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Some Chemical and Biological Additives With Hyperaccumulator Plants for Amendment the Sandy Soil Contaminated for Long Term by Sewage Water

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Abstract: Long- term irrigation with sewage water in the sandy soils cause severe adverse contamination consequences with potentially toxic elements (PTEs), potential organic pollutants (POPs) and/or enteric pathogens that lead to degradation of these lands. Cairo is now served by six large wastewater treatment works, which produce significant quantities of sewage water. The preferred option is to use this in agriculture, particularly in reclaimed desert land, which is inherently deficient in organic matter, nutrients and trace elements. However, there are concerns about the long-term accumulation and potential effects of heavy metals and pathogenic organisms. Abou Rawash Farm is reclaimed desert land west of Cairo. For combating this type of contamination a field experiment was conducted at Abu-Rawash sewage farm where the soil has been irrigated with water sewage for 32 years and there are concerns about the contamination of the site by potentially toxic heavy metals (The contamination index of PTEs was 633.9) and enteric pathogens. The site provides a possible model of the potential long-term effects of heavy metals and other pollutants on crops in the soils irrigated with sewage water in Egypt. This work is a part of the bioremediation project funded by Egyptian Academy of Scientific Research and Technology (Science and Technology Development Fund, STDF) and implemented by NRC. During the previous tasks implemented throughout the project for the bioremediation of sewage soils, some treatments gave pronounced effectiveness and hence it was tried in this field experiment to innovate a proper bioremediation protocol for sewage soils using successive chemical, microbial and phytoremediation treatments. The hyperaccumulator plant used for decontamination of these pollutants was Indian mustard (*Brassica juncea* Czern). Six treatments including fallow soil (irrigated without growing crop plants), cultivated control, inoculated with arbusculer mycorrhiza (AM), soil inoculation with *Thiobacillus* (a mixture of *Thiobacillus ferrooxidant* and *Thiobacillus thiooxidant*), soil treated with a mixture of 250 gm bentonite plus 250 g rock phosphate and inoculated with PDB and soil treatment with all the biological and chemical treatments. The contaminated soil was chemically or biologically treated two months prior sowing the Indian mustard crop. Soil quality criterion index (Zn equivalent model) was numerically expressed for the levels of PTEs toxicity. Plant samples from each treatment were taken at vegetative and seed maturity stages and the growth criteria as well as yield and yield attributes were determined. The PTEs, POPs and enteric pathogens were determined at three sampling occasions. Plant analysis and PTEs accumulation as well as the level of dehydrogenase in soil rhizosphere were determined. Statistical analysis was applied using SAS software. The results indicated that soil inoculation with AM and the integrated treatments to the cultivated land with Indian mustard plants seems to be more effective in achieving the phytoremediation process. Safety seed yield of Indian mustard plants were significantly increased by 16.6% when bentonite was used while with the application of *Thiobacillus*, the integrated and AM treatments caused a reduction in the safety seed yield by 18.8, 6.6 and 0.4%, respectively. However, the plant weight was increased in most cases. A cumulative effect was recorded when the three tested remediative amendments were applied together, where the aerial parts and total plant weight were increased by 81.4 and 81.6%, respectively in comparison to the plants grown without

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additives. The data provide assurance about the minimal risk to the environment from PTEs, POPs and enteric pathogens when using hyper-accumulator plants with the integrated treatment of chemical and biological methods prior sowing the hyper-accumulator Indian mustard plants and this approach could be effective in decontamination of such polluted soils and gave a safe product from Indian mustard oils in such long-term contaminated soils.

Key words: Desertification · Decontamination · Indian mustard · Hyperacumulator · PTEs · Bentonite

INTRODUCTION

Cairo is now served by six large wastewater treatment works, which produce significant quantities of sewage water. The preferred option is to use this in agriculture, particularly on reclaimed desert land, which is inherently deficient in organic matter, nutrients and trace elements. Sewage effluent was used in farming, since distant past, however at a restricted magnitude. Early in 1531 sewage farming started in Germany and thereafter progressively distributed in other parts of the world. In Egypt the first sewage farm was installed in 1930 at El-Gabal Al-Asfer near Cairo and sewage farms gradually disseminated later on in other regions. At the time being, sewage effluent represents one of the considerable natural water resources in Egypt that should be well managed, rather than discarded. Sewage effluent, however, is impregnated with huge amount of contaminates, particularly PTEs, that accumulate in sewage soils and could easily enter the food chain, ensuing crucial adverse environmental and public health difficulties. Certainly, safe and sustainable reuse of sewage effluent in farming necessitates the removal of soil contaminants. The concept of phytoremediation of contaminated sewage soils had been recently highlighted using certain hyper-accumulator plants capable to remove PTEs contaminants from them, or to at least assist in their degradation to less toxic forms [1]. Not only are the biological activity and physical structure of soils maintained after phytoremediation but also this technique is potentially cheap, visually unobtrusive and offers the possibility of bio-recovery of PTEs [2, 3].

Irrigation with sewage effluent, however, must be applied under proper precautions to protect human health and the environment. However, there are concerns about the long-term accumulation and potential effects of heavy metals and pathogenic organisms. Long-term irrigation with sewage water in the sandy soils may cause severe adverse contamination consequences with potentially toxic elements (PTEs), POPs and/or enteric pathogens, which lead to degradation of these lands. For combating this type of contamination a field experiment was conducted at Abu-Rawash sewage farm where the soil has been irrigated for more than three decades with sewage water. The hyper-accumulator plant used for decontamination of these pollutants was Indian mustard (*Brassica juncea* Czern). One of the most famous hyper-accumulators plants is Indian mustard [4, 5].

Abou Rawash Farm is reclaimed desert land west of Cairo. It has been irrigated with sewage for over 30 years and there are concerns about the contamination of the site by potentially toxic heavy metals and enteric pathogens. The site provides a possible model of the potential long-term effects of heavy metals and other pollutants on crops for sewage sludge-treated soils in Egypt. Purakayastha *et al.* [6], in a pot culture experiment, grew five different species of Indian mustard as possible accumulators of PTEs in soils sewage for more than two decades. In general, they found that the highest concentration and uptake of PTEs was observed in shoots compared to roots or seeds of the different species of Indian mustard. Total uptake of PTEs was negatively correlated with available as well as the total soil PTE concentrations. Among the root parameters, root length emerged as the powerful parameter to dictate the uptake of PTEs by Indian mustard.

Microbial remediation of sewage soil is a well welcomed technique. It is well known that microorganisms perform an urgent mode in the devastation of PTEs in agricultural ecosystems and several researchers had been attempted to develop means of using microorganisms and microbial enzymes for decontaminate sewage soils. However, as far as PTEs are not biologically degradable and persist in indefinitely soil ecosystem Glick [7] stated that once they accumulate in the soil, they inversely affect the microbial compositions, particularly plant growth promoting rhizobacteria (PGPR) in the rhizosphere and their metabolic activities. He added that the elevated concentration of PTEs in soils and their uptake adversely affect plant growth, symbiosis and consequently crop yields through disrupting photosynthesis, inactivating respiration, protein synthesis and/or carbohydrate metabolism.

Phytoremediation efficiency is often limited by many factors such as contaminants bioavailability in soil, plant root development and the level of tolerance of the plant to each particular contaminant [8]. Given the fact that there were a large number of variables in the current experiment, it isn't possible to distill this information and come up with one ideal set of conditions for all PTEs contaminant phytoremediation experiments. These variables include plant type, soil composition, endogenous bacteria, concentration and range of the contaminants, temperature range and type and physiological state of bio-additives. This complexity notwithstanding, careful examination of the data suggests that certain considerations might facilitate the phytoremediation of soil contaminants. Chelate ion exchange resin, cation - exchange resin, ferrous sulfate and silica gel, lime, gypsum and naturally occurring clay minerals such as bentonite, Kaolin, Zeolite and green sand have been found to immobilize heavy metals [9]. Achakzai *et al*. [10] also explained that AM inoculation promoted both the vegetative growth and the hyperextraction of heavy metals (*viz.,* Cu, Ni, Pb and Cd) from oil contaminated and AM inoculated soils, but inhibited by soils treated with AM (Glomus mosseae) inoculation only.

Phytoremediation can be Hyper accumulating plants could defined as "use of green plants and their associated microbiota soil amendments and agronomic technique; to remove, contain or render harmless the environmental for contaminants [11]. Recently it was not overlooked that coupling phytoremediation with other techniques such as microbial, physical and/or chemical treatment could be viable in many cases.

Therefore, the aim of this present work was to evaluate the decontamination of the expected pollutants by hyper-accumulator plant species treated with some chemical or micro-organisms.

MATERIALS AND METHODS

The contaminated soil was chemically or biological treated two months prior sowing the hyper-accumulator plant: the Indian mustard (*Brassica juncea* Czern). Six treatments including fallow soil, cultivated control, inoculated with Mycorrhyzal (AM) conidia, soil inoculation with a mixture of *Thiobacillus thiooxidant and Thiobacillus ferrooxidant*, soil treated with a mixture of 250 gm bentonite plus 250 g rock phosphate kg^{-1} soil and inoculated with (PDB phosphate dissolving bacteria) and soil treatment with all the biological and chemical treatments. The microbiological organisms used were determined. Soil pH, PTEs (Potential toxic elements in soil and plant materials were estimated. Soil quality criterion index (Zn equivalent model) was numerically expressed for the levels of PTEs toxicity. Plant samples from each treatment were taken at vegetative and seed maturity stages and the growth criteria as well as yield and yield attributes were determined. The PTEs, POPs and enteric pathogens were determined at three sampling occasions. Plant analysis and PTEs accumulation as well as the level of dehydrogenase in soil rhizosphere were determined. Statistical analysis was applied using SAS software.

At Abu-Rawash sewage farm a completely randomized field experiment with four replicates were carried out during the winter season 2011-2012 using hyper-accumulator plant, Indian mustard, to decontaminate the most polluted sewage soil at the farm, after being bio-remediated with the most effective chemical and biological treatments reached during the previous phases of the study. Chemical analysis of the field experiments soil was mentioned in Table 1.

The experiment consisted of 24 plots (3m x 3.5m $=10.5m^2$ = 1/400 fed. with 5 ridges (70cm) irrigated with sewage effluent using furrow irrigation. The contaminated sewage soil was chemically and biologically bioremediated for two months before sowing by six treatments including uncultivated control, cultivated control, inoculated with AM, inoculated with a mixture of *Thiobacillus thiooxidant & Thiobacillus ferrooxidant*, soil treated with a mixture of 250 gm bentonite plus 250 g rock phosphate per kg soil and inoculated with phosphate dissolving bacteria and soil treated with all the aforementioned remediative amendments.

Seeds of Indian mustard (*Brassica juncea* Czern) were sown on 1st November at 70 cm ridge width x 20cm between plants. During the experimental period the expected average of day and night temperatures ranged between 30 and 38°C, respectively and light duration were ranges between 12-14 h light/24 h. Plant samples from each treatment were harvested at the maximum vegetative growth stage; before plant flowering (Harvest 1), while the rest were kept until seed production stage (Harvest 2). Soil samples were collected initially and at both vegetative and mature stages. All treated and control treatment did not receive any mineral fertilizers. Plants were harvested by cutting the shoots at the soil surface and roots were carefully separated from the adjacent soils. The PTEs, POPs and enteric pathogens were analyzed at three tested intervals. The following characteristics were measured for

Table 1: Total PTE contents in Abou-Rawash farm soils ** (ppm oven dry basis)

Soil No.	Soil depth (cm)	Sewage farming started since	Landscape	Cd	Cu	Fe	Mn	Pb	Zn	Ni	Zn equivalent
30	0-30	30	Sandy soil was	-	35.65	596.0	45.19	57.7	400.6	18.0	633.9
31	30-60		cultivated with artichoke	-	33.50	850.0	73.34	63.2	94.55	14.0	287.6
32	0-30	30	Loamy sand soil was	14.95	111.7	758.5	160.0	48.2	73.05	18.8	458.4
33	30-60		cultivated with artichoke	13.45	112.1	761.0	227.0	49.3	75.05	10.0	389.2

** El-Ashry *et al.* [45]

the vegetative sample, plant height, dry weight of aerial parts, dry weight of roots, dry weight of plant, plant analysis and PTEs accumulation. The following characteristics were measured at the harvest stage, plant height, dry weight of aerial parts, dry weight of roots, dry weight of plant, number of pods plant^{-1}, pods weight, berries yield, biological yield, plant analysis and PTEs accumulation.

Microbiological Methods: To develop a microbial decontamination process in sewage soils, a steady supply of efficient microorganisms was ensured in a microbial culture collection containing highly active isolated pure cultures.

PDB Inoculation: Bacillus megatherium var phosphaticum: Phosphate dissolving bacteria (PDB) were isolated on Bunt and Rovira medium [12] as modified by Lauw and Webley [13]. The medium is composed of 0.40 gm KCl, 0.50 $(NH_4)_2SO_4$, 0.50 gm $MgSO_4.7H_2O$, 0.01 gm $FeCl_3.8H_2O$, 0.10 gm $CaCl_2$, 1.0 gm peptone, 1.0 gm yeast extract, 5.0 gm glucose, 20 gm agar, 250 ml soil extract and 750 ml water and with a pH of 6.8. To 10 ml portions of the melted medium 0.5 ml sterile K_2HPO_4 (10%) solution was added and followed by 1.9 ml of sterile 10% $CaCl_2$ solution and thoroughly mixed directly before pouring in plates. PDB was detected by clear zones, isolated and grown on nutrient broth.

Thiobacillus

Acidithiobacillus Ferrooxidans: DSMZ medium 882 [14] was used to isolate and grow *Acidithiobacillus ferrooxidans*.

Acidithiobacillus Thiooxidans: A pure culture of the sulfur-oxidizing bacterium *Acidithiobacillus thiooxidans*, active in a wide pH range was isolated from soil moistened and enriched with elemental sulfur and incubated at 30°C for 15 days. Five gram portions of the sulphur enriched soil was placed a 250-ml Erlenmeyer flask containing 100 ml of modified Waksman medium [15, 16] and incubated for three weeks at 30°C. Modified Waksman medium is composed of 3.0 g/l K_2HPO_4, 0.1 g/l $MgSO_4$•$7H_2O$, 0.3 g/l $CaCl_2$•$2H_2O$, 0.01 g/l $FeSO_4$•$7H_2O$ and 10 g/l S as an energy source and with a pH of 4. The mixed culture obtained in this enrichment medium was re-inoculated in fresh modified Waksman medium and was again incubated under the same conditions. From the culture broth, 10 ml aliquot was sampled every 7 days during incubation to determine its pH until reaching 2. The obtained cell suspension of *Acidithiobacillus thiooxidans* was used in inoculating the sewage soil.

Mycorrhyzal (AM) Conidia: AM fungi spores were extracted from soil by wet sieving and sucrose density gradient centrifugation [17]. Soil sample was placed in a plastic bag and air-dried (with the tops of the bags rolled down) for 24 hours under cover, then brushed through a 2mm mesh and stored at 4°C. The procedure included passage of 25 g of air-dried soil or 30 cm^3 of harvested trap culture substrate through 1,000-, 500-, 125- and 32-µm sieves. The 1,000-µm sieves were checked for spores adjacent to or inside roots, while the 500-µm sieves was checked for larger spores, spore clusters and sporocarps. The contents of the 125- and 32-µm sieves were layered onto a water-sucrose solution (70% wt/vol) gradient and centrifuged at 900 µg for 2 min. The resulting supernatant was passed through the 32-µm sieves, washed with tap water and used to inoculate the sewage soil. Mycorrhyzal is not specific in terms of the partner plant they choose, which means that the same fungus could be grown on a large number of plant species.

Microbial Cultivation and Fortification: All microorganisms used in the bioremediation trails were grown in Bioflo and Celligen fermentor/bioreactor, each in its specific medium, to reach 10^6 CFU. Each microbial suspension was impregnated on a proper mordant at the rate of 20 ml microbial suspension per 100 gm mordant oven dried soil. Sewage soils were solely inoculated with a single microorganism at the rate of 100 gm impregnated mordant/400 gm soil.

Chemical Methods

Soil pH: The pH value in the soil was measured using the glass electrode method in a 1: 2.5 soil water suspension [18].

PTEs: Potential toxic elements in soil and plant materials were estimated by the ammonium bicarbonate DPTA as described by Cottenie *et al.* [19] using Atomic Absorption according to A.O.A.C [20].

Soil Quality Criterion Index (Zn Equivalent Model): Was numerically expressed for the levels of PTEs toxicity according to the following equation (ppm): Zn concentrationX1+Cu concentration X2+Ni concentration X8. A quality criterion index over 250 units indicated a risky situation necessitating remediation for sustainable farming management [21].

Statistical Analysis: Statistical analysis was done using SAS software (1985).

RESULTS

Plant's Growth Evaluation: Results did not indicate any inhibition in the germination percent of Indian mustard plants and the growth habitations of plants was normal without any obvious symptoms of PTEs toxicity in the contaminated sewage soils as previously showed by Marchiol *et al*. [22] and Achakzai *et al*. [10]. Nanda Kumar *et al.* [23] reported that the following concentrations were not phytotoxic to *Brassica juncea* when added to soil mixtures: 2 mg/L Cd^{2+}, 100 mg/L Ni^{2+}, 50 mg/L Cr^{3+}, 500 mg/L Pb^{2+}, 3.5 mg/L Cr^{6+}, 100 mg/L Zn^{2+} and 10 mg/L Cu^{2}. The results in Table 2 indicated that Indian mustard plants positive response to the addition of remediative amendments to the highly decontaminated sewage soil. Application of bentonite, AM, Thiobacillus and the all remediative amendments under study produced healthy indian mustard plants and caused an increments of the root weight by 41.3, 68.4, 78.4 and 83.9%, respectively relative to that plants growing in soil without additives. More or less the aerial parts take the same direction. Indian mustard plants was significantly increased by 47.6, 53.3 and 73.4%, when bentonite, AM, *Thiobacillus* were used, respectively. A cumulative effect was recorded when the three tested remediative amendments were applied together, where the aerial parts and total plant weight were increased by 81.4 and 81.6%, respectively in comparison to the plants grown without additives.

Insignificant differences were noticed in the total plant weight between all the additives and *Thiobacillus spp.* treatments as well as between bentonite + rock phosphate and Mycorrhyzal (AM) conidia treatments (Table 1).

Yield and Its Attributes: Application of Mycorrhyzal (AM) conidia as a remediative amendment, *Thiobacillus spp.* significantly increased the root weight of Indian mustard plant as well as all other remediative amendments also caused significant increase of Indian mustard root weight by 80.0, 69.4, 27.3 and 26.1% than that of control, bentonite + rock phosphate, *Thiobacillus spp.* and All remediative amendments treatments, respectively (Table 3).

Insignificant difference was also recorded in the root weight between *Thiobacillus spp.* and all remediative amendments treatments. The shoot weight of Indian mustard plants was significantly affected by the remediative amendment treatments (Table 3), where application of Mycorrhyzal (AM) conidia and all remediative amendments treatments caused a significant increase of shoot weight by 25.5 and 16.7 %, while application of Bentonite + rock phosphate and *Thiobacillus* spp. treatments significantly decreased the shoot weight by 11.1 and 15.4%, respectively, if compared to untreated treatment Similar trend was noticed with plant weight criteria (Table 3). Growing Indian mustard plants in contaminated sewage soil without additive produced more number and weight of pods plant^{-1} than those of plants growing in the plots treated with one of the remediative amendments treatments. These results were true except with Mycorrhyzal (AM) conidia treatment, where the plants gave insignificant increase of pods weight than that of control. Concerning the seed yield plant^{-1} and per hectare, the data in Table (3) indicated that application of Bentonite + rock phosphate to the contaminated sewage soils gave the lowest seed yield of Indian mustard plants which significantly about the half of untreated treatment. This result may be attributed to that using Bentonite + rock phosphate gave the lowest number and weight of pods per plant^{-1}.

Insignificant difference in the seed yield (Table 3) was noticed between the two treatments of Mycorrhyzal (AM) conidia and all remediative and the untreated treatment. Application of *Thiobacillus spp.* as remediative amendment caused a reduction in the seed yield of Indian mustard plants by 12.8% relative to the control.

As shown in Table 3, in comparison to control all the four tested treatments exhibited reduction in the pod weight plant^{-1} and all the plants showed highly significant ($P<0.005$) reduction in the seed yield plant^{-1}. Insignificant difference was noticed in the seed yield plant^{-1} between Mycorrhyzal (AM) conidia treatment and control as well as among Bentonite + rock phosphate,

Table 2: Plant dry weight of Indian mustard (at vegetative growth stage) grown in highly contaminated sewaged soils in the presences and absence of some remediative amendments

	Indian Mustard		
Characters Treatments	Root Fresh weight (g plant^{-1})	Shoot Fresh weight (g plant^{-1})	Plant Fresh weight (g)
Without addition	45.3	481.8	527.1
bentonite + rock phosphate	64.0	714.0	778.0
Mycorrhyzal (AM) conidia	76.3	731.5	807.8
Thiobacillus spp.	80.8	833.3	914.1
All remediative amendments	83.3	873.8	957.1
LSD at 0.05	8.5	65.3	72.3

Table 3: Yield and Plant dry weight of Indian mustard grown in highly contaminated sewaged soils in the presences and absence of some remediative amendments

Characters Treatments	Root Fresh weight (g plant^{-1})	Shoot Fresh weight (g plant^{-1})	Plant Fresh weight (g)	Pods number Plant^{-1}	Pod weight (g)	Seed weight (g plant^{-1})	Seed yield (kg ha^{-1})
Without addition	23.3	367.7	391.0	551.7	111.7	41.0	2322
Bentonite + rock phosphate	24.8	326.8	351.6	386.5	63.4	20.6	1167
Mycorrhyzal (AM) conidia	42.0	461.3	504.4	503.5	113.8	40.8	2319
Thiobacillus spp.	33.0	311.0	344.0	397.9	91.2	33.5	2024
All remediative amendments	33.3	428.3	461.6	381.7	93.0	38.3	2178
LSD at 0.05	4.5	44.1	40.4	45.9	9.8	4.7	171.3

Table 4: Concentration of PTE (mg/kg soil) in the soil after harvested Indian mustard plants

	Concentration of PTE (mg/kg soil)				
Treatments PTEs	Control	Indian mustard	Reduction (%)	Indian mustard + AM	Reduction (%)
Zn	237.0	158.0	33.3	211.00	11.0
Mn	26.0	24.6	5.4	21.75	16.4
Cu	43.0	32.5	24.4	35.00	18.6
Ni	3.0	2.5	16.7	1.30	56.7

Table 5: Heavy metals content in the different organs of Indian mustard plants (at maturity stage) grown in highly contaminated sewaged soil in the presence and absence of AM inoculation (average of the replicates in ppm oven dried basis)

		Heavy metals								
		Mn			Cu			Zn		
Plant part	Conc.	HCSS	HCSS + AM	PML	HCSS	HCSS+ AM	PML	HCSS	HCSS+ AM	PML
Roots	Conc. (ppm)	144.20	46.70	30	14.1	12.50	20	218.3	284.3	50
	SD	6.60	2.27		4.62	4.20		0.2	0.5	
Shoots	Conc. (ppm)	94.10	119.30		20.8	27.40		351.7	208.5	
	SD	5.95	13.34		8.52	4.92		3.0	2.5	
	SD	2.80	5.02		1.53	1.71		0.2	0.5	
Plant	Conc. (ppm)	286.00	252.90		39.5	62.20		602.7	536.9	
Seeds	Conc. (ppm)	47.70	86.90		12.5	12.80		23.7	44.1	
	SD	2.80	5.02		1.53	1.71		0.2	0.5	

Abbreviations: Conc.; Concentration, HCSS; Highly contaminated sewaged soil, PML; permissible maximum limits [46]

Thiobacillus spp. and all remediative amendments treatments. The three previous treatments gave the same seeds weight plant^{-1}, which lowers than the control by 62.5% (Table 3).

Heavy Metals in the Soil and Indian Mustard as Affected by the Addition of AM Inoculation: Data presented in Table 4 showed that using Indian mustard plants as a hyperacumulators of PTEs from the contaminated sewage soils led to 33.3, 5.4, 24.4 and 16.7% reduction in the concentration of Zn, Mn, Cu and Ni metals in the soil, respectively. Inoculation of AM increased the decontamination of Mn an Ni metals by 11.6 and 48.0%, respectively if compared to plants without VAM inoculation. The results in Table 5 revealed that, in general, Zn mineral was found in different tissues of Indian mustard and its sequence concentration was root > shoot > seeds.

On the contrary, concentrations of Mn and Cu metals were in the sequence shoot > seeds > root. This may be due to that more Cu metal was transported and concentrated in the straw of the plants as shown in Table (5). In most cases, roots, shoots and seeds of Indian mustard contained less Mn, Cu and Zn content (mg kg^{-1}) in the presence of AM inoculation, except for Cu and Zn in the shoots and the seeds, respectively. AM inoculation resulted in enhancing phytoremediation of Mn and Cu in the shoots of Indian mustard by 26.8 and 31.7%, respectively in comparison to plants grown without AM inoculation.

However there was a slight decrease in Zn concentration in the seeds of the plants inoculated with AM. It's worthy to mention that Mn concentrations in the root, shoot and seeds of Indian mustard plant were found to be above the maximum permissible limits in plants. While the concentration of Cu in the seeds and all plant organs are lower than the Permissible Maximum Limits levels (WHO standards). Also, Zn concentration in the Indian mustard seeds was under the Permissible Maximum Limits levels (Table 5). It's worth to mention that undetectable of heavy metals was found in the volatile oil of Indian mustard plant, therefore the data excluded.

DISCUSSION

The present study is seeking for evaluating the ability of Indian mustard as a hyper-accumulator plant in decontamination of PTEs from contaminated sewage soils and hence to combat their adverse health and environmental impacts. Most of the traditional methods of PTEs decontamination from swage soils are either extremely costly (i.e., excavation, solidification and burial) or simply involve the isolation of the contaminated sites.

Some methods, such as soil washing, can pose an adverse effect on soil biological activity and fertility. On the other hand, phytoremediation is an emerging biotechnology for cleaning contaminated sewage soils that is attractive due to its low cost and versatility. It showed a tremendous potential in several field applications for PTEs decontamination in soils [3]. A special advantage of phytoremediation is that soil functioning is maintained and life is soil reactivated. Phytoremediation has its drawbacks as it is a long-term, slow complex biological process and PTEs specific besides lacking strict measures of efficiency to reach a solid conclusion [24]. *Brassica juncea* (Indian mustard) - a high-biomass plant that can accumulate Pb, Cr (VI), Cd, Cu, Ni, Zn, 90Sr, B and Se [23, 1]. Cu^{2+} (6 mg/L) and Zn^{2+} (100 mg/L) were accumulated in Indian mustard roots with a bioaccumulation coefficient of 490 and 131after 24 hours, respectively [25].

In the current work Zn, Cu and Mn were selected as indicator PTEs in plant and soil samples to follow the effect of phytoremediation on PTEs decontamination. This selection based on the adverse action of these PTEs on the microbial activity in sewage soil. In addition, the concentration of Mn in the sewage soil was higher than the normal rage compared to other PTE's calculated. Concerning other PTE's, it was found that their content in the sewage soil was very low. Nickel was estimated in the sewage soil because it is a major parameter needed in calculating the Zn equivalent equation.

In the present work inoculation with VAM generally enhanced phytoremediation of PTEs with Indian mustard. VAM establishes mutual symbiosis with the majority of higher plants, providing direct links between fungi and roots Sharda and Adholeya [26]. VAM exerts direct and indirect protective effects on the host plants under conditions of soil PTEs contamination [27]. Direct effects include PTEs bending and immobilization in VAM structure and indirect effects include VAM contribution to balanced plant mineral nutrition leading to increased plant growth and enhanced PTEs tolerance. The effect of VAM inoculation might facilitate plant growth and lower and less toxic levels of PTEs increased the amount of PTEs taken up by the plant [28]. Also siderophores might help the making some PTEs in the soil more bioavailable [29, 30]. Audet and Charest [31] reported that VAM had a key role in the regulation of soil PTEs bioavailability through bio-sorption processes and in the alleviation of plant PTEs toxicity and nutrient imbalances [27].

The data obtained in our experiment confirmed that Indian mustard plants are tolerant to heavy metals. Similar results were reported by Lim *et al*. [32] and Purakayastha *et al*. [6]. AM inoculation resulted in enhancing phytoremediation of Mn and Cu in the shoots of Indian mustard by 26.8 and 31.7%, respectively in comparison to plants grown without AM inoculation. However, there was a slight decrease in Zn concentration in the seeds of the plants inoculated with AM. Similar results were found by Medina and Azcón [33] and Achakzai *et al*. [10] who reported that the beneficial effectiveness of this symbiosis with suitable AM fungi in *A. niger*-treated agro-waste residue-amended soil can also be regarded as a successful biotechnological tool for reclamation of H M-contaminated soils.

Midarkodi [34] reported that the ratio of metals between soil and plant parts (transfer coefficient) is an important criterion for the selection of plant species for phytoremediation of soils contaminated with high level of heavy metals. The metal ratio ranged from 6.9 to 7.5 for *Brassica jun*cea revealed its high accumulation capacity [35]. Reisinger *et al.* [36] reviewed the literature and reported that plants have evolved a number of mechanisms to cope with heavy metal stress. These include the synthesis of the S-rich metal chelators glutathione (GSH) and phytochelatins (PCs) [37]. GSH, the most abundant low-molecular-weight thiol compound in plants [39].

Zn mineral was found in different tissues of Indian mustard and its sequence concentration was root > shoot > seeds. On the contrary, concentrations of Mn and Cu metals were in the sequence shoot > seeds > root. This may be due to that more Cupper metal was transported and concentrated in the straw of the plants as shown in Table 5. Similar results were reported by Purakayastha *et al*. [6] and Achakzai *et al*. [10]. They showed that *Brassica juncea* extracted the largest amounts of Cu from the soil. In general, the highest concentration and uptake of PTEs was observed in shoots compared to roots or seeds of the different species. Total uptake of PTEs by *Brassica* spp. correlated negatively with the available as well as the total soil PTE concentrations. Among the root parameters, root length emerged as the powerful parameter to dictate the uptake of PTEs by *Brassica* spp. Soil inoculation with AM that has a distinct advantageous action with higher plants was done to increase the surface absorption area of plant roots to guarantee maximum nutrient uptake by plants for the most part from the targeted PTEs. This is a natural biological process taking place for all time in all cultivated soil ecosystems.

Soil treatment with bentonite aimed to increase the adsorptive power of the sewage soil ecosystem. The application of bentonite seemed to have some ecological and health adverse impacts as mentioned before. Bentonite contains some PTEs and some radioactive sources that might faultily adverse soil ecosystem. Parallel to that it might cause some health impacts to those who came in close contact with this amendment. The bioremediation process implemented in the current work raised bentonite content in the soil ecosystem, however being not that much to put forth severe adverse impacts. However precautious and imitative measures have to be cared about on the basis of the periodical monitoring results of the sewage soil ecosystem. Despite of that, the risks associated with bentonite application are acceptable. The expected adverse risks associated with bentonite use, when interacted with the receiving sewage soil ecosystem are considered direct, immediate, cumulative, predicted, medium, indeterminate, sever, fast acting and directed to many species.

It's worth to mention that undetectable of heavy metals was found in the volatile oil of Indian mustard plant. Similar finding was mentioned by Hussein *et al.* [40] who reported that no detectable amount of the potential toxic elements was recorded in the essential oils of the aromatic plants. In the same line, Zheljazkov *et al.* [41] mentioned that peppermint, basil and dill can be grown in soils enriched with Cd, Pb and Cu medium without risk for metal transfer into the oils and without significant alteration of essential oil composition that may impair marketability. They added that results support the use of aromatic plants as alternative crops for Cd, Pb and Cu enriched soil. Also, Bağdat and Eid [42] and Chand [43] came to the similar findings. Bağdat and Eid [42] concluded that the tested cultivars of peppermint and corn mint could be successfully grown in highly heavy metal polluted areas, as in the area around NFMC near Plovdiv, without contamination of the end product - the essential oils. Despite yield reduction (up to 14%), due phytoremedation behaviour of some medicinal and aromatic plants to various pollutants to heavy metal contamination, mint still remained a very profitable crop and it could be used as substitute for the other highly contaminated crops. Blagojeviæ *et al*. [44] reported that the general view on heavy metals content in the sage essential oil showed that the concentration of toxic heavy metals Pb and Cd, as well as Zn, was bellow detection limits of used analytical technique.

Phytoremediation by rotation of crops with certain accumulators or hyper-accumulators is a very real option. Recovery of PTEs from vegetation had centered on incineration and recovery from ash or wet extraction techniques. Even if it were not practicable to recover the PTEs from plant biomass or ash, they would be concentrated into a much smaller volume for ultimate disposal.

CONCLUSIONS

The results showed promising effectiveness in decontaminating of PTEs, POPs and enteric pathogens through the bioremediation or chemo-remediation of such contaminated soils. The results indicated that soil inoculation with AM to the cultivated land with Indian mustard plants seems to be more effective in achieving the phytoremediation process. Generally it could be concluded that using the integrated treatment of chemical, biological prior sowing the hyper-accumulator Indian mustard plants could be effective in decontamination of such polluted soils and gave a safe product from Indian mustard oils. These data provide assurance about the minimal risk to the environment from trace elements and PTEs when using hyper-accumulator plants in such a long-term sludged soils.

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