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EFFECT OF HEAVY METALS ON SOIL MICROBIAL COMMUNITY AND MUNG BEANS SEED GERMINATION

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Abstract

Pollution of the biosphere by heavy metals has caught our attention in finding new ways to sustain, restore and manage the environment**.** In this context, the study was carried out to assess and determine the cumulative effect of heavy metals on soil microorganisms and seed germination, which ultimately contribute serious threats to the ecosystem. We attempted to isolate stress tolerant bacteria and fungi from the soil amended with varying concentrations of heavy metal salts including silver (Ag), zinc (Zn) and lead (Pb). The procedure describes the selection of heavy metal salts, characterization of soil, simulated pollution of soil, isolation, identification, characterization, screening and assessment of heavy metal tolerance by bacteria and fungi. Effects of varying concentrations of heavy metals on the germination of seeds (bioassay) were also taken into consideration. The data suggested that lead and silver were found to be toxic for the growth of microorganisms. The amendment of zinc in the soil up to 50mM concentration facilitated the growth of bacteria and fungi. An overall change in the microbial communities was observed in comparision with the control. Germination of seeds was favoured with increasing concentrations of zinc (50mM), which indicated the essentiality of zinc to plants. More deleterious effects on the germination of *Vigna radiata* (mung beans) were observed in case of silver, while moderate toxicity was observed in case of lead. Silver was found to be extremely toxic since the minimum concentration (10mM) resulted in the complete inhibition of seed germination. The seed germination in the presence of 10 and 25 mM concentration of silver was delayed for a period of 10 days but subsequent exposure to same concentration for 15 days resulted in the germination of seeds.

Introduction

The quality of life on earth is linked undeniably to the overall quality of the environment (Anon., 1995). Pollution of the biosphere by heavy metals due to industrial, agricultural and domestic activities has created a serious problem for the safe and rational utilization of soils (Srivastava *et al*., 2005; Avery, 2001; Igwe *et al*., 2005; Kandori *et al*., 1993; Volesky, 1990). Industrial inputs and the agronomic application of fertilizers, pesticides and metal- contaminated sewage continue to contribute the metal accumulation in the soil (Herland *et al*., 2000). The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be naturally degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain ((Igwe *et al*., 2005; Smejkalova *et al*., 2003). Metal toxicity may affect all forms of life including microorganisms, plants and animals, but the degree of toxicity varies for different organisms. Physical, chemical and biological processes may combine under certain circumstances to concentrate metals rather than dilute them (Igwe *et al*., 2005). Several researchers using isolation-based techniques have demonstrated that heavy metal contamination can cause shifts in microbial populations (Barkay *et al*., 1985; Doelman *et al*., 1994; Gringell *et al*., 1976; Roane & Kellogg, 1996). The immediate toxicity of metals to soil organisms is moderated by metal immobilization by soil colloidal components however, heavy metals may be mobilized by local and global changes in soil conditions i.e., changes in physical and chemical conditions of soil environment, including decrease in pH, redox potential and enhanced decomposition of organic matter (Gupta, 1992, Hattori, 1996; Kelly *et al*., 2003). Heavy metals exert toxic effects on soil microorganism (Pawlowska & Charvat, 2004) hence results in the change of the diversity, population size and overall activity of the soil microbial communities (Smejkalova *et al*., 2003; Gupta, 1992; Hattori, 1996; Kelly *et al*., 2003). Gasper *et al*., (2005) reported that the after-effect of the observed heavy metal (Cr, Zn and Cd) pollution influenced the metabolism of soil microbes in all cases. In general, an increase of metal concentration adversely affects soil microbial properties e.g. respiration rate, enzyme activity, which appears to be very useful indicators of soil pollutions (Brookes, 1995; Szili-Kovacs *et al*., 1999). Microorganisms, including bacteria, algae, fungi and yeast, serve as very constructive models for studying the harmful effects of metals at the cellular level (Avery, 2001). These microorganisms can also be used to remove toxic-metals from contaminated sites as they can efficiently accumulate heavy metals and radionuclides from their external environment (Ali & Wainwright, 1995). In the present study, effects of different heavy metals on the diversity and extent of soil microorganisms have been determined. The effects of metal toxicity on the germination of mung bean seeds were used as a model in this study, since seeds are well protected against various stresses which soon after imbibitions and subsequent vegetative developmental processes, become stress-sensitive in general (Weiqianjgli *et al*., 2005).

Materials and Methods

Metals used in this study included lead sulphate (Pb SO_4) and lead acetate (Pb $(CH_3COO)_2.3H_2O$) , silver nitrate (Ag NO_3), zinc sulphate ($ZnSO_4$). Garden soil was collected from the Department of Microbiology, University of Karachi. Physicochemical characteristics of the soil i.e. colour, structure, pH, organic matter, electric conductivity (ECe), water holding capacity (WHC) and moisture content were determined using the methods described by Gupta, (2004). The soil was filled into clay pots (200g/ pot) and was amended with different concentrations (10, 25 and 50 mM) of these metal salts. Controls were run side by side without any metal amendment. All the tests and controls were run in triplicate. Rossi & Cholodney's buried slide technique was performed to determine the effect of heavy metals on microorganisms in their native environment (Alexander, 1961; Waksman, 1957). Isolation was carried out after 15 days of incubation and standard plate count (SPC) was performed as given by Pelczar *et al*., (2003). Ten-fold serial dilutions were made up to 10^{-6}. An amount of 0.1 ml from the diluted samples (dilution10^{-4} for fungi and 10^{-6} for bacteria) was spread on the respective culture media plates using a glass spreader. These plates were then incubated at ambient temperature for 24 h for bacteria and 4-5 days for fungi. Soil extract agar (pH 7) was used for bacteria, colonies were counted and CFU/g was calculated for bacteria. Soil extract agar (pH 5), Potato dextrose agar (pH 5.0) and Malt agar (pH 5.0) were used for the isolation of fungi. The isolates were maintained on respective media slants and were identified (Barnett & Hunter, 1998; Barnett, 1960; Thom & Raper, 1945). The isolates were inoculated into soil extract broth (pH 7 and pH5) with 0.1% glucose amended with heavy metal for the assessment of heavy metal tolerance. Heavy metals were filter sterilized and the solutions were added into double strength soil extract broth to get the required concentrations of 10, 25 and 50mM heavy metals in broths. In order to determine the effects of heavy metals on germination, mung bean (*Vigna radiata*) seeds were immersed in 3% formaldehyde/deionized water and then washed with deionized water to eliminate the exogenous microbial contamination. These seeds were embedded 1.0 cm deep in soil pots amended with different concentrations of metals and germination rate was determined. The data was statistically analyzed by one-way analysis of variance (ANOVA) using SPSS (10) to determine the statistical differences between means of treatments.

Results and Discussion

The soil collected was dark brown, silty clay, porous with small aggregates, organic matter 54 g Kg^{-1}, ECe 178.5 μScm^{-1} (micro Siemens cm^{-1}), WHC 23 ml and moisture content of 2.13 % (w/w) and pH 7.2. Bacteria and fungi were the dominant microorganisms observed in the soil by Rossi & Cholodney's buried slide technique. Bacteria were observed in different morphological forms, cocci and rods with variety of arrangements. Fungi were found to be present as mycelial mats, rhizomorphs or as various types of spore forms. Abundant cyst forms, scattered long rods, cocci in bunches and spore forming rods were observed in control soil without heavy metal amendment.

Increasing concentrations of silver (25 and 50mM) in the soil resulted in the decreased growth of some groups of microorganisms. The overall decrease in the growth of microorganisms was observed, due to increased concentrations of heavy metal contamination, which is in agreement with the findings of Kikovic, (1997) and Smejkalova, *et al.,* (2003). However, in the present study there was a marked increase in the number of cocci (in bunches and scattered forms) with the increasing concentration of silver. The overall profile of microorganisms remained the same with that of control. Bacterial count (CFU) was markedly decreased with the increasing concentrations silver (Table 1), which is similar to the findings of Smejkalova *et al*., (2003). Predominance of Gram negative bacteria over Gram positive has been found in silver contaminated soil by Frostegard *et al*., (1993) and similar results were obtained in the present study. Germination of seeds was adversely affected and delayed at lower concentration of silver (10mM) and was completely inhibited at 50mM concentration (Figs. 1 and 2).

Large rods were observed in zinc-amended soil. Scattered cocci and filamentous fungi were observed in 10 mM concentration of zinc. There was a slight increase in the bacterial growth when zinc concentration was increased to 20 mM and 50mM. Protozoan cysts and rods were also observed in this soil. In our study the highest concentration of zinc (50mM) did not result in suppression in the growth of microorganisms but zinc was found to have favourable effects on the growth. Munzuroglu & Geckil (2002) have reported that some heavy metals such as Cu, Zn and Ni are essential micronutrients for plants but are toxic to organisms at high concentrations. Bacterial count was also found to increase with the increasing concentrations of zinc than the control (Fig. 3). Seed germination was also favoured with the increasing concentrations of zinc that indicated its essentiality to the plants (Figs. 1 and 2). Peletra *et al*., (2000) have found that Zn did not significantly reduce the seed germination, even at concentration of 40ppm ($p<1\%$).

Table 1. Enumeration of viable microorganisms in heavy metal contaminated soil.

Heavy metals	Number of heterotrophs i.e., (mean ± SD) CFU/g for:			
	0 mM(control)	10 mM	25 mM	50 mM
Zn	$(4.489 \pm 0.1) \times 10^8$	$(6.71 \pm 0.14) \times 10^8$	$(8.033 \pm 0.13) \times 10^8$	$(8.575 \pm 0.14) \times 10^8$
Pb	$(4.489 \pm 0.1) \times 10^8$	$(2.278 \pm 2.0) \times 10^8$*	$(1.784 \pm 2.7) \times 10^8$*	$(1.095 \pm 1.8) \times 10^8$*
Ag	$(4.489 \pm 0.1) \times 10^8$	$(2.038 \pm 2.3) \times 10^8$*	$(1.203 \pm 1.9) \times 10^8$*	$(8.32 \pm 1.8) \times 10^7$*

*, $p < 0.01$; values compared with control.

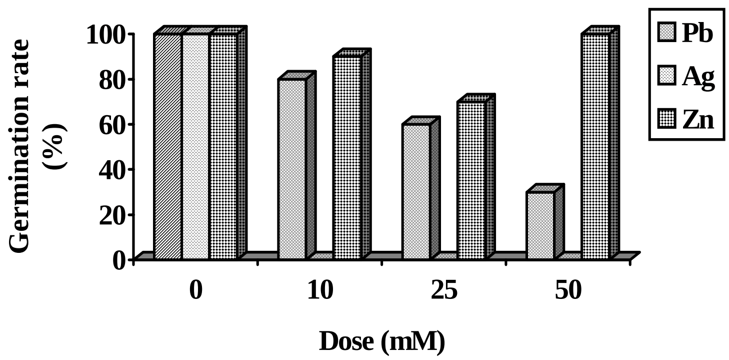

Fig. 1. Mung bean seed germination after 10 days of exposure to heavy metals.

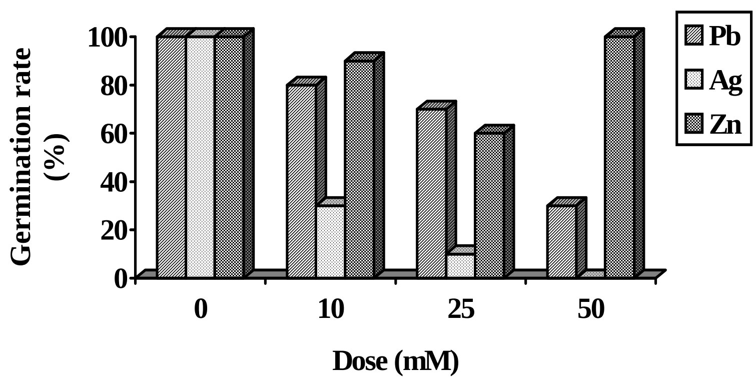

Fig. 2. Mund bean seed germination after 15 days of exposure to heavy metals.

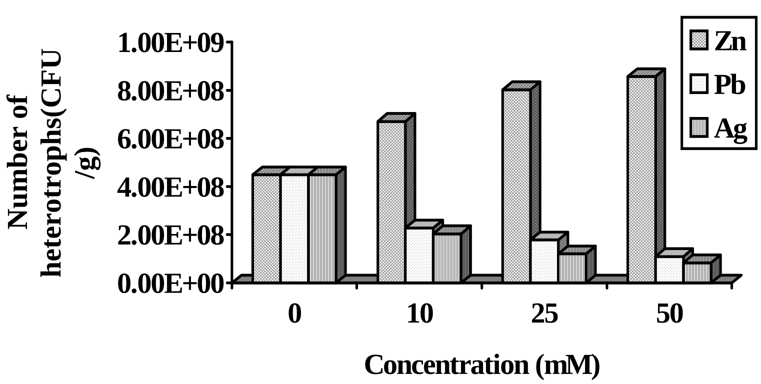

Fig. 3. Enumeration of viable microorganisms in heavy metal contaminated soil.

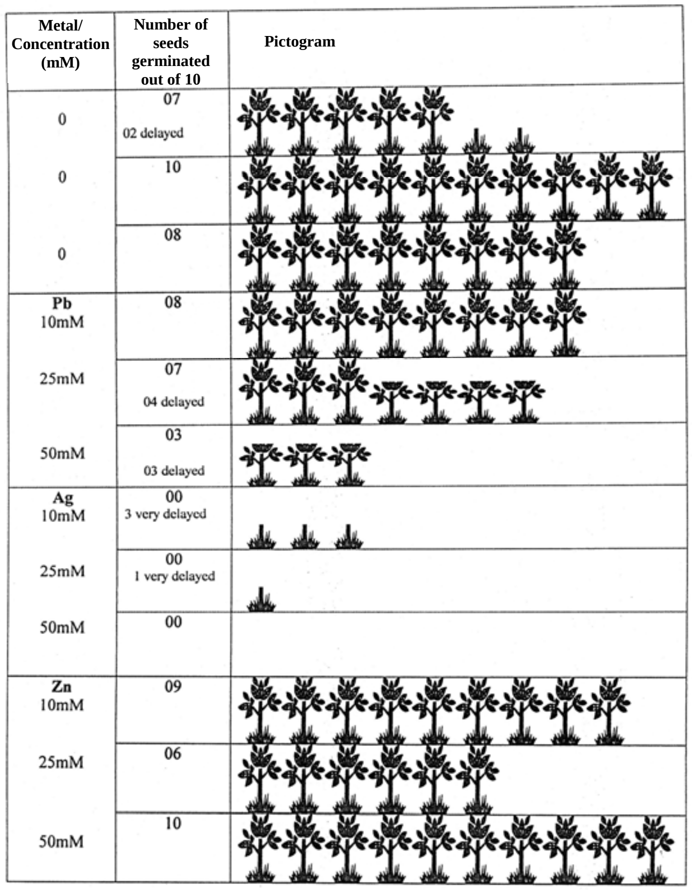

Fig. 4. Effect of heavy metals on the germination of mung bean seeds.

In case of soil contaminated with lead (Pb) slight change was observed in the soil microbial profile. Cocci were observed in aggregates, bunches, pair and scattered arrangement. Endospore forming rods were observed in scattered arrangement. Growth of fungi was obvious in the form of mycelial mats. Bacterial count was markedly decreased with the increasing concentrations of lead. Delayed germination of the *Vigna radiata* resulted from the relative toxicity of lead being added into the soil. Kelly *et al*., (2003) have studied the change in the population structure of the soil microbial communities resulting from metal contamination and a recovery of several microbial populations resulting from remediation. Seed germination was gradually delayed in the presence of increasing concentration of lead (Pb). It may be due to prolong incubation of the seeds that must have resulted in the neutralization of the toxic effects of lead by some mechanisms eg., leaching, chelation, metal binding or/and accumulation by microorganisms. Results obtained after 10 and 15 days of inoculation of seeds revealed that at lower concentrations, lead posed little or no harm to seed viability but at higher concentration, germination was retarded (Figs. 1 and 2). This is in accordance with the earlier reports by Jaja & Odoemena (2004). Order of toxicity of these heavy metals was found to be Ag > Pb > Zn which is in accordance with earlier reports of Horsfall (1956).

Table 2. Identified microbial isolates from soil contaminated with heavy metals.

Isolate #	Scientific name
RT101	*Aspergillus sulphureus*
RT102	*Aspergillus niger*
RT103	*Aspergillus niger*
RT104	*Tritiractium album*
RT105	*Aspergillus variecolar*
RT106	*Fusarium solani*
RT107	*Aspergillus fumigatus*
RT108	*Aspergillus niger*
RT109	*Cladosporium cladosporioides*
RT110	*Aspergillus parsiticus*
RT111	*Alternaria longipes*
RT112	*Aspergillus wentii*
RT113	*Aspergillus temera*
RT114	*Fusarium solani*
RT115	*Acremonium murorum*
RT116	*Alternaria alternata*
RT117	*Aspergillus panamensis*
RT118	*Aspergillus unguis*
RT119	*Fusarium solani*
RT120	*Cladosporium cladosporioides*
RT121	*Arthrobacter* sp.
RT122	*Bacillus cereus*
RT123	*Pseudomonas* sp.
RT124	*Bacillus megaterium*
RT125	*Corynebacterium* sp.
RT126	*Bacillus pumilus*
RT127	*Bacillus subtilis*
RT128	*Staphylococcus* sp.

Those seeds, which were adapted, showed resistance through their delayed germination. It might be the case that prolong incubation of the seeds resulted in the neutralization of the toxic effects by some mechanism eg., leaching, chelation, metal binding, accumulation by microorganisms, paradoxical effect (Schatz *et al*., 1965). Consequences of the presence of toxic metals lead to delay in germination of seeds that in turn affect the crop yield. If plant tissues accumulate these heavy metals then it can ultimately affect the human health in the form of poisoning eg., lead poisoning.

Most of the fungi and bacteria were found to be resistant to the change in pH and they could grow in a wide range of pH. Fungi were found to be more resistant to heavy metals than bacteria when allowed to grow in the liquid media amended with heavy metals. Fungal resistance to heavy metals has also been reported by Hiroki (1992), Jordan & Lechevalier (1975).

The bacteria and fungi isolated and identified (Table 2) may prove to be potential candidate in the bioremediation of metal contaminated soils/ sites and recovery of precious metals (silver) from the contaminated soils and industrial effluents. The presence of toxic metals in soil lead to the decreased value of that land with reference to its productivity and ultimately affect the human health in the form of poisoning eg., lead poisoning.

Heavy metal contamination of soil via industrial effluents, sewage influx, contaminated ground waters etc., can induce serious problems to soil, cropping, vegetation and in turn human health. Heavy metal accumulation by plant tissues, its presence in the soil persistently or its presence in ground waters is not a healthy sign for the environment. So cleaning of our environment from these pollutants, such that they are no longer toxic to life, is of great importance.

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