6.591	0.253
Phaseolus lunatus	6.00	3.17	2.17	4.83	1.67	3.17	12.423	0.029
Lens culineris	5.83	3.33	1.00	5.17	2.83	2.83	14.579	0.012

Table 2b. Percentage of seed germination of wild (or naturalized) species by Friedman Test.

species	Mean Rank						Chi-square	Sig.
	Control	T1	T2	T3	T4	T5		
Cynodon dactylon	5.00	2.00	2.17	5.00	1.83	5.00	13.407	0.020
Azadirachta indica	5.67	2.33	1.83	3.50	2.50	5.17	11.823	0.037
Parkinsonia aculeata	6.00	2.67	2.50	3.33	3.33	3.17	7.935	0.160
Prosopis Juliflora	5.83	3.00	3.50	4.67	1.00	3.00	12.577	0.028
Lactuca remotiflora	5.50	3.67	2.00	4.83	3.67	1.33	11.856	0.037

Note: T1=fiber industry sludge, T2= fiber industry sludge-ash, T3=chemical industry sludge, T4= chemical industry sludge-ash, T5= marble waste powder

Table 3a. Speed of germination percentage (crop species).

Species	Control %	T1 %	T2 %	T3 %	T4 %	T5 %
Vigna radiata	28.61	7.94	5.83	7.01	2.28	6.77
Pisum sativum	25.27	8.05	4.36	6.34	2.22	3.08
Vigna mungo	17.88	6.80	2.75	4.55	2.36	3.16
Phaseolus lunatus	16.17	3.55	2.22	5.95	2.47	2.61
Lens culineris	22.77	4.47	2.33	9.77	2.22	3.17

Table 3b. Speed of germination percentage (wild or naturalized species).

Species	Control %	T1 %	T2 %	T3 %	T4 %	T5 %
Cynodon dactylon	27.22	5.5	7.56	9.84	3.95	12.23
Azadirachta indica	14.02	3.05	1.38	2.2	1.58	6.05
Parkinsonia aculeata	8.92	4.41	4.16	2.61	1.52	3.11
Prosopis Juliflora	10	6.72	6.14	4.56	5.8	0.83
Lactuca remotiflora	13.39	3.14	1.38	5.25	0.55	0.83

Table 4. Mean number of fungal colonies recorded from different treatments.

Treatments	Fungal Species	
	Aspergillus niger	***Aspergillus flavus***
Control	7±1.00a	9±1.52abc
T1	6±0.66a	13±1.73a
T2	5±1.80a	5±0.88c
T3	5±1.45a	12±3.21ab
T4	4±1.00a	5±1.80c
T5	4±0.57a	6±1.73bc
LSD	**3.50**	**6.01**

Note: T1=fiber industry sludge, T2= fiber industry sludge-ash, T3=chemical industry sludge, T4= chemical industry sludge-ash, T5= marble waste powder, Numbers followed the same letters in the same column are not significantly different.

The analysis of the chemical industrial sludge showed that it contains Pb > Cr > Zn > Mn > Ni >Cu in that order, while fiber industry sludge contained Fe > Mn > Co > Zn > Ni = Cu in that order (Table 1) which gave adverse effects on seed germination and speed of germination in the form of ash because ash becomes more concentrated regarding heavy metals due to incineration of organic matter. The results showed that the ashes of fiber industry and chemical industry reduce the seed germination and speed of germination respectively. Heavy metals have been shown to reduced growth of trees in a number of studies (Kelly *et al.,* 1979 and Uzair *et al.,* 2009). Shaukat *et al*., 1999 have studied that toxic effect of Cd, Cr and Pb on germination and growth a tree species and a crop plant. The seed germination is a critical reproductive stage which may be sensitive to high acidity or high

concentration of trace elements (Iqbal and Shazia, 2004). The response of heavy metals varies from species to species according to Iqbal and Rehman (2002). Heavy metal toxicity in the environment is of great concern because of its toxic effect on plant growth. Some of the test species were found less sensitive, while others were found highly sensitive. The reduced germination of seeds under heavy metal stress could be a depressive effect on the activity of embryo (Zeid, 2001). This also explains how various species in an industrial area respond variably in terms of sensitivity. To determine the tolerance capability the plants germination tests were performed.

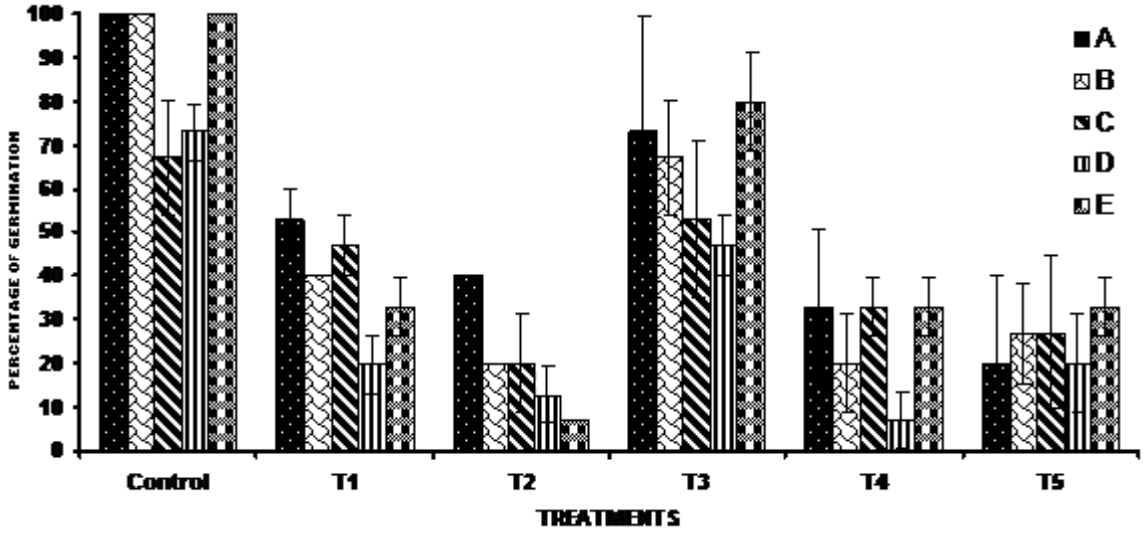

Fig.1. Percentage of seed germination of crops species with treatments.
Note A= *Vigna radiata,* B= *Pisum sativum,* C= *Vigna mungo,* D= *Phaseolus lunatus,* E= *Lens culineris*

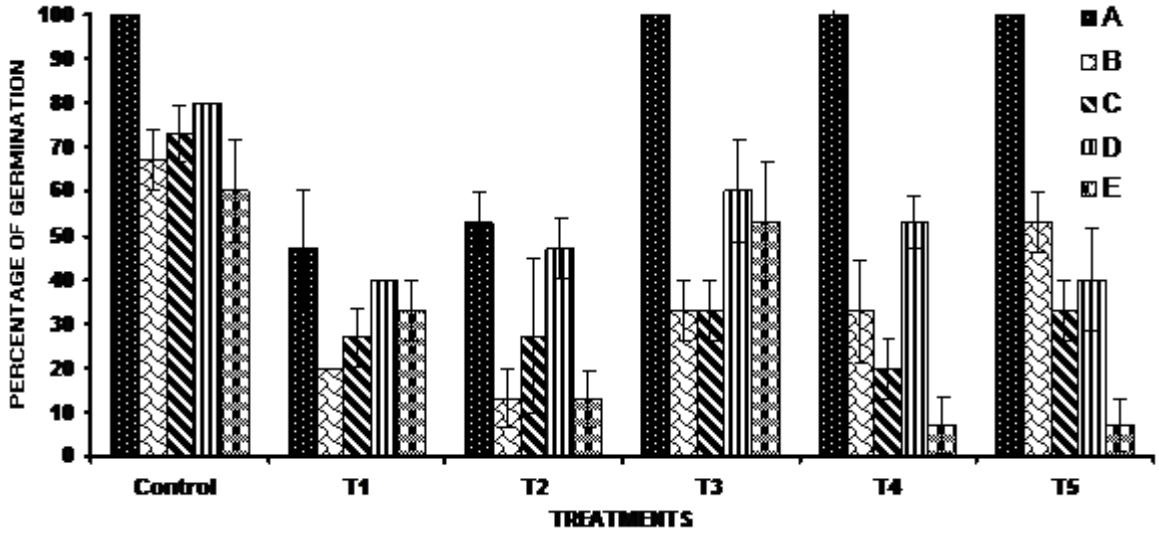

Fig. 2. Percentage of seed germination of wild (or naturalized) plants species with treatments.
Note: A= *Cynodon dactylon,* B= *Azadirachta indica,* C= *Parkinsonia aculeata L.,* D= *Prosopis Juliflora,* E= *Lactuca remotiflora*
T1=fiber industry sludge, T2=fiber industry ash, T3=chemical industry sludge, T4=chemical industry ash, T5=marble waste powder.

There were two widely distributed fungal species *Aspergillus niger* and *Aspergillus flavus* that were examined during microbial analysis. The analysis of variance ANOVA showed non-significant results for number of colonies of both species among all treatments (Table 4). Duncan's multiple range tests showed that the mean values of both species did not exhibit any difference with controls. This is contradiction against the earlier statement. However the number of colonies of *Aspergillus flavus* was significantly reduced 5±0.88 in fiber industry sludge-ash compared to fiber industry sludge 13±1.73. Similar results were observed in chemical industry sludge-ash where number of colonies of *Aspergillus flavus* 5±1.8 was recorded in ash treatment compared with chemical industry sludge treatments 12±3.21. These results showed that organic matter of a particular industrial sludge supports the growth of *Aspergillus flavus* while its ash suppressed the growth of the same species. These results suggested that the treatments were highly contaminated and the presence of these two species give an idea about the pollution resistance of these fungal species.

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