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Metal decontamination of tannery solid waste using *Tagetes patula* in association with saprobic and mycorrhizal fungi

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Abstract A greenhouse trial was conducted to investigate the role of mycorrhizal and resistant fungi on heavy metal phytoextraction from different concentrations of tannery solid waste amended soil (10, 20, 50, and 100%) by *Tagetes patula*. The four treatments included were, the control (C) without any inoculum, mycorrhizal (M) inoculated with strongly mycorrhizal roots of *Cynodon dactylon*, fungal (F) inoculated with *Trichoderma pseudokoningii* and the combined inoculation with both mycorrhizal and fungal inocula (M + F). The dual inoculation increased plant biomass and phytoextraction ability of plant for metals like Cd, Cr, Cu, and Na. Plants given only fungus (F) and only mycorrhizal (M) treatment also showed significant growth rate as compared with control treatment. The statistical analysis of data indicated synergistic interaction between mycorrhizal and fungal inoculum promoting high biomass and enhanced metal phytoextraction. Thus using more than one group of rhizosphere fungi in association with a high biomass producing plant may be employed for rendering tannery solid waste free of metals.

Keywords Phytoextraction · Toxic metals · Arbuscular mycorrhizal fungi · Saprobic fungi

1 Introduction

The leather industry is one of the major sources of environmental pollution in Pakistan. The worst condition is that of Kasur, being the biggest tanning concentration per unit area in the country. Tanning is a complicated and laborious process that can involve over 130 different chemicals, depending on the type of raw material used and the finished product. The discharge of untreated effluents from tanneries is a growing problem in Pakistan (Khan 2001). The presence of excess sodium and chromium in tannery waste is very dangerous for human health, which enters through the food chain. It is, therefore, essential to remove the contamination before disposal.

Presently, there is no proper solid waste disposal system in the tanneries and even at the Kasur Tannery Waste Management Agency (KTWMA) treatment plant situated at Depalpur Road, Kasur, Pakistan. The solid waste obtained from the tanning industries is being dumped into a landfill site without taking the environment into consideration. This method has so far been considered a low-cost solution. However, it merely shifts the contamination problem elsewhere along with all the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into the adjacent environment and making the land useless.

The contamination of surface and groundwater by the leaching of solid wastes generated by industrial activities as a result of water runoff and rainfall is another matter of great concern. The leachates from tannery solid waste (TSW), a major environmental pollutant, were examined by Chandra et al. (2004) who found that the chief constituents of this type of waste were chromium and other metals, which is thought to cause genetic abnormalities.

Management techniques such as isolation, cleansing, and inerting are three conventional treatments for metal-contaminated soils. Isolation may involve the removal of the top soil and then covering with concrete or non-contaminated soils. Cleansing involves the leaching of pollutants with acids while inerting is the addition of other

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chemicals to the soil that bring the pollutants into a non-toxic form. These conventional procedures are expensive and may leave the soil infertile or cause further pollution by leaching, being a temporary solution (Robinson et al. 2000).

Using hyperaccumulator plants for phytoremediation has been proposed as an environmentally friendly, low-cost technology for decreasing heavy metal contents of highly contaminated soils and materials (McGrath et al. 2002). It has been reported that *Tagetes erecta*, (Malarkodi et al. 2008) and *Tagetes patula* (Li et al. 2007) have substantial potential for soil metal remediation. The ideal plant for phytoremediation should possess multiple traits such as fast growth with high biomass production, a deep rooting system that is easily harvestable, and able to tolerate and accumulate a wide range of heavy metals in its aerial and harvestable parts (Clemens et al. 2002). The natural phytoremediators often lack these qualities, therefore scientists have been forced to research the effectiveness growth-promoting microorganisms.

According to Khan (2001), the protection provided to roots by the AM fungi and enhanced capability for greater uptake of minerals results in greater biomass production, which is a prerequisite for successful remediation. These fungi are important as they play a vital role in metal tolerance and accumulation (Zhu et al. 2001; Jamal et al. 2002). A greater volume of metals is also stored in the mycorrhizal structures in the root and in the spores (Chen et al. 2001). Associated microorganisms have been studied for their ability to degrade a number of contaminants (Suresh and Ravishankar 2004; Macek et al. 2004).

Development of a successful technology to treat the tannery waste combined with effective solid waste disposal may provide the tanning industry in the country with the needed boost to stay in business profitably. Currently, there is no other practical alternative for the solid waste disposal other than the landfill. The main theme of this research is to enhance the plant growth and its ability to uptake heavy metals including other pollutants, by using rhizosphere fungi in plants.

2 Materials and methods

Tannery solid waste (TSW) was collected from the solid waste landfill site at a depth of 15 cm, constructed by Kasur Tannery Waste Management Agency (KTWMA) at Kasur, Pakistan, in a tannery waste-water treatment plant. The solid waste transported to the Department of Botany, University of the Punjab, Lahore, was air-dried in metallic troughs under sunlight. It was crushed and then sieved through a sieve of mesh size 1 mm^2.

Three types of TSW amended soils (10, 20, and 50%) were prepared by mixing crushed and sieved solid waste with soil collected from the Botanical Garden, University of the Punjab, Lahore, Pakistan. Crushed and sieved TSW (100%, without soil) was also used in preliminary experiments.

The soil saturation extract was prepared by the method given by Rhoades (1982) and was used to determine the pH, conductivity, and sodium chloride percentage of the soil samples by using an auto ranging portable waterproof microprocessor EC/TDS/NaCl/°C meter (Model HI 9835). Carbonates, bicarbonates, and chlorides were determined using the titremetric methods given by Saeed (1980).

The determination of sodium, was done on a flame photometer (Model: PFP7&PFP7/C, England) while the determination of Cr, Cd, Cu, and Pb was done on an atomic absorption spectrophotometer (Model: AA-1275/VARIAN, Australia). Soil samples were acid digested by the method given by Mench et al. (1994). Harvested plants were dried at 60°C for 24 h and crushed in a grinding machine. The roots and shoots were digested separately according to the method given by Greenberg et al. (1992).

Certified seeds of *T. patula* L. were collected from Pulse Centre, NIAB Faisalabad, Pakistan. A preliminary experiment was conducted to check the tolerance of *T. patula* at different concentrations of TSW amended soils (10, 20, 50, and 100%). The four sterilized soil types were filled up in pots and seed germination was checked. The seed even failed to germinate in 50 and 100% amendments.

In the actual experiment, two solid waste amendments were selected based on the tolerance level of plants in the first experiment, i.e., 10 and 20% and normal soil. The roots of *Cynodon dactylon* Pers. for mycorrhizal inoculum were given in experimental pots by placing 3 inches below the soil surface in pots. For this purpose, 1 g of roots per 100 g of soil was used. The saprobic fungus, *Trichoderma pseudokoningii* Rifai isolated from TSW, in the form of conidial suspension was also used as inoculum. *T. pseudokoningii* was grown in 500-ml conical flasks containing potato dextrose broth for 8 days. The cultures were then filtered through Whatman no. 1 filter paper and the mycelial mat was macerated using a Waring blender for 1 min and mixed with 250 ml of 0.1 M $MgSO_4 \cdot 7H_2O$ solution. Ten ml of this inoculum containing 5×10^4 c.f.u./ml was used for inoculating each pot. The experiment was set in a wire house having a glass roof in a completely randomized design. The experiment consisted of five replicates for each treatment.

The plants were harvested after 50 days until the maturation of seeds. The growth and metal content of the plants was analyzed. The plants were acid digested by the nitric acid and perchloric acid method for metal analysis.

The mycorrhizal assessment of roots was expressed in terms of extent of infection while the activity of the saprobic fungus was estimated by the colony-forming units (c.f.u) from the soil.

Fresh root samples were stained using 0.05% Trypan Blue as described by Phillips and Hayman (1970) and the

percent root colonization was estimated by adopting the grid-line intersect method of Giovanetti and Mosse (1980). Extramatrical chlamydospores in root-zone soil samples were enumerated using the wet sieving and decanting method of Gerdemann and Nicolson (1963). The population of *T. pseudokoningii* in the root zone soil was determined by a dilution plate method using 2% MEA.

Statistical analyses were applied on the results of morphological and elemental assays. The software Analyse-it Free, available on the Web was used to determine the values of two-way ANOVA for different fungal and soil treatments.

3 Results

Five different combinations of soil and solid waste samples were prepared. The soil taken from Botanical Garden and soil with TSW amendments in the ratio of 1:10, 1:5, 1:1 ratio of TSW: Soil (w/w) and TSW only. All the soil treatments showed a clear variation in physico-chemical parameters when analyzed. pH, conductivity values, NaCl (%) and metals were significantly higher in TSW and showed an increase with increasing amendment in soil (Table 1). Carbonates were found to be totally absent. The increase in metal content was also in accordance with the increase in concentration of TSW. The amount of all metals was considerably higher in 100% TSW but amount of the Cr was the highest (25,534 mg kg^{-1}). The order of arrangement of soil samples on the basis of quantification of metals and other pollution parameters was in the order 100% TSW > 1:1 > 1:5 > 1:10 > simple soil.

Table 1 Some physico-chemical properties of soil and different concentrations of tannery solid waste (TSW)

Parameters	Soil	1:10 (TSW)	1:5 (TSW)	1:1 (TSW)	TSW
pH	7.1	8.0	8.1	8.3	8.9
ECe (dS/cm)	0.02	0.31	0.81	1.42	2.89
NaCl (%)	2.9	52.3	125.7	255.1	421.5
Bicarbonates (mg l^{-1})	103.7	152.5	189.1	262.3	359.9
Carbonates (mg l^{-1})	–	–	–	–	–
Chlorides (mg l^{-1})	62.1	255	812	2,233	3,118
Cd (mg kg^{-1})	55	6,580	8,750	9,720	10,097
Cr (mg kg^{-1})	110	10,250	15,510	19,520	25,534
Cu (mg kg^{-1})	600	2,100	5,250	7,510	10,554
Na (mg kg^{-1})	995	2,515	4,645	6,350	9,440

TSW = Tannery solid waste (dried, crushed, and sieved)

1:10 = Soil contaminated with 10% TSW

1:5 = Soil contaminated with 20% TSW

1:1 = Soil contaminated with 50% TSW

The maximum growth of 50-day-old plants of *T. patula* was observed in case of soil, being relatively less in 1:10 and 1:5 as indicated by growth parameters (Tables 2, 3). The statistical analysis of the data showed significant growth in all parameters in lower TSW concentration in soil followed by a decrease at higher (1:5) concentration. However, the maximum increase in values was found in F + M treatment over their controls M and F, for each of the corresponding soil treatments.

The maximum metal extraction efficiency was found in plants growing on soil samples supplemented with of F + M inoculum as compared to soil samples inoculated with either F or M. However, inoculation with the saprobic fungus (F) showed better metal uptake than M. The accumulation of all metals in the plant tissues increased with the corresponding increase in the TSW ratio in soil. The roots showed a higher metal bioaccumulation level as compared to shoots (Tables 4, 5). The order of treatments on the basis of metal accumulation was established as M + F > F > M > control in both shoots and roots.

The highest amount of chromium was observed in case of F + M treatment of 1:5 amendment, being 3,950 mg kg^{-1} dry weight of roots and 1,450 mg kg^{-1} dry weight of shoots. The minimum value was recorded for Cd figuring 840 mg kg^{-1} dry weight of roots. The F treatment showed higher metal uptake as compared to either M or control. In 1:10 amendment, Cr was found to be the highest in F + M being 3,100 mg kg^{-1} dry weight of roots while Cu was recorded to be least in F treatment with a value 510 mg kg^{-1} dry weight of roots and 450 mg kg^{-1} dry weight of shoots.

In the case of 1:10 concentration, Cr was also found to be the highest in F + M, 1,210 mg kg^{-1} dry weight of shoots. The minimum uptake was recorded for Cu in control reaching barely to 310 mg kg^{-1} dry weight of shoots.

Table 6 shows the percentage reduction of these metals in soil from where 50-day-old plants of *T. patula* were harvested. Maximum reduction (62%) was observed for Cu in 1:10 concentration in F + M treatment. Other metals like Cr, Cd and Na were also reduced in sufficient amounts.

The results of assessment of fungal inoculum both in soil and in plants are shown in Table 7. The control treatment (without any inoculum) plants showed 0% AM infection and no c.f.u for *T. pseudokoningii* and no spores in soil. In 10% concentration of solid waste, M + F showed maximum percentage of infection, 95% while minimum infection, 35% was observed in 20% concentration in F treatment. Similarly, maximum c.f.u/g of soil was observed in 10% conc. in F + M treatment 1.4×10^5 c.f.u. of *T. pseudokoningii* per gram of soil and the minimum value, 0.16×10^5 was observed for M treatment. The maximum spore number of 256 spores per 50 g of soil was

Table 2 Various morphological parameters observed in 50-day-old plants of *Tagetes patula* growing in different concentrations of tannery solid waste amended soils

Treatments	Parameters								
	Shoot length (cm)			Root length (cm)			Seedling length (cm)		
	Soil	1:10	1:5	Soil	1:10	1:5	Soil	1:10	1:5
Control	45.1 ± 0.11	44.2 ± 0.17	25.9 ± 0.45	30.1 ± 0.23	29.00 ± 0.24	18.9 ± 0.36	75.4 ± 0.28	73.4 ± 0.34	44.8 ± 0.21
M	50.5 ± 0.21	42.5 ± 0.45	31.2 ± 0.12	35.2 ± 0.55	21.5 ± 0.25	15.2 ± 0.25	86.0 ± 0.16	64.2 ± 0.52	46.5 ± 0.47
F	60.2 ± 0.20	43.9 ± 0.52	34.5 ± 0.41	39.4 ± 0.44	25.4 ± 0.27	17.5 ± 0.16	99.7 ± 0.34	69.4 ± 0.36	52.1 ± 0.23
M + F	68.0 ± 0.21	65.10 ± 0.35	40.3 ± 0.30	40.1 ± 0.21	31.6 ± 0.28	21.4 ± 0.27	108.2 ± 0.66	97.2 ± 0.17	61.9 ± 0.23
LSD ($P = 0.05$)									
For conc.	8.7			6.4			13.9		
For treatments	7.5			5.5			12.0		

LSD = Least significant difference

± = Standard error

Control: Without any treatment

M: Mycorrhizal treatment

F: Fungal treatment

F + M = Fungal + Mycorrhizal treatment

1:10: Tannery solid waste amended soil (10% tannery solid waste mixed with soil)

1:5: Tannery solid waste amended soil (20% tannery solid waste mixed with soil)

Table 3 Various morphological parameters observed in 50-day-old plants of *Tagetes patula* growing in different concentrations of tannery solid waste amended soils

Treatments	Parameters											
	No. of leaves			No. of roots			Fresh weight (g)			Dry weight (g)		
	Soil	1:10	1:5	Soil	1:10	1:5	Soil	1:10	1:5	Soil	1:10	1:5
Control	14 ± 0.11	7 ± 0.34	6 ± 0.09	21 ± 0.12	13 ± 0.12	6 ± 0.16	26.5 ± 0.17	19.2 ± 0.36	12.4 ± 0.34	10.4 ± 0.12	8.1 ± 0.22	5.2 ± 0.41
M	16 ± 0.21	9 ± 0.23	8 ± 0.14	20 ± 0.22	11 ± 0.56	8 ± 0.13	29.1 ± 0.16	18.4 ± 0.16	11.3 ± 0.29	9.5 ± 0.13	8.5 ± 0.52	6.1 ± 0.12
F	18 ± 0.42	10 ± 0.63	9 ± 0.12	24 ± 0.43	14 ± 0.31	10 ± 0.48	30.7 ± 0.08	19.1 ± 0.46	12.1 ± 0.34	10.1 ± 0.65	8.9 ± 0.38	6.7 ± 0.37
M + F	21 ± 0.33	13 ± 0.41	10 ± 0.54	27 ± 0.32	16 ± 0.45	12 ± 0.27	35.2 ± 0.36	24.4 ± 0.24	14.2 ± 0.22	13.5 ± 0.34	9.7 ± 0.27	7.1 ± 0.26
LSD ($P = 0.05$)												
For conc.	1.48			2.10			3.0			1.6		
For treatments	1.28			1.82			2.6			1.3		

LSD = Least significant difference

± = Standard error

Control: Without any treatment

M: Mycorrhizal treatment

F: Fungal treatment

F + M = Fungal + Mycorrhizal treatment

1:10: Tannery solid waste amended soil (10% tannery solid waste mixed with soil)

1:5: Tannery solid waste amended soil (20% tannery solid waste mixed with soil)

Table 4 Metal content of roots in 50-day-old plants of *Tagetes patula* growing in different concentrations of tannery solid waste amended soils

Treatments	Amount of Cd (mg kg^{-1})			Amount of Cr (mg kg^{-1})			Amount of Cu (mg kg^{-1})			Amount of Na (mg kg^{-1})		
	Soil	1:10	1:5	Soil	1:10	1:5	Soil	1:10	1:5	Soil	1:10	1:5
Control	150	710	840	3	1,500	2,500	110	510	910	710	750	940
M	190	850	950	2	2,550	3,150	90	640	1,100	850	950	1,150
F	210	890	990	4	2,910	3,410	125	590	1,250	890	1,000	1,210
M + F	290	1,050	1,190	3	3,100	3,950	140	890	1,390	995	1,240	1,310
LSD ($P = 0.05$)												
For conc.	99.28			784.2			218.9			94.8		
For treatments	85.98			679.1			189.6			82.1		

LSD = Least significant difference

± = Standard error

Control: Without any treatment

M: Mycorrhizal treatment

F: Fungal treatment

F + M = Fungal + Mycorrhizal treatment

1:10: Tannery solid waste amended soil (10% tannery solid waste mixed with soil)

1:5: Tannery solid waste amended soil (20% tannery solid waste mixed with soil)

Table 5 Metal content of shoots in 50-day-old plants of *Tagetes patula* growing in different concentrations of tannery solid waste amended soils

Treatments	Amount of Cd (mg kg^{-1})			Amount of Cr (mg kg^{-1})			Amount of Cu (mg kg^{-1})			Amount of Na (mg kg^{-1})		
	Soil	1:10	1:5	Soil	1:10	1:5	Soil	1:10	1:5	Soil	1:10	1:5
Control	110	410	520	1	1,050	1,110	85	310	450	440	550	810
M	100	590	590	3	990	1,190	90	425	510	510	610	850
F	120	620	610	4	1,120	1,345	110	490	550	525	650	910
M + F	140	710	850	5	1,210	1,450	125	610	690	690	760	950
LSD ($P = 0.05$)												
For conc.	154.3			159.7			115.1			56.1		
For treatments	133.6			138.3			99.6			48.6		

LSD = Least significant difference

± = Standard error

Control: Without any treatment

M: Mycorrhizal treatment

F: Fungal treatment

F + M = Fungal + Mycorrhizal treatment

1:10: Tannery solid waste amended soil (10% tannery solid waste mixed with soil)

1:5: Tannery solid waste amended soil (20% tannery solid waste mixed with soil)

observed in M + F treatment, while the minimum number of 50 spores per 50 g of soil was observed for 20% conc. in M treatment.

4 Discussion

Phytoremediation includes plant-based technologies that reduce, remove, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications.

In the present study, TSW was used to prepare different concentrations with soil. Analysis of all concentrations of samples showed a high degree of pollution with metals, especially cadmium, chromium, copper, and sodium. Higher values of pH, conductivity, chlorides and bicarbonates are other aspects of pollution. Increasing toxicity

Table 6 Percentage reduction of metals in different concentrations of TSW-amended soil observed after harvesting of plants of *Tagetes patula*

Treatments	Percentage reduction of:							
	Cd		Cr		Cu		Na	
	1:10	1:5	1:10	1:5	1:10	1:5	1:10	1:5
Control	18	15	25	24	40	26	32	28
M	22	18	35	27	51	31	36	34
F	23	19	40	31	52	35	46	38
F + M	27	24	43	35	62	40	52	45
LSD ($P = 0.05$)								
For conc.	1.13		7.2		6.86		3.81	
For treatments	0.79		9.47		4.85		5.40	

LSD = Least significant difference

Control: Without any treatment

M: Mycorrhizal treatment

F: Fungal treatment

F + M = Fungal + Mycorrhizal treatment

1:10: Tannery solid waste amended soil (10% tannery solid waste mixed with soil)

1:5: Tannery solid waste amended soil (20% tannery solid waste mixed with soil)

was observed with increasing amount of both samples. Physico-chemical soil properties are fundamental for soil quality, with soil structure being one of the most influential factors (Buscot 2005). The higher values of Cr and other metals in TSW are a result of using 'The Chrome Tanning Method' due to its high speed and cost-effectiveness in the processing of leather production.

Arbuscular mycorrhizal (AM) fungi can be used as potential biotechnological tools for enhancing phytoremediation of heavy-metal-contaminated soils (Gaur and Adholeya 2004). The majority of bioaugmentation studies of historically contaminated sites have been conducted with allochthonous fungi (Pointing 2001; Šašek 2003). However, the use of autochthonous species for the cleanup of a contaminated site has been shown to be a successful approach (Atagana 2004; Garon et al. 2004). Such findings have prompted the use of indigenous fungi to clean up TSW.

The results clearly indicate that the saprobic fungal strain of *T. pseudokoningii* abetted *T. patula* in terms of increased plant biomass and metal accumulation. Fungi are effective in the accumulation of heavy metals such as chromium, copper, lead, mercury, and zinc, and the ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them for bioremediation (Alexander 1994). The plants given only saprobic culture showed more promising results than Mycorrhizal or the control treatments.

The morphological results indicated that there is a great analogy between these two types of fungi, as maximum growth of seedling, greater dry weight was observed in F + M treatment in comparison with only F or M

Table 7 Influence of AM fungi and *Trichoderma pseudokoningii* on mycorrhizal root colonization, spore number, and population in the root zone soil of *Tagetes patula* grown in different concentrations of tannery solid waste in soil

Treatments	Parameters								
	% age infection of AM fungi (in roots)			*T. pseudokoningii* ($\times 10^5$ c.f.u. g^{-1} soil)			Spore No 50 g^{-1} soil		
	Soil	10%	20%	Soil	10%	20%	Soil	10%	20%
Control	–	–	–	–	–	–	–	–	–
M	86.0	88.0	55.0	0.30	0.16	0.20	190.0	185.0	170.0
F	60.0	64.0	35.0	0.96	1.11	1.6	64.0	60.0	50.0
M + F	90.0	95.0	59.0	1.20	1.40	3.5	256.0	250.0	188.0
LSD ($P = 0.05$)									
For conc.	18.03			1.21			32.46		
For treatments	15.61			1.05			28.40		

Control = without any inoculum

M = Mycorrhizal inoculum

F = Fungal inoculum only

M + F = Combined mycorrhizal and fungal inoculum

10% = soil contaminated with 10% TSW

20% = soil contaminated with 20% TSW

TSW = Tannery solid waste (Dried, crushed and sieved)

c.f.u = Colony forming units

LSD = Least significant difference

inoculum. According to Gryndler (2000) microbial populations in the rhizosphere are known either to interfere with or to benefit from the establishment of mycorrhizal symbioses. The symbiotic relationship between mycorrhizal and autochthonous fungi (*T. pseudokoningii*) was confirmed by significantly greater growth and earlier flowering in treatment with dual inoculum. Flowering stage was not observed in control treatment and the plants did not show good morphological conditions. The synergistic effects of different fungi and bacteria have been studied by Srinath et al. (2003). The enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mosseae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans* has been shown. Similarly, enhanced plant biomass of Marigold as a result of inoculation with *Glomus intraradices* and *Trichoderma aureoviride* compared to inoculation with *G. intraradices* alone has been reported (Calvet et al. 1993). In this study, though the maximum biomass was observed in uninoculated control plants, dual inoculation by saprobic fungus (F) and mycorrhizal fungi (M) also caused significant production of biomass.

Among the heavy metals, Cd, Cu, and Cr are highly toxic pollutants as they not only affect growth and germination but also cause a significant reduction in seed and fruit production of plants (Nasralla and Ali 1985). The findings conform to the higher concentrations of TSW-amended soil having higher concentration of these metals affecting the growth and yield of plants. But microorganisms can play a great role in this scenario. The elemental assays showed that the amount of Cr uptake was highest in both saprobic and mycorrhizal fungal (F + M) treatment. The amount of Cr uptake in F treatment was more significant as compared to mycorrhizal treatment. The coefficient of extraction showed that the plants treated with both fungal and mycorrhizal infections can uptake a significant amount of heavy metals from the contaminated soil. A correlation was observed within treatments and the order of phytoextraction co-efficient was on the order of F + M > F > M > control.

There are researchers who believe that plants for phytoextraction should accumulate metals only in the roots (Salt et al. 1995; Flathman and Lanza 1998). In the present study, a high uptake of metals was observed in roots of plants. A significantly high amount of metals was observed in F + M in higher concentrations of TSW-amended soils followed by F treatment in all experiments but in some cases M treatment showed better uptake of metals than F treatment. The concentration of metals in the plants was found to increase with increasing concentrations of TSW in soil. These findings are in accordance with Gupta and Sinha (2006), who showed that the accumulation of heavy metals (Cr, Ni, and Cd) in the plants was found to increase with increase in tannery sludge concentration in the soil.

The potential of hyperaccumulator plants for phytoremediation application relies upon their growth rates as well as metal accumulation rate (g metal per kg of plant tissue). The two primary reasons that limit global application of the phytoremediation are the slow growth rates exhibited by most naturally occurring metal hyperaccumulators and the limited solubility of metals in soils (Prasad 2003). By using these organisms on a large scale the polluted soil can be decontaminated, and many plants that cannot tolerate the harsh environment of the contaminated soil can be made to grow under these conditions. Already known hyperaccumulators may be further improved for their hyperaccumulation capabilities by selecting the appropriate associated rhizosphere microbes.

Tagetes patula has an excellent potential for metal phytoremediation because of high biomass production and good tolerance and accumulation of metals. This plant (in association with rhizosphere fungi) can be used to clean up soil moderately contaminated with TSW, and after 2–3 crops of this plant, the soil can become organically rich and will be safe for cultivation of crop plants. There is greater reduction of metals like Cr, Cd, Cu, and Na in case of lower TSW concentration in plants having dual inoculation. Although a greater uptake of these metals was observed in higher concentration (1:5 TSW), percentage reduction of these metals was higher in lower concentration (1:10 TSW). Thus after sufficient plant growth and metal accumulation, the plants can be harvested and removed, resulting in the permanent removal of metals from the site. As with soil excavation, the disposal of contaminated material is a concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal (Kumar et al. 1995). So the plants that have accumulated metals can be incinerated and a much lower volume of ash can be disposed off in a safe land having a lining of impervious material. The future of phytoremediation lies in integrated management of soil microbial populations for phytoextraction of heavy metals from industrial wastes.

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