Journal of Oilseed Brassica, 10 (2) : 87-91, July 2019

M. Sc. Thesis Award

Enhancing metal solubility and phytoextraction in soil under Indian mustard

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(Received: 20 January 2019; Revised: 22 January 2019; Accepted: 4 February 2019)

Abstract

In phyto-extraction slow removal rate of metals by plant is the main constraint in the way of making effective use of this green technique in managing contaminated site. Hence, an attempt has been made to study the effect of selected organic and inorganic amendments on enhancing efficacy of phytoextraction of Zn, Cd and Pb in contaminated soil using Indian mustard (*Brassica juncea* cv. Pusa Vijay) as a test crop. Results indicated that Cd availability in soil was increased from 38.7 to 96.7% under different amendments, whereas Pb availability enhanced from 53.8 to 94.0%. All the amendments enhanced the EDTA and DTPA-extractable Zn, Cd and Pb in soil. Cadmium removal by shoot of mustard was enhanced to the extent of 125, 62.5, 175, 175 and 212% under green manure, EDTA, S+S oxidizing bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria treated soil, respectively over control. Root parameters *(i.e.* root length, diameter, surface area) also improved under different amendments. Application of green manure and metal solubilizing bacteria was the most efficient in enhancing the Zn, Cd and Pb removal by Indian mustard and phytoextraction from practical point of view.

Key words: *Biomass yield, metal removal, mustard, phytoextraction*

Introduction

Indian mustard (*Brassica juncea* L.) is an important oilseed crop in India, contributes maximum in domestic edible oils and being cultivated in 6.28 Mha with 7.46 Mt production and 1188 kg ha^{-1} of its productivity (Ministry of Agriculture, 2015). Indian mustard is one of the best heavy metal accumulators among the flowering plants. Mustard as a high biomass accumulating crop can remove high amount of different heavy metals like Pb, Cr, Se, Hg *etc*. (Kaur *et al.,* 2015). The expression of several metallothioneins and GSH gene, compartmentalization, biochemical degradation or inactivation from the active transport system by mustard makes it useful for phyto-extraction of different heavy metals. Heavy metal pollution has been considered as a serious problem. Release of metals from ores during the extraction has been considered as the key source, which enriches the environment. With the industrial development and disruption of natural biogeochemical cycles, problem of metal pollution is on the rise. Seriousness of metal pollution can be envisaged from the fact that unlike other organic pollutants, metals are not biodegradable. Mining, smelting, electroplating, use of pesticide and phosphate fertilizer, bio solids, industrial discharge *etc.* are some of the anthropogenic sources of metals (Rattan *et al.,* 2009). The accumulation of heavy metals in soil and water poses a risk to the environmental and human health by getting into the food chain (Meena *et al*., 2013). These elements accumulate in the body tissue of living organisms (bioaccumulation) and their concentrations increase as they pass from lower trophic levels to higher trophic levels (a phenomenon known as bio-magnification). In soil, heavy metal cause toxicological effects on microbes, this may lead to a decrease in their numbers and activities.Many heavy metals and metalloids are toxic and can cause undesirable effects and severe problems even at very low concentrations (Rattan *et al.,* 2009). Phytoremediation basically refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environments (Salt *et al.,* 1998). This approach of remediation of metal contaminated sites includes Phytoextraction, Phytodegradation, Rhizofiltration, Phytostabilization and Phytovolatilization *etc* (Table.1). Phytoextraction is a sub process of phytoremediation in which plants are used for uptake of contaminants from soil by plant roots and their translocation and accumulation in aboveground biomass, one of major problems associated with phytoextraction is lower metal removal rate (Blaylock, 2000). Phytoremediation, by using metal-accumulating plants like *Brassica* sp., including Indian mustard (*Brassica juncea*) for toxic metal removal from soil has been proposed as a possible solution to this problem. Hence, enhancing phytoextraction efficiency by using mustard as test crop constituted the frontier area of research.

Materials and Methods

For the present study, bulk surface soil (0-15 cm) was collected from Godwa, Udaipur district (Aridsol, alluvial), Rajasthan. The collected bulk soil was used for greenhouse experiment using Indian mustard (*Brassica juncea* cv. Pusa Vijay) as a test crop to evaluate the effect of green manure, EDTA, S+S oxidizing bacteria, metal solubilizing bacteria and green manure+ metal solubilizing bacteria on solubility and bioavailability of Zn, Cd and Pb in soil. Treatments comprised of control, where no amendment was applied, green manure (S*esbania)* @ 10 g per kg soil, EDTA @ 10.0 mM per kg of soil, elemental sulphur (S) @ 0.11 g per kg of soil + sulphur oxidizing bacteria (*Thiobacillus spp*.), Metal solubilising bacteria (*Pseudomonas spp*) and Metal solubilising bacteria + green manure (S*esbania*) both the microorganism were applied in solution form @ 2 ml per seed. All the treatments were replicated thrice under complete randomized block design. All the other agronomical practices followed uniformly in all the pots. Indian mustard was harvested after 60 days of sowing (DAS). Soil and plant samples were collected after harvesting of the crop. Post-harvest soil samples were processed and analysed for different parameters using standard methodology like pH and electrical conductivity (Jackson, 1973), organic carbon (Walkley and Black, 1934), dehydrogenase activity (Klein *et al.* 1971), as well as DTPA-extractable Zn, Cd and Pb (Lindsay and Norvell, 1978). Plant and root samples were processed and digested with HNO_3 using microwave digestion.

Computation of phytoextraction efficiency

The efficiency of phytoextraction can be determined by computing bio-concentration factor (Zhuang *et al.,* 2007)

$$Bioconcentration\ factor = \frac{Concentration\ of\ metal\ in\ harvested\ tissue}{concentration\ of\ metal\ in\ soil}$$

Here concentration of metal in harvested tissue indicate metals content in above ground plant parts and concentration of metals in soil indicate total concentration of metals in soil.

Results and Discussion

Initial characteristics of experimental soil

Collected bulk surface soil was characterized for important parameters, and results indicated that pH and electrical conductivity (EC) of experimental soil were 8.10 and 1.96 dS m^{-1}, respectively. Mechanical analysis of the soil indicates that experimental soil belongs to sandy clay loam textural class. Organic carbon content and cation exchange capacity (CEC) were 0.92% and 22.8cmol (p^+) kg^{-1}, respectively. Available N, P and K content in soil were 246, 20.0 and 100 kg ha^{-1}, respectively. Available S content in soil was recorded as 5.01 mg kg^{-1}. DTPA-extractable Zn, Cd and Pb were 12.2, 0.08 and5.3 mg kg^{-1}, respectively.

Biomass yield of mustard

After harvest of mustard, shoot and root dry matter were recorded. Dry matter yield of mustard was increased as 223, 113,167, 166 and 183% under green manure, EDTA, S+S oxidizing bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria treated pots, respectively over control (Figure. 1), with the corresponding figures for root as 135,87,120,141 and 158% (Figure 2). The highest shoot biomass was recorded in green manure treated pots, whereas the lowest shoot biomass was recorded with control pots. This may be explained based on the fact that plant growth was positively affected by release of growth promoting substances/ nutrients from added organic manure and microorganism (Shi *et al.,* 2016; Meena *et al.* 2017; Meena and Patel, 2018).

Metal removal

Zinc, Cd and Pb content were determined in above ground biomass and calculated the total removal by mustard at 60 DAS. Zinc removal by Indian mustard was enhanced by 277, 202, 213, 243 and 284% under green manure, EDTA, S+S oxidizing bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria, respectively over control (Figure 3). Cadmium removal by mustard was enhanced to the extent of 125, 62.5, 175, 175 and 212 % in green manure, EDTA, S+S oxidizing bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria treated soil, respectively over control.More or less similar trend with different magnitude were observed for Pb also.

Root parameters

Whole root system was removed from the each pot after harvest of the mustard, and wash properly, and root parameters were recorded using root scanner. Results revealed that root length was 23.1, 120, 116, 161, 91.4 and 71.0 cm when mustard grown on control, green manure, EDTA, S+S oxidizing bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria treated pots, respectively (Table. 2). The corresponding value of total surface area were 43.2, 74.5, 64.6, 73.9, 78.9 and 89.2 cm^2 respectively. The projected surface area were 13.8,

Table 1: Different phytoremediation processes

Process	Mechanism	Contaminant	Typical plant
Phytoextraction	Hyperaccumulation	Metals (Pb, Cd, Zn, Ni, Cu) with EDTA addition for Pb, Se	Sunflower, Indianmustard, rapeseed plant
Rhizofiltration	Rhizosphere accumulation	Metals (Pb, Cd, Zn, Ni, Cu) Radionuclides(137Cs, 90Sr, 238U), Hydrophobic organics	Aquatic plants : (pond weed, duckweed); *Hydrilla*
Phytostabilization	Complexation	Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U), Hydrophobic organics (PAHs, PCBs, dioxins, furans, pentachlorophenol, DDT)	Phreatophytetrees totranspire large amounts of water for hydraulic control
Phytovolatilization	Volatilization byleaves	Mercury (Hg), selenium (Se), Tritium ($_1H^3$)	Poplar, Indianmustard,canola
Phytodegradation	Degradation in plant	Herbicides (atrazine,alachlor), Aromatics(BTEX), Chlorinated aliphatics	Phreatophyte trees,poplar, willow, sorghum, clover

Source: Mukhopadhyay and Maiti (2009)

Table 2: Effects of EDTA, elemental sulphur, microorganisms and green manure on different root parameters

Treatment	Root length (cm)	surface area (cm^2)	projected area (cm^2)	volume (cm^3)	average diameter (mm)
Control	23.1	43.2	13.8	6.43	5.95
Green manure	120	74.5	23.7	6.92	1.98
EDTA	116	64.6	20.6	2.76	1.71
S+S oxidizing bacteria	161	73.9	23.5	2.89	1.46
Metal solubilizing bacteria	91.4	78.9	25.1	5.43	2.75
Green manure + metal solubilizing bacteria	71.0	89.2	28.4	8.92	4.00

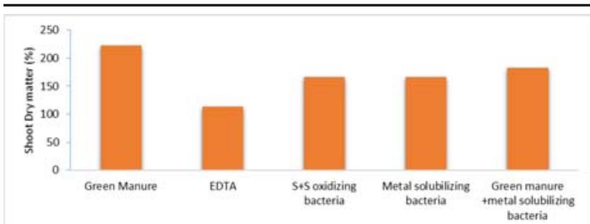

Figure 1. Per cent improvement in shoot dry matter of Indian mustard over control

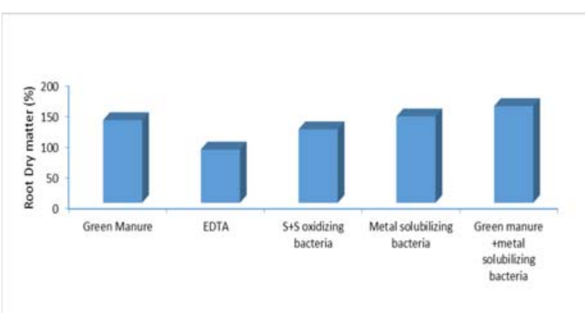

Figure 2. Per cent improvement in root dry matter of Indian mustard over control

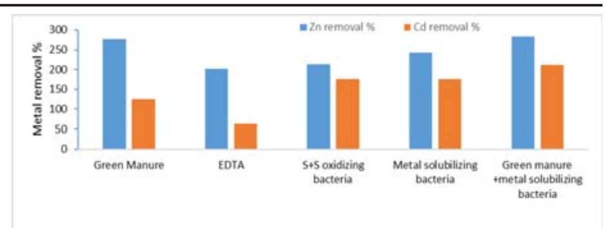

Figure 3. Per cent removal of zinc and cadmium by Indian mustard over control

Table 3: Effects of EDTA, elemental sulphur, microorganisms and green manure on dehydrogenase activity in soil

Treatments	Dehydrogenase activity (µg TPF g^{-1} $24h^{-1}$)
Control	79.8^{e}
Green Manure	129^{c}
EDTA	108^{d}
S+S oxidizing bacteria	131^{c}
Metal solubilizing bacteria	140^{b}
Green manure +metal solubilizing bacteria	155^{a}
LSD (P = 0.05)	7.63

23.7, 20.6, 23.5, 25.1 and 28.4 cm^2 respectively. Root volume and diameter varies between 2.76 to 8.91 cm^3 and 1.46 to 5.94 mm, respectively. Highest root length was found in S+S oxidizing bacteria treatment and root diameter was highest in control.

Soil parameters

Dehydrogenase activity (DHA)

Dehydrogenase activity (DHA) in soil after the harvest of Indian mustard was significantly affected due to application of all the amendments used in order to enhance the solubility of metals in soil. Dehydrogenase activity increased from 79.8 (in control) to 129, 108, 131, 140 and 155 µg TPF g^{-1} $24h^{-1}$ in soil due to addition of green manure, EDTA, S+S oxidizing bacteria, metal solubilizing bacteria and green manure +metal solubilizing bacteria, respectively (Table. 3). Usually organic compounds undergo biological oxidation through dehydrogenation process. Specific dehydrogenase system govern the dehydrogenase activity of soil which are integral part of microorganism therefore assessment of dehydrogenase activity in soil shows average activity of microbial population. This results show that activity of microorganism in amended soils was enhanced where ever organic manure was added. Besides, amended soils showed where ever population of microorganism was enhanced by adding microbial culture, there was concurrent increased in dehydrogenase activity as well (Abhilash *et al.*,2012). Addition of EDTA also enhanced the dehydrogenase activity in soil probably due to reduction of metal toxicity through formation of metal chelate complex. Another plausible reason may be related to addition of organic substance through EDTA itself leading to proliferation of microorganisms.

DTPA-extractable metals in soil after harvesting of Indian mustard

DTPA-extractable Zn was found as 167, 322, 171, 123 and 160% in soil under green manure, EDTA, S+S oxidizer bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria in treated soil, respectively; the corresponding value of Cd were 200, 427,172, 163, and 172% (Figure 4). DTPA extractable Pb content in soil were 147, 197, 120, 118 and 131% in green manure, EDTA, S+S oxidizer bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria, respectively. This is in line with the results of obtained by various researchers (Ray *et al.,* 2013; Mishra *et al.,* 2019).

Bio-concentration factors

Bio-concentration factor (BCF) indicates the efficiency of a plant species in accumulating a metal into its tissues from the surrounding environment. The value of BCF for Zn varies from 0.23 to 0.14, highest value of BCF obtained in EDTA treatment (0.41) followed by green manure + metal solubilizing bacteria (0.36). BCF for Cd and Pb varies from 0.93 to 1.59 and 0.02 to 0.06 respectively (Table 4). The results from the present study showed that all tested plants had low Pb bio-concentration factor values, indicating that plants had difficulties in mobilizing Pb in the root zone in comparison with Zn and Cd (Meena et al. 2016).

Table 4: Effect of EDTA, elemental sulphur, microorganisms and green manure on bio-concentration factor of zinc, cadmium and lead grown in pot experiment

Treatments	Zn	Cd	Pb
Control	0.23	0.93	0.02
Green Manure	0.32	1.06	0.02
EDTA	0.41	1.37	0.06
S+S oxidizing bacteria	0.30	1.54	0.04
Metal solubilizing bacteria	0.34	1.59	0.05
Green manure + metal solubilizing bacteria	0.36	1.57	0.05

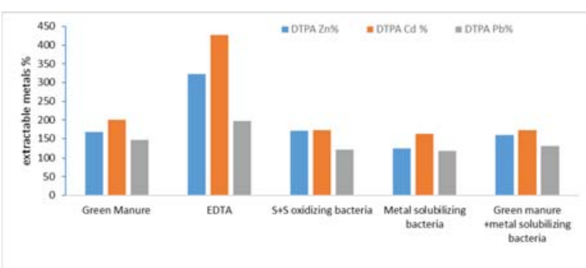

Figure 4: Effect of EDTA, elemental sulphur, microorganisms and green manure on enhancement (%) in DTPA-extractable zinc, cadmium and lead in soil over control

Conclusion

Addition of amendments, *viz*. green manure, S + S oxidizing bacteria, metal solubilizing bacteria and green manure + metal solubilizing bacteria could improve biomass yield, plant metal uptake and improve DTPA extractable metal and soil biological quality in terms of dehydrogenase activity.

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