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Integrated micro-biochemical approach for phytoremediation of cadmium and zinc contaminated soils

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ABSTRACT

The integrated potential of oilcake manure (OM), elemental sulphur (S^0), *Glomus fasciculatum* and *Pseudomonas putida* by growing *Helianthus annuus* L for phytoremediation of cadmium and zinc contaminated soils was investigated under pot experiment. The integrated treatment (2.5 g kg^{-1} OM, 0.8 g kg^{-1} S^0 and co-inoculation with *G. fasciculatum* and *P. putida* promoted the dry biomass of the plant. The treatment was feasible for enhanced cadmium accumulation up to 6.56 and 5.25 mg kg^{-1} and zinc accumulation up to 45.46 and 32.56 mg kg^{-1} in root and shoot, respectively, which caused maximum remediation efficiency (0.73 percent and 0.25 percent) and bioaccumulation factor (2.39 and 0.83) for Cd and Zn, respectively showing feasible uptake (in mg kg^{-1} dry biomass) of Cd (5.55) and Zn (35.51) at the contaminated site. Thus, authors conclude to integrate oilcake manure, S^0 and microbial co-inoculation for enhanced clean-up of cadmium and zinc-contaminated soils.

1. Introduction

Heavy metals cause environmental pollution as a result of anthropogenic activities such as mining, smelting, electroplating, energy and fuel production, power transmission, sludge dumping, military operations and un-hygienic approach of rapidly growing population such as application of heavy metal containing fertilizers (i.e. superphosphate) or pesticides in agricultural soil (Rahimi et al., 2013; Thawornchaisit and Polprasert, 2009). They cause risk for primary and secondary consumers and ultimately to humans. Due to high Cd^{2+} mobility in the soil–plant ecosystem it can easily enter into food chain ultimately become hazardous to human beings, animals, plants and the whole environment (Pinto et al., 2004). Soil pollution by heavy metals is a global environmental problem as it has affected about 235 million hectares of arable land worldwide (Bermudez et al., 2012).

1.1. Remediation of heavy metals

Contaminated soils may be remediated by physical, chemical or biological techniques but the physical or chemical methods can be very costly and also destructive to the soil ecosystem. Phytoextraction or hyperaccumulation, one of the biological techniques, has been proposed as an eco-friendly in situ remediation technology for the contaminated soils (Tandy et al., 2006). However, the technique has several advantages and disadvantages as well (Erakhrumen and Agbontalor, 2007; Ghosh and Singh, 2005). Low biomass production in hyperaccumulator plants and susceptibility of root to high metal concentration lead to extension of researches on the use of microorganisms in order to develop application of phytoextraction and to make this method more feasible and economical (Glick, 2003; Ansari and Malik, 2007). The adequate restoration of the environment requires cooperation, integration and assimilation of biotechnological approaches along with traditional and ethical wisdom to conserve our natural resources (Mani and Kumar, 2014).

1.2. Microbe assisted phytoremediation

The use of rhizospheric microbes such as arbuscular mycorrhizal fungi (AMF) in phyto/bioremediation of heavy metal contaminated soils has attracted more attention recently. AMF provide direct links between soil and roots and consequently may have an essential contribution to plant growth by improving mineral nutrition and enhancing plant tolerance to stress (Gaur and Adholeya, 2004; Juwarkar and Singh, 2010; Banni and Faituri, 2013). Furthermore, AMF also affect metal uptake by plants from soil and translocation from root to shoot, however, mycorrhizal effects may depend on elements, plant and fungal species/ecotypes

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(Li and Feng, 2001). AMF *Glomus mosseae* and *G. intraradices* are effective in accumulation heavy metals in the root system and its phytotoxic effects, contributing to the phytoremediation of contaminated soils (Whitfield et al., 2004; Sivasankari et al., 2013). Chacko et al. (2009) found that *Pseudomonas putida* (*P. putida* MTCC 6809) exhibited the characteristics of plant growth promoting rhizobacteria (PGPR). It showed reasonably good production (4.2 $\mu g\ ml^{-1}$) of plant growth hormone indole acetic acid (IAA) and siderophore and was also found positive for the production of ammonia and catalase. The bacterium exhibited stimulation of root and shoot growth in presence of heavy metals. The organism is also tolerant to a number of heavy metals at higher levels. These characteristics make *P. putida* MTCC 6809 an excellent candidate for field application in contaminated soil (Pollmann et al., 2006; Spaepen et al., 2007).

1.3. Oilcake manure as bioresource tool

Earlier synthetic chelates were utilized for the clean-up of the contaminated environment (Bennedsen et al., 2012), however, the method had some limitations causing negative effects on the chemical, physical and biological properties of the soil, or might lead to groundwater pollution through leaching. To avoid some of these constraints, the use of elemental sulphur (S^0) to decrease soil pH and increase the solubility of heavy metals in soils was suggested (Kayser et al., 2000). However, organic inputs have always been preferred on account of greener, economic and more eco-friendly views (Mani and Kumar, 2014). Composting can enhance organic matter degradation and humification process, and consequently it can reduce the toxicity of metals (Singh and Kalamdhad, 2013). Oilcake manures (having N content >2 percent) are richer source of NPK than that of composts. It is partially humified and mineralized under the action of soil microflora which contributes to physical and biophysical components of soil fertility (FAO, 2006). Its biotechnological applications also include production of vitamins and antioxidants (Ramachandran et al. 2007; Vastag et al., 2011) in plants. Recently Mani et al. (2014a) prepared a hyperaccumulator oilcake manure which was used as an alternative for chelate induced phytoremediation of cadmium and lead from soils. In another study combined application of sulphur and vermicompost caused enhanced phytoremediation of lead through *Chrysanthemum indicum* L (Mani et al., 2014b).

1.4. Objective of the study

With the aforesaid perspectives regarding problems associated with cadmium and zinc contamination and its possible remediation through integrated micro-biochemical approach for the clean-up of the contaminated soil, the present study was implemented with an aim to explore the possibility of cleaning-up the Cd and Zn-contaminated soils through growing a field crop (oilseed plant), *Helianthus annuus* L (*H. annuus* L) during the year 2011–2013 at Sheila Dhar Institute Experimental Farm, Allahabad, India. The objectives of the present study were (1) to compare the dry biomass production and accumulation of Cd and Zn under different treatment combinations comprising of oilcake manure, S^0, AMF and *P. putida*, (2) to investigate the potential of *H. annuus* L for the enhanced phytoremediation of Cd and Zn through the different treatment combinations, and (3) to investigate the physico-chemical properties of the investigated soils after phytoremediation under the integrated micro-biochemical approach.

2. Material and methods

2.1. Plant material and experimental layout

The experimental sites are located between latitudes 25°20′–20°57′N and longitudes 81°52′–81°86′E and belong to the Indian tropical sub-humid region of Indo-Gangetic plain Allahabad, Uttar Pradesh, India. The soils of Gangetic plain are Alluvial Entisols having some recent origin. The mean texture of the experimental soil was silty clay loam (sand 49.06 ± 5.65 percent, silt 28.36 ± 6.53 percent and clay 22.45 ± 4.39 percent). The details of site-wise physico-chemical properties are given in Table 1a. Two sites namely Sheila Dhar Institute Farm uncontaminated soil (S_1) and Naini contaminated soil (S_2) are located in the agricultural farms receiving sewage-irrigation for more than 25 years spread around 36 km^2 of the urban and sub-urban area of Allahabad.

Applied oilcake manure was prepared from mustard seed 30 percent, rape seed 30 percent and sunflower seed 40 percent (Table 1b) (Mani et al., 2014a). Mustard cake, rapeseed cake and sunflower cake were purchased from market and finely crushed in a blender before use. The optimum moisture content of the investigated soil was maintained at 64 percent using sprinkler irrigation which is suitable for the enhanced enzymatic activities in soils. The de-oiled cake poses petroleum residue of about 0.2 percent. The oilcake manure was used in powdered form in order to reduce the residual petroleum substantially. It was incubated at 55 °C for 96 h prior to its application in pots.

The soil was ground to pass through a 2 mm sieve. Soil was sterilized by steam (at 121 °C) and used as a cultivation bed. Plastic

Table 1a
Physico chemical properties of experimental soils before and after phytoremediation with the integrated treatment.

Parameters	S_1		S_2	
	Before phytoremediation	After phytoremediation with integrated treatment (T_7)	Before phytoremediation	After phytoremediation with integrated treatment (T_7)
pH	7.8 ± 0.2	7.52 ± 0.13	7.6 ± 0.1	7.42 ± 0.11
EC ($dS\ m^{-1}$) at 25 °C	0.28 ± 0.03	0.27 ± 0.026	1.24 ± 0.03	1.19 ± 0.036
Organic Carbon ($g\ kg^{-1}$)	3.45 ± 0.15	4.89 ± 0.40	4.76 ± 0.15	5.73 ± 0.27
CEC [C mol (p^+) kg^{-1}]	19.6 ± 0.64	29.3 ± 0.79	22.1 ± 1.64	29.8 ± 0.79
Total N (%)	0.07 ± 0.01	0.08 ± 0.026	0.08 ± 0.01	0.09 ± 0.021
Total P_2O_5 (%)	0.036 ± 0.004	0.04 ± 0.007	0.038 ± 0.006	0.043 ± 0.005
Total Cd ($mg\ kg^{-1}$)	0.12 ± 0.01	0.082 ± 0.002	2.2 ± 0.10	1.36 ± 0.11
Total Zn ($mg\ kg^{-1}$)	19.6 ± 0.45	16.42 ± 0.38	39.4 ± 1.25	31.86 ± 1.08
Sand (%)	54.48 ± 2.46	54.51 ± 1.24	49.5 ± 3.25	49.8 ± 1.38
Silt (%)	20.86 ± 1.25	20.89 ± 0.57	32.8 ± 2.45	32.5 ± 0.76
Clay (%)	24.65 ± 2.76	24.6 ± 0.65	17.7 ± 1.75	17.7 ± 0.96

Note: S_1=Sheila Dhar Institute uncontaminated soil, S_2=Naini contaminated soil; ± values indicate standard errors having three replications, N=nitrogen, P_2O_5=phosphorus, EC=electrical conductivity, CEC=cation exchange capacity, Cd=cadmium, and Zn=zinc.