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Phytoremediation Potential of Few Natural Plants to Decontaminate Distillery Spent Wash Polluted Soil

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ABSTRACT: Due to complexation of Endocrine-Disrupting Chemicals (EDCs) and mixture of organic pollutants sugarcane dependent distillery discharge is a threat to environment for its safe disposal. The distillery sludge contains mixture of complex organic pollutants as well as high quantity of Pb, Mn, Zn, Fe, Cu and Ni which enhance the toxicity of discharge to the environment. Concentrations of nine metals *i.e.*, Mg, Cd, Mn, Cr, Fe, Cu, Ni, Zn and Pb in soils and natural herbs species *Cannabis sativa* and *Argemone maxicana* collected from distillery spent wash discharge sites were investigated. This indicated high accumulation and translocation potential of these plants. The bioaccumulation and transfer of metals from soil to plants was evaluated in terms of Bioconcentration Factor (BCF). Further, the bioconcentration factor and translocation factor (TF) for various metals were found greater than one for these plants. This has provided robust evidence for a hyper accumulatory tendency for these plants as well as, indicating the heterogeneous adaptive properties of these plants grown in a highly polluted site. The polluted site became a challenge for the survival of human being and also the environment. Thus, our aim was to define that which plant species exhibit the accumulation potential and evaluate whether these species could be usefully employed for phytoremediation. Phytoremediation is an economic, efficient and unique eco-friendly modern technology. Our results indicated that both *Cannabis sativa* and *Argemone maxicana* are accumulator for the studied heavy metals.

Keywords: Distillery discharge, Phytoremediation, Translocation Factor, Bioconcentration Factor, Bioaccumulation.

INTRODUCTION

In the modern world, the large-scale industrialization and manufacturing of a number of chemicals has created a global deterioration of the quality of environment. In India, major cities have seen an increase in pollution over the last few decades due to an increase in the number of people and industrial activities, which is likely to result in an increase in the amount and number of pollutants discharged into the surrounding environment. There are over 397 distilleries in India that produce molasses based on sugarcane. Each of these distilleries releases around 3.5 – 1013 kilolitres (kL) of spent wash per year. On average, 1500 tons of sludge are produced per day during the anaerobic breakdown of spent wash (Chandra *et al.*, 2018). This figure reflects the level of environmental pollution generated by the waste produced by the distillery sector across the country. It is regulated to treat this waste water prior to disposal; however, due to the high cost of effluent treatment and sophisticated physico-chemical techniques, only partial treatment is done and large amount of the waste water is either stored in lagoon dams, unlined tanks, or discharged into surface water bodies and streams, compromising the quality of water resources and the environment (Karanam & Joshi 2010). It is a matter of serious concern for the metal to persist in soil for much longer duration than in the other parts of the biosphere. According to Beyermann and Hartwig (2008) heavy metals like Cd, Cr, Ni, Pb setc., have been classified as carcinogenic to humans and animals. Recently, a lot of work has been done to find low-cost technologies to clean up soil contaminated by heavy metals. (Chatterjee *et al.*, 2011). Therefore, plants can be used to ameliorate heavy metal pollutants from the soil. This cost-effective approach is called phytoremediation which also referred as green solution (Butcher, 2009). Currently, phytoremediation has become a matter of public and scientific interest as well as a topic of many researches. So, vegetation plays an increasingly important ecological and sanitary role for chemically polluted lands (Antonkiewicz and Jasiewicz 2002).
Several authors include the bioaccumulation factor (BAF or BCF) as an element for classification of species as a hyper accumulator species. According to

Antonsiewicz *et al.* (2008); Yoon *et al.* (2006), phytoremediation should favor native plants because native plants are more likely to survive, grow and reproduce under stress compared to imported plants. This is why the search for native heavy metal tolerant plants is so important. Few studies evaluated the potential for phytoremediation of native plants (Ginocchio and Baker 2004) under field conditions. Khairia M. Al-Qahtani (2012) found that the heavy metals concentration in soils have the order of (Fe > Zn >Cr >Cu > Pb > Ni > Cd) while in plants (*Calotropis procera*, *Cyperus laevigatus* and *Argemone maxicana*) the tendency was (Fe > Zn >Cu >Cr > Ni > Pb > Cd). This concept, combined with the public's concern about soil contamination due to heavy metals in industrial areas in the city of Riyadh in Saudi Arabia, necessitates the need to look for plant species that can be used for phytoremediation, as there are currently no reports of plant species that are tolerant to heavy metals and that are hyperaccumulators of heavy metals that can be used in this area. Therefore, two aims of this study were: first to evaluate the concentrations of Cd, Cr, Cu, Fe, Pb, Ni and Zn in soils and two plant species (*Cannabis sativa* and *Argemone maxicana*) and second to evaluate whether these species could be usefully employed in phytoremediation.

MATERIALS AND METHODS

The most valuable industries in western U.P. are: metal industries, food industries and agro-based industries such as paper mills, distilleries etc. Soil samples were collected at nearby discharge outlet from three sites each of, Daurala distillery, Bajaj Hindustan distillery & Central distillery of district Meerut separately at 10cm, 20cm & 30cm depth. Soil samples of each site with same depth were mixed and replicates were used. The plant sampling was done at the peak of the season around each distillery discharge site. Three plants of the species were selected at a time from each distillery and the mean of three observations was considered as final. The samples were dried for nearly 48 hours at 85°C in an oven and the reduced to powder in electric grinder. Then they were kept in polythene bags to avoid any loss in fresh weight.

Heavy Metal Analysis. Heavy metals were then analyzed by direct aspiration of the soil and plant sample solution into an Atomic Absorption Spectrophotometer (AAS). The Bioconcentration Factor (BCF) was calculated as metal concentration ratio of plant root to soil as given in the following formula

BCF = Metals in plant/Metals in soil.

RESULTS AND DISCUSSION

Metal levels fall as the depth of the soil increases. Distillery spent wash contains these metals due to metalliferous ore smelting, pesticides, fertilizers and municipal waste. Chandra *et al*., (2018) also found that distillery sludge contains high quantity of Fe, Zn, Cu, Mn, Ni and Pb. As indicated by Kumar *et al.* (2011) the pH of soil was acidic to slight alkaline in nature and varies from 6.7-7.6. The contamination of soil with heavy metals showed several issues, including phytotoxic effects of certain metals such as Cu, Pb, Cd, and Zn (also known as micronutrients) and if these are beyond the critical endogenous level these cause several phytotoxicities (Susarla *et al*., 2002; Chehregani *et al*., 2005). Another and even more serious issue is the accumulation of potentially harmful elements in food or forage plants and their transportation through the food chain and ultimately to humans. At high concentration all the heavy metals have robust toxic effects and are stared as environmental pollutant. One of the emerging technologies is the use of different plants for environmental remediation. In this technique, plants with high metal content are grown in polluted soil (Lasat, 2002). Interest in phytoextraction has significantly grown following the identification of metal accumulator plants.

Table 1: Metal composition in soil at distillery discharge site in relevant seasons.

Site	Cu	Cr	Pb	Fe	Mn	Zn	Cd	Mg	Ni
DDS	2.976 ±0.142	—	1.985 ±0.135	27.820 ±2.115	23.083 ±2.321	8.208 ±0.821	0.238 ±0.012	172.407 ±8.421	0.790 ±0.021
CDS	2.906 ±0.135	0.521 ±0.012	2.557 ±0.143	29.269 ±2.135	27.520 ±2.561	10.656 ±0.921	0.299 ±0.018	188.952 ±9.878	0.724 ±0.027
BHDS	2.998 ±0.121	—	—	27.432 ±2.005	24.996 ±2.009	9.568 ±1.081	0.325 ±0.021	113.891 ±6.782	0.654 ±0.024
DDW	2.588 ±0.154	—	1.972 ±0.132	25.332 ±1.980	22.632 ±1.980	6.666 ±0.342	0.168 ±0.009	154.785 ±8.910	0.748 ±0.035
CDW	2.632 ±0.134	0.416 ±0.018	1.956 ±0.121	25.868 ±1.421	25.810 ±2.086	8.961 ±0.256	0.186 ±0.012	180.837 ±10.521	0.536 ±0.012
BHDW	2.870 ±0.209	—	—	25.516 ±2.009	24.573 ±1.891	6.956 ±0.412	0.316 ±0.027	111.045 ±5.621	0.530 ±0.021

DD=Daurala Distillery, CD=Central Distillery, BHD=Bajaj Hindustan Distillery, S= Summer, W=Winter

The analysis of soil and plant exposed that the accumulation is noticeably the consequence of type of elements (Dermirezen, 2002). *Cannabis sativa* was found dominant in summer season at all the sites. This plant shows highest concentration of available Mg (240.481 ppm) followed by Fe (39.833 ppm), Mn (38.550 ppm), Zn (18.102 ppm), Cu (5.410 ppm), Lead (4.950 ppm), Ni (1.229 ppm) and Cd (1.009 ppm) with Bioconcentration factor of 1.395, 1.432, 1.67, 2.205, 1.828, 2.494, 4.239 and 1.556 respectively at Daurala distillery site. *Argemone maxicana* has highest concentration of available Mg (221.819 ppm) followed by Fe (34.059 ppm), Mn (30.241 ppm), Zn (15.196 ppm), Cu (4.556 ppm), Lead (3.473 ppm), Ni (0.991 ppm) and Cadmium (0.788) with Bioconcentration factor of 1.433, 1.344, 1.336, 2.279, 1.760, 1.761, 1.325

and 4.690 respectively in winter season at this site. Thus, *Argemone maxicana* also has highest Bioconcentration factor for cadmium. The Bioconcentration factor in *Argemone maxicana* for Cd is followed by zinc and lead.

During winter season at Central distillery *Argemone maxicana* has highest concentration of available Mg (255.382 ppm) followed by Fe (36.271 ppm), Mn (34.716 ppm), Zn (17.24 ppm), Cu (5.586 ppm), Pb (3.994 ppm), Cr (1.841 ppm), Ni (1.450 ppm) and Cd (1.253 ppm) with Bioconcentration factor of 1.412, 1.402, 1.345, 1.924, 2.122, 2.042, 4.425, 2.705 and 6.736 respectively. During summer season at Central distillery *Cannabis sativa* has highest concentration of available Mg (277.61 ppm) followed by Fe (42.656 ppm), Mn (41.695 ppm), Zn (22.502 ppm), Cu (6.195 ppm), Pb (4.775 ppm), Ni (1.655 ppm), Cr (1.318 ppm) and Cd (1.248 ppm) with Bioconcentration factor 1.469, 1.457, 1.515, 2.112, 2.132, 1.867, 2.286, 2.574 and 4.174 respectively.

Argemone maxicana has highest concentration of available Mg (173.273 ppm) followed by Fe (38.882 ppm), Mn (32.613 ppm), Zn (13.818 ppm), Cu (5.122 ppm), Ni (0.957 ppm) and Cd (0.715 ppm) with Bioconcentration factor 1.56, 1.524, 1.327, 1.986, 1.785, 1.805 and 2.263 respectively at Bajaj Hindustan distillery. During summer season *Cannabis sativa* was found dominant at Bajaj Hindustan distillery with highest concentration of available Mg (187.142 ppm) followed by Fe (41.795 ppm), Mn (39.223 ppm), Zn (18.817 ppm), Cu (5.557 ppm), Ni (1.273 ppm) and Cd (0.994 ppm) with Bioconcentration factor of 1.643, 1.524, 1.569, 1.967, 1.853, 1.946 and 3.058 respectively. This plant has highest Bioconcentration factor for Ni (3.024) followed by Zn (2.023) at this site.

The results of Malik *et al.* (2010) showed that majority of plant species have accumulated higher concentration of Pb, Zn and Cu than usual limits in the shoots. The concentrations of the investigated heavy metals in soil dominated with *Cannabis sativa* as well as in plant also the sequence is Fe >Mn >Zn >Cu>Pb>Ni>Cr> Cd. However, the concentrations of the investigated heavy metals in soil dominated with *Argemone maxicana* possess the sequence like Fe >Mn >Zn >Cu> Pb >Ni>Cr>Cd while in plants the trend was slightly different (Fe >Mn >Zn >Cu >Pb>Cr> Ni > Cd). Chandra *et al*., (2018) also found the phytoextraction potential in *Argemone maxicana*.

Table 2: Metal compositions in *Cannabis sativa* and *Argemone maxicana* at distillery discharged sites during relevant seasons.

Site	Dominant species	Cu	Cr	Pb	Fe	Mn	Zn	Cd	Mg	Ni
DDS	*C. sativa*	5.410 ±0.842	-	4.950 ±0.695	39.833 ±2.652	38.550 ±3.658	18.102 ±1.951	1.009 ±0.012	240.481 ±9.056	1.229 ±0.053
CDS	*C. sativa*	6.195 ±0.781	1.318 ±0.235	4.775 ±0.521	42.656 ±2.891	41.695 ±3.891	22.502 ±1.927	1.248 ±0.021	277.61 ±10.612	1.655 ±0.062
BHDS	*C. sativa*	5.557 ±0.921	-	-	41.795 ±3.021	39.223 ±2.391	18.817 ±1.821	0.994 ±0.027	187.142 ±8.0521	1.273 ±0.045
DDW	*A. maxicana*	4.556 ±0.342	-	3.473 ±0.215	34.059 ±1.890	30.241 ±1.226	15.196 ±1.252	0.788 ±0.024	221.819 ±8.987	0.991 ±0.009
CDW	*A. maxicana*	5.586 ±0.217	1.841 ±0.128	3.994 ±0.321	36.270 ±1.996	34.716 ±2.009	17.024 ±1.121	1.253 ±0.038	255.38 ±9.0524	1.450 ±0.036
BHDW	*A. maxicana*	5.122 ±0.233	-	-	38.882 ±2.095	32.613 ±1.651	13.818 ±1.009	0.715 ±0.033	173.273 ±6.045	0.957 ±0.048

DD=Daurala Distillery, CD=Central Distillery, BHD=Bajaj Hindustan Distillery, S= Summer, W=Winter

Table 3: Bioconcentration factor in *Cannabis sativa* and *Argemone maxicana* at distillery discharge sites during relevant seasons.

Site	Dominant species	Cu	Cr	Pb	Fe	Mn	Zn	Cd	Mg	Ni
DDS	*C. sativa*	1.818	-	2.494	1.432	1.670	2.205	4.239	1.395	1.556
CDS	*C. sativa*	2.132	2.530	1.867	1.457	1.515	2.112	4.134	1.469	2.286
BHDS	*C. sativa*	1.854	-	-	1.524	1.569	1.967	3.058	1.643	1.947
DDW	*A. maxicana*	1.760	-	1.761	1.345	1.336	2.280	4.690	1.433	1.325
CDW	*A. maxicana*	2.123	4.425	2.042	1.402	1.345	1.900	5.661	1.412	2.705
BHDW	*A. maxicana*	1.785	-	-	1.524	1.327	1.985	2.263	1.560	1.806

DD=Daurala Distillery, CD=Central Distillery, BHD=Bajaj Hindustan Distillery, S= Summer, W=Winter

Cd is a toxic element found in nature in association with Zn. The concentration of Cd in soils is generally low. The highest concentration of Cd was found at a site that was dominated by *Argemone maxicana*. This is due to the high pH value which increases the precipitation of Cd at this site (El-Rayis and El-Sabrouti 1997). These author found that highest uptake of Cd was accomplished by *Calotropis procera* stem followed by *Argemone maxicana*.

Chromium (Cr) is not an essential element for plant growth, and it is possible that plants have no specific mechanism and transport for Cr (Shanker *et al.*, 2005). Usually, soils of the selected spots in the area under investigation acquired absence of Cr except central distillery spent wash discharge site associated with *Cannabis sativa* and *Argemone maxicana*. Outcomes from the present study displayed that root of all plants achieved higher Cr concentrations. This may be due to the fact that Cr is stable in the root cell vacuoles and exhibits reduced translocation, making it less toxic. This may be a neutral toxicity response of the plants (Macnicol and Beckett 1985). According to him the noxious levels of Cr range from 1 to 10 µg/g dry weight in plants.

Although, Copper (Cu) is an indispensable element for plants and animals but, extreme concentrations are measured to be highly toxic. Cu concentrations in plants above 10-30 μg/g d.w. are regarded as poisonous (Macnicol and Beckett 1985). Iron (Fe) is an essential micronutrient for plants and animals (Kunze *et al.*, 2001). Over-abundance of Fe can cause toxicity. The highest concentration was found in the soil of the central distillery site. Industrial discharges from the nearby industrial complex may affect the concentration of Fe in the soil. Plant analysis showed that roots of all plant species are highly susceptible to Fe accumulation. According to Allen (1989), iron concentration above 40-500 μg/gd.w.is considered as toxic for plants. The capability of roots to reduce Fe^{+3} to Fe^{+2} is believed to be fundamental in the absorption of this cation by most plants (Tinker, 1981). Higher concentrations of Fe in the roots of the investigated species could be due to its precipitation in iron- plaque on the root surface (Tanner, 1996; Batty *et al.*, 2002).

The least mobile heavy metal is lead (Pb). Lead is not a necessary heavy metal, but it is poisonous to plants. Studies have shown that Pb can be transported and absorbed within a plant under specific conditions (Meers *et al.,* 2005). Blaylock and Huang (2000), also observed that shoot Pb concentrations were found to be comparable to the concentration of intact roots in the same species when submerged in a nutrient-rich Pb solution. In general, soil and plant Pb concentrations were significantly higher at different sites. This may be attributed to airborne deposition of Pb from a high-traffic road that impacted the open area. Ross (1994) found that 30-300 μg/g Pb concentrations are considered to be toxic to plants. The higher the translocation of Pb, the higher the Pb concentration in the shoot. Plants that translocate more Pb will produce higher Pb concentrations in shoot. These plants are considered to be "potential candidates" for Pb phytoextraction programs. This is because only shoots are harvested during Pb phytoextraction, which emphasizes the importance of selected species as accumulators of Pb (Huang *et al.*, 1997).

Zn is an important trace element, but high levels of Zn can have adverse health consequences. The highest concentrations of Zn found in soil in the area were between 6.666 and 10.656 parts per million (ppm) at these locations. The highest toxic levels found in various plants were between 100 to 500 parts per million d.w. (Waganov and Nizharadze 1981). The highest Zn concentration in the root (22.502 ppm) was attained by *Cannabis sativa* of central distillery unit. The rootsare thought to be important for zinc uptake (Aubert and Pinata 1997). It was also found that the highest levels of zinc found in the roots of the two native plants (including *Cannabis sativa*) were also associated with high levels of zinc in the soils at the site. Previous studies on the deposition of different metal ions in native plants have demonstrated that the majority of metals were deposited in the roots compared to other parts of the plant (Zaranyika and Ndapwadza 1995; Chandra and Kulshreshtha 2004). The results obtained by Aboulroos *et al.* (1996) showed that Zn content of plant amplified with increasing levels of Zn in the soils. The research done by Kandil *et al.* (2003) found highly noteworthy correlations between the soil content of micro as well as macro-nutrients and heavy metals and their accumulation in the root of plants.

The manganese is required for photosynthesis and other process of plants. The concentration of Mn was found highest at CD (27.520 ppm) and lowest at DD (22.632 ppm). *Cannabis sativa* shows higher concentration of this metal. Devarajan (l996) found that the available micronutrients *viz.,* Zn, Fe, Cu and Mn of the post-harvest soil were increased from 2.2 to 3.9 ppm, 22.9 to 31.6 ppm, 4.1 to 7.3 ppm and 15.5 to 25.8 ppm respectively due to fertigation with 10 times diluted distillery effluent. Baskar (2001) reported that the available micronutrients *viz.*, Fe, Mn, Zn and Cu were progressively increased by the graded levels of distillery effluent and the availability being maximum with the application of distillery effluent @ 2.5 lakh litres per acre. They also stated that increased availability might be due to direct contribution from the effluent as well as solubilization and chelation effect of organic matter supplied by the effluent.

Nickel is widely used as a catalyst in chemical and food industries, as a prime material for paint and battery reduction, and in electroplating. According to Kabata-Pendias and Pendias (1984), The normal Ni content in land plants growing in non-contaminated soils ranged from 0.1 to 3.7 Ug/g. Our results showed that the Ni concentration of the investigated species was higher than that of the normal plant. This indicates that these plants have a high tolerance for this element. The heavy metal concentrations in soil growing *Cannabis sativa* and *Argimone maxicana* increase in the pattern of "Fe> Mn> Zn>Cu>Pb> Ni> Cr> Cd". However, the heavy metal concentration in roots of the investigated plants increases in the pattern Fe> Mn> zn> Cu> Pb> Cr> Ni> Cd. This may suggest that all metals originate from the same source of contamination. Plaque increased the concentration of 5 metals (Cu> Cr> Zn> Pb> Ni> (Sundby *et al.*, 1998) with high pH conditions (> 7.0) which enhanced metals uptake into roots (Weis and Weis, 2004).

Bioconcentration factor. The amount of selected metals a plant accumulates varies significantly from species to species and the extent to which a plant absorbs an element is largely dependent on the species, its natural controls and the quality of the soil (Chunnilal *et al.*, 2005). There are a large number of factors that influence metal buildup and bioavailability in soil and climate, in plant genotype, and in agronomic control, such as: active/passive transfer mechanisms, sequestration, speciation, oxidation states, plant root system, and how plants respond to elements in terms of seasonal cycles (Kabata-Pendias and Pendias 1984). Sediment structure has also been identified as a critical factor that influences the amount of metals the plants absorb. Soil metal solubility is largely determined by soil pH and system oxidation state (Ghosh and Singh 2005). According to Sherene (2010) the factors influencing mobility and adsorption of heavy metals in

soil are pH, organic matter, soil texture, pore structure, temperature, ionic strength, phenolic substances etc.
The results showed that the soil of study area was sandy in texture and neutral in composition with a pH > 6.7. Neutral and high pH can stabilize soil toxic elements resulting in reduced leaching of the soil toxic elements. Toxins may also stabilize due to slightly base soil pH which results in less element concentration in soil solution which may limit absorbality of elements from soil solution into plant tissues.

Majority of plant species under investigation had BCF >1, though the heavy metals remained below 1000 ppm concentration. In general, BCF values of Cd, Cu, Ni and Zn were higher as compared to other metals. If you have soil that's been contaminated with heavy metals, you can use species that are tolerant to heavy metals and have a high bioconcentration factor (BCF) which help to keep the soil healthy. This is because the metals stay in the roots and stop them moving from the roots to the shoots once they've been absorbed by the roots (Cui *et al.*, 2007).

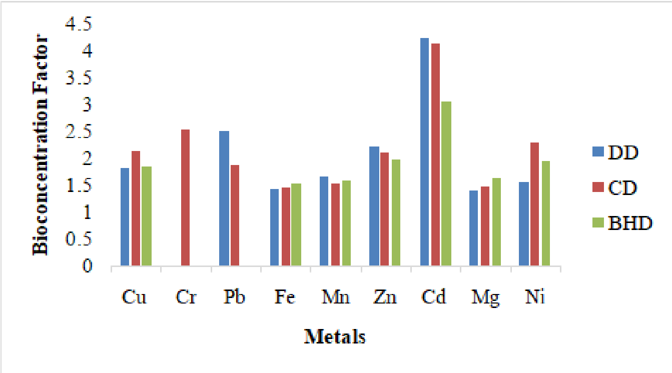

Fig. 1. Graph showing comparative Bioconcentration Factor in *Cannabis sativa* during summer season at three sites.

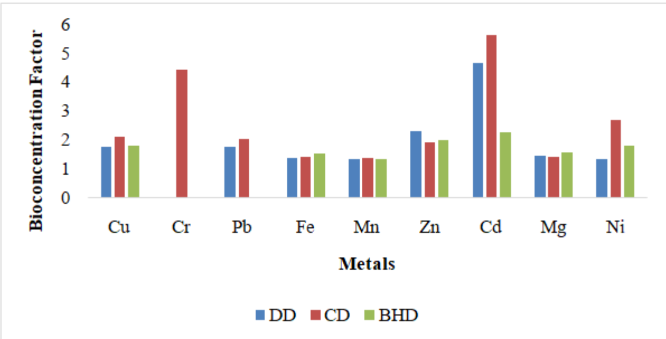

Fig. 2. Graph showing comparative Bioconcentration Factor in *Argemone maxicana* during winter season at three sites.

High metal concentration can be explained by a well-defined detoxification pathway that involves the sequestration of high metal ions in the vacuoles by attaching them to suitable ligands (*e.g.*, organic acids, protein and peptides) in the presence of enzymes capable of working at high concentrations of metallic ions (Cui *et al.*, 2007) and metal elimination approaches of plant species (Ghosh and Singh 2005). Plant species having high transfer values were found suitable for phytoextraction usually requires translocation of heavy metals in easily harvestable plant parts *i.e.*, shoots (Yoon *et al.*, 2006 and Berjuei *et al.*, 2015). According to Ghosh and Singh (2005) phytoextraction isa process to eradicate the contamination from soil without abolishing soil structure and fertility. Chandra *et al.*, (2018) found that BCF valueis more than one for various metals in *Argemone maxicana.* The results of this study indicated that both plants have relatively low BCF for Fe (2.4–4.0) compared with other metals. The high concentration of Fe in the roots of the investigated plants and the low translocation in the above ground parts indicated that these plants are suitable for phytostabilizing this element in the area studied.

CONCLUSIONS

Outcomes indicated that *Cannabis sativa* and *Argemone maxicana* are accumulator for the studied heavy metals. In soils as well as in both the plants the concentrations of heavy metals have the sequence of “Fe> Mn> Zn >Cu >Pb> Ni >Cr> Cd. Except Cd, roots of both the plants show high concentrations of all these metals, and thus are the best biomonitor’s for heavy metal pollution in the site under investigation.BCF (Bioconcentration Factor) values were highest for Cd and Zn, and lowest for Pb and Ni. These plant species could be considered hyper-acumulators and phytoextracted. However, they had relatively low BCFs for Fe compared with other metals. The high concentration of Fe in the roots of the studied plants and the low translocation in the above ground parts indicated that they are suitable for phytostabilizing this element in the area studied. The results of this study suggest that these plant species may be suitable options for phytoremediation.

Biotechnologically and genetically engineered approaches can be employed to improve naturally occurring plants to decontaminate hazardous compounds. The authors have the opinion that use of natural plants for depolluted contaminated site must be investigated further in favor of environment.

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