Phytoremediation of Mixed Contaminated Soils - Effects of Initial Concentrations

Reshma A. Chirakkara[1], Krishna R. Reddy[2], F. ASCE

[1] Graduate Research Assistant, University of Illinois, Department of Civil and Material Engineering, 842 West Taylor Street, Chicago, Illinois 60607, USA; rac2@uic.edu
[2] Professor, University of Illinois, Department of Civil and Material Engineering, 842 West Taylor Street, Chicago, Illinois 60607, USA; kreddy@uic.edu

ABSTRACT: Numerous contaminated sites exist worldwide that contain a mixture of organic and heavy metal contaminants. Very few technologies are proven to be efficient to address the problem of such mixed contamination. Most of these technologies are energy-intensive and expensive and they can disturb the natural ecosystem. Phytoremediation has potential to be a green and sustainable approach to decontaminate and restore the contaminated sites, maintaining the biological activity and physical structure of the soils. However, its effectiveness for mixed contaminants is not well understood. This study presents series of laboratory experiments conducted to investigate the effects of initial contaminant concentration on phytoremediation of mixed contaminated soils. A silty clay (typical field soil) was spiked with naphthalene, phenanthrene (representative organic contaminants), lead, cadmium and chromium (representative heavy metals), in different concentrations. Two plant species, specifically *Avena sativa* (oat plant), and *Helianthus annuus* (sunflower), were grown in these contaminated soils as well as in uncontaminated soil for comparison purposes. Results showed that the increase in contaminant concentrations in the soil negatively influenced the growth and biomass of the plants. *Helianthus annuus* showed lower germination, survival, growth rates, and biomass under increasing contaminant concentrations compared to *Avena sativa.*

INTRODUCTION

Many sites worldwide are contaminated with a mixture of organic and heavy metal contaminants. Since many remediation technologies aim to degrade or immobilize only a particular type of contaminant, remediation of sites co-contaminated with organic and heavy metal contaminants can be a difficult task. Many of the methods used for mixed contaminated soils are energy intensive or expensive. For large sites with shallow and moderate contamination, phytoremediation can be a practical option to remediate mixed contaminants. Phytoremediation is a low cost method which has the potential to treat both organic and inorganic contaminants. Phytoremediation is an

emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water (Sharma and Reddy, 2004). This technology has been receiving attention lately as an innovative and cost-effective alternative to more established treatment methods used at hazardous waste sites (USEPA, 2000). The inherently aesthetic nature of planted sites makes phytoremediation an attractive option compared to other cleanup methods (ITRC, 2009). The nature of on-site contaminants and the concentrations of these contaminants are governing factors in phytoremediation (Kranner and Colville, 2011, Henner et al. 1999). Higher phytotoxicity of the chemicals at higher concentrations can negatively affect the germination and survival of the plants, which in turn affect the phytoremediation efficiency. Understanding the contaminant concentrations above which the plants are expected to survive better is important in phytoremediation implementation.

BACKGROUND

Many historically industrialized former wetland and grassland sites in Chicago have been found to be contaminated with a mixture of organic and heavy metal contaminants. Naphthalene, phenanthrene, lead (Pb), cadmium (Cd), and chromium (Cr) are observed to be the most common contaminants at many of the sites (City of Chicago, 2005). The 100 % concentrations used in this study are the concentrations similar to the maximum concentrations found at the sites considered.

This study has attempted to understand the range of contaminant concentrations above which plant survival and growth are considerably affected by the initial contaminant concentrations. This is expected to give better understanding of site contaminant concentrations, below which phytoremediation can be effectively implemented.

EXPERIMENTAL METHODS

Selected Plant Species

The plant species for the study were selected based on biomass and capability of survival in mixed contaminated soil based on some previous results. The plants selected were *Avena sativa* (oat plant), and *Helianthus annuus* (sunflower). *Avena sativa* was studied for its phytoremediation efficiency for heavy metal (Ebbs and Kochian, 1998) and organic contaminants (Miya and Firestone, 2001) in the past. *Helianthus annuus* species was also involved in phytoremediation studies of both organic (Rosado and Pichtel, 2004) and heavy metal (Meers et al. 2005. contaminants.

Soil Selected

Clean gray silty clay, which represents typical Chicago glacial till, was obtained from a field site in Chicago, IL, and was used for the pot experiments. The aim is to use the experimental results for the phytoremediation of some sites with mixed

contamination in Chicago. The physic-chemical properties of the soil used in the study are presented in Table 1.

Table 1: Physico-chemical properties of soil used for the experiments

Soil water content	0.95 %
Soil organic content	2.3 %
Specific gravity	2.7
Liquid limit	33.1 %
Plastic limit	18.91 %
Plasticity index	14.19 %
Clay (< 0.002mm)	42 %
Silt (0.002 - 0.05mm)	42 %
Sand (0.05 – 2 mm)	14.3 %
USCS Classification	CL
USDA Classification	Silty clay

Soil Spiking Procedure

The reference uncontaminated soil was prepared by mixing the soil with 15 % water. The contaminated soil was prepared by spiking the soil with naphthalene, phenanthrene, Pb, Cd and Cr. For that, measured amount of naphthalene and phenanthrene were dissolved in hexane by mixing using a magnetic stirrer. The hexane containing naphthalene and phenanthrene was mixed with dry soil. The mixed soil was kept for 3 to 4 days in the fume hood for drying and to ensure that all hexane evaporated. Soil was mixed once every day during drying to ensure uniformity. Measured amounts of $PbCl_2$, $K_2Cr_2O_7$ and $CdCl_2$.½ H2O were mixed in water (to yield approximate water content of 15 % in soil) for one hour using magnetic stirrer. The solution was added to the soil, previously spiked with naphthalene and phenanthrene. The soil was mixed well to ensure homogenous distribution of contaminants. By varying the amount of chemicals in the mixture, soils with different contaminant concentrations were prepared. The maximum contamination used here is taken as 100 % contamination. The amounts of contamination for different percentages mentioned here are listed in Table 2.

Table 2: Contaminant Concentrations Used in the Experiment

Notation Used	**Concentration of Contaminants in Soil (mg/kg)**				
	Pb	Cd	Cr	Naphthalene	Phenanthrene
100 %	500	50	200	50	100
50 %	250	25	100	25	50
25 %	125	12.5	50	12.5	25
10 %	50	5	20	5	10

Measured properties of the contaminated and uncontaminated soil at the time of seeding are presented in Table 3.

Table 3: Properties of soil at the time of seeding

Property	Soil Type				
	Clean soil	Contaminated soil			
	(0 %)	10 %	25 %	50 %	100 %
pH	7.622	7.642	7.874	7.546	7.341
Oxidation reduction potential (mV)	-36.8	-37.6	-51.3	-32.4	-20.2
Electrical conductivity (mS/cm)	0.239	0.216	0.128	0.283	0.454
Water content (%)	15.2	14.1	17.3	15.5	13.4

The prepared soil samples were filled in pots of 8 cm diameter and 9 cm height for seeding the two plant species. Three contaminated pots were prepared for each plant species, for each concentration selected. The seeds were placed in the pots approximately a half inch below the soil surface. Each pot was kept on separate trays to ensure that the leachate did not get mixed up. The pots were placed under grow lights (metal halide lamps; average light intensity of 400 μmols/m^2/s) which were hung ~ 12 inches above the plants to obtain the desired light intensity. A timer was set to provide 16 hours of light per day. The hanging height was adjusted as the plants started growing taller to reduce the heat stress caused by the hanging lamps to the plants. The temperature below the grow lights, at the height of the plants was measured as 25 °C. Fans were used to control the temperatures of the grow lights.

Pots Setup and Monitoring

The plants were watered and were grown for 65 days. The locations of the pots were rotated periodically to ensure uniform light intensity to all the pots. Total 100 ml of nutrient solution with NPK 20-20-20 was applied to each pot, once in a week. Weekly monitoring was done by counting the number of plants in each pot and measuring the plant height. Photographs were also taken every week to record the plant growth and biomass production. Soil samples were taken at the beginning and end of the plant growth period to test for metals and organic contaminants.

At the end of the plant growth period, roots of the plants were separated out from shoots and washed in deionized water. The roots, shoots and soil were dried in an oven at 60 °C for 6 days (until it attained constant weight). The dry weights of roots and shoots are noted as root biomass and shoot biomass.

Analytical Testing

Testing of physical properties of the soil viz. water content (ASTM D2216), pH (ASTM D4972) and grain size analysis (ASTM D422) was done as per ASTM standards.

RESULTS AND DISCUSSION

It was observed that all the plant species had delayed germination and reduced rate of germination, survival and growth in contaminated soil compared to the control. Different plant growth characteristics and the soil contaminant concentrations were analyzed in clean and contaminated soil. Germination percentages of the plants in contaminated and uncontaminated soils are plotted in Fig. 1. Here germination is explained as the appearance of a green shoot/leaf above the soil. Germination of oat plant did not seem to be much affected by increasing contamination in the soil. But sunflower showed decrease in germination rate with increase in contamination in the soil. Different germination rates in different species may be due to the difference in seed coat permeability of the plant species (Wierzbicka and Obidzinska, 1998).

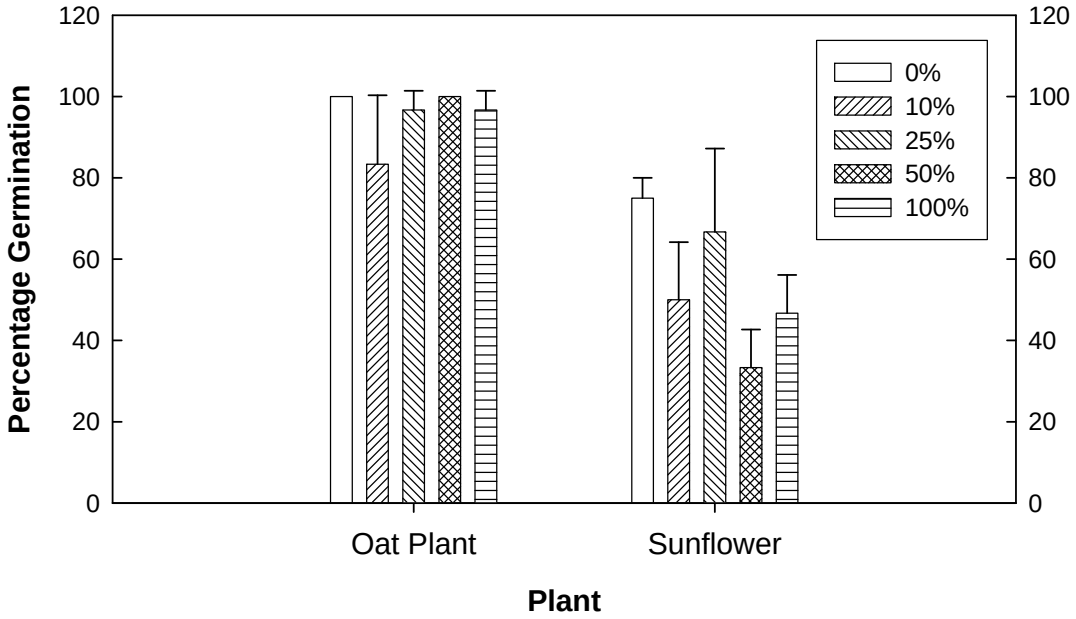

FIG. 1. Percentage Germination of Oat Plant and Sunflower for Soils with Different Percentages of Contamination

All the plants which germinated did not survive by the end of the experimental period. Some plants in contaminated soils showed phytotoxicity symptoms like yellowish color and reduced growth and eventually dried up. Leaf health and quality were affected in the plants in contaminated soil compared to the plants in control soil for these plants. Here survival is expressed as the presence of green/live plant in the pot at the end of the test period. Percentage survival is the number of surviving plants as percentage of the number of seeds germinated. Fig. 2 shows the percentage survival of oat plant and sunflower in soils with varying contamination. The percentage survival of oat plants seemed to be better than that of sunflower plants in contaminated soils. Sunflower showed diminishing rate of survival with increasing contamination in soil. Survival rate of 50 % and 100 % contaminated soils were considerably less compared to the control (0 %) soil. Increase in plant heights with

time for oat plant and sunflower are plotted in Fig. 3 and Fig.4 respectively. It is evident from the plots that growth rates decreased with increasing contamination for both the plant species.

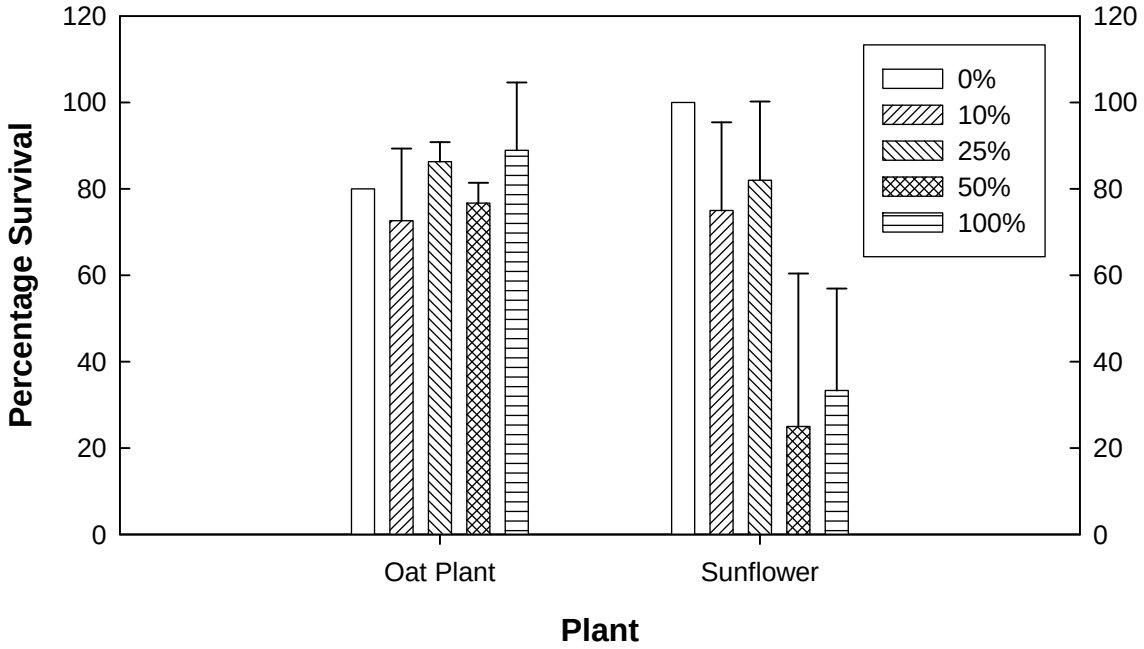

FIG. 2. Percentage Survival of Oat Plant and Sunflower for Soils with Different Percentages of Contamination

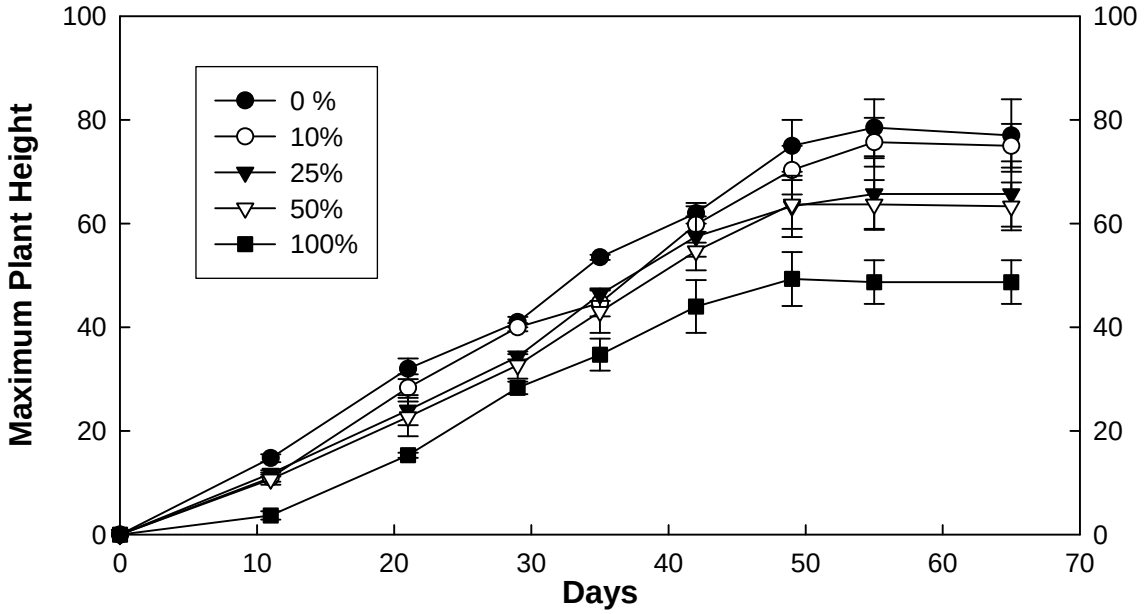

FIG. 3. Increase in Plant Height with Time for Oat Plant in Soils with Different Percentages of Contamination

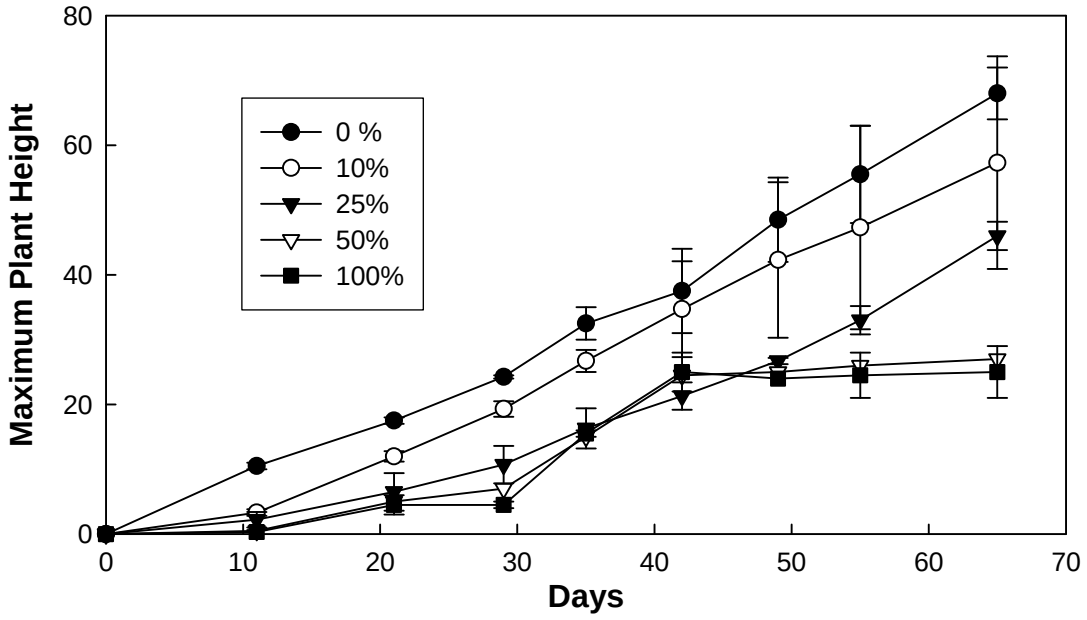

FIG.4. Increase in Plant Height with Time for Sunflower in Soils with Different Percentages of Contamination

Fig. 5 shows the final (after 65 days) maximum plant height of plants.

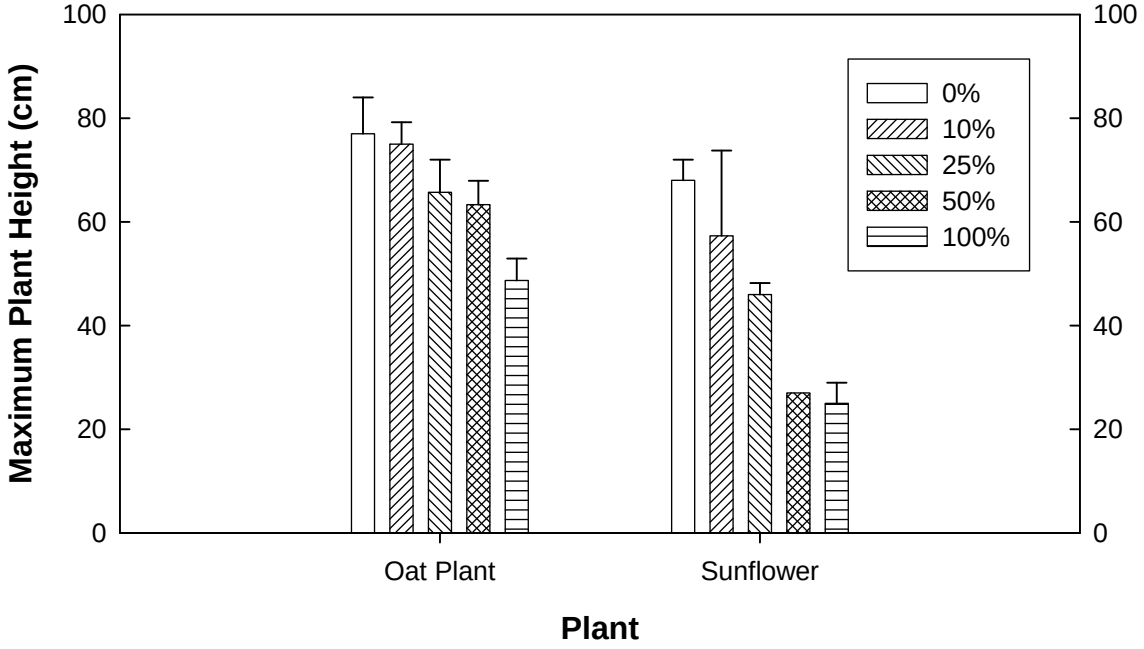

FIG.5. Final Maximum Plant Height for Oat Plant and Sunflower in Soils with Different Percentages of Contamination

The reduction in maximum plant height in contaminated soils compared to control for both the plant species are presented in Table 4. Sunflower showed higher reduction in plant height compared to control in all contaminated soils. The reduction in plant height was considerable in the case of 50 % and 100 % contaminated soils for sunflower.

Table 4: Average Values of Percentage Reduction on Maximum Plant Height for Contaminated soils Compared to Control

Plant	Percentage of Contamination			
	10	25	50	100
Oat plant	2.6	14.7	17.7	36.8
Sunflower	15.7	32.4	60.3	63.2

Average root and shoot biomass of plants in clean soil and contaminated soil are given in Table 5. Percentage reduction of biomass in 10 % contaminated soil was lesser for sunflower compared to oat plant. But for all other contaminated soils, sunflower had higher biomass reduction than oat plant.

Summarizing the growth characteristics, it can be observed that the germination, survival, plant height and final biomass were greatly influenced by the combined contamination conditions. The phytotoxicity to the plants by the combined contamination condition can be due to the metals or organic contamination or the combination of both. If the growth characteristics of both species are compared, oat plant seem to have better growth and survival rates in higher contamination soils compared to sunflower.

Table 5: Average Values of Root Biomass, Shoot Biomass and Total Biomass for Oat Plant and Sunflower for Soils with Different Percentages of Contamination

Biomass	Plant	Percentage of Contamination				
		0	10	25	50	100
Root Biomass (g)	Oat plant	5.5	3.2	2.2	1.2	1.3
	Sunflower	3.7	1.7	1.0	1.1	1.0
Shoot Biomass (g)	Oat plant	5.5	2.9	2.7	2.4	1.6
	Sunflower	7.9	6.5	2.0	0.7	0.8
Total Biomass (g)	Oat plant	11.0	6.1	4.9	3.6	2.9
	Sunflower	11.6	8.2	3.0	1.8	1.9

The final (after plant growth) soil samples from all the pots, were analyzed for pH, electrical conductivity and oxidation-reduction potential. Table 6 shows the average values of pH, electrical conductivity and oxidation-reduction potential for the soil samples after the plant growth period. According to this, pH, electrical conductivity and oxidation-reduction potential are not significantly affected by contamination or by the presence of plants. This can be taken as a positive result for remediation, as microbial activities responsible for the rhizodegradation and rhizostabilization are

dependent on the soil pH to a great extent (Atagana et al. 2003). The pH is expected to play an important role in metal availability for plants and the data will be correlated with heavy metal concentrations at later stages of the study.

Table 6: Average Values of pH, Oxidation Reduction Potential and Electrical Conductivity for Oat Plant and Sunflower for Soils with Different Percentages of Contamination

Value	Plant	Percentage of Contamination				
		0	10	25	50	100
pH	Oat plant	8.0	8.0	8.1	8.0	7.9
	Sunflower	8.0	8.0	7.9	7.8	7.7
ORP (mV)	Oat plant	-57.6	-56.8	-67.0	-58.4	-52.1
	Sunflower	-58.3	-59.2	-54.1	-45.1	-39.6
EC (mS/cm)	Oat plant	0.11	0.11	0.07	0.11	0.13
	Sunflower	0.10	0.10	0.12	0.17	0.22

CONCLUSIONS

The mixed contamination soil had significant effect on the growth characteristics of oat plant and sunflower. All plants showed delayed germination, reduced germination and survival rates in contaminated soil compared to the control. Oat plant had a better germination rate in all contaminated soils, compared to sunflower. The survival rate of oat plants did not seem to depend upon the magnitude of contaminant concentrations in the soil. The survival rate of sunflower was comparable to that of oat plant at lower concentrations. But the survival rate of sunflower plants were greatly affected at 50 % and 100 % contaminant concentrations. Both species showed reduced growth rate and biomass with increasing contaminant concentration. The results suggest that oat plant has better survivability in higher contamination soil, compared to sunflower. It may not be appropriate to use sunflower plants in contaminations above 50 % concentrations considered here since the biomass of plants are reduced by more than 80 % in that case. Soil amendments to increase the biomass and survivability of sunflower plants may also be considered to improve phytoremediation efficiency.

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