AIMS Environmental Science, 4(2): 187-205.
DOI: 10.3934/environsci.2017.2.187
Received: 19 December 2016
Accepted: 14 February 2017
Published: 23 February 2017

http://www.aimspress.com/journal/environmental

Research article

Assessment of repeated harvests on mercury and arsenic phytoextraction in a multi-contaminated industrial soil

Martina Grifoni[1], Francesca Pedron[1], Gianniantonio Petruzzelli[1,*], Irene Rosellini[1], Meri Barbafieri[1], Elisabetta Franchi[2] and Roberto Bagatin[2]

[1] Institute of Ecosystem Studies, National Council of Research, Pisa, Italy
[2] Eni S.p.A., Renewable Energy & Environmental Laboratories, S.Donato Milanese (MI), Italy

* **Correspondence:** Email: petruzzelli@ise.cnr.it; Tel: +39-050-3152489; Fax: +39-050-3152473.

Abstract: Mercury is widely distributed throughout the environment. In many contaminated soils other contaminants are present along with mercury; of these, arsenic is one of the most frequently found metals. In the presence of mixed contamination of this kind, remediation technologies must overcome many difficulties due to the different chemical characteristics of the various contaminants. In this study, repeated assisted phytoextraction cycles with *Brassica juncea*, were conducted on a laboratory scale to evaluate the removal efficiency of mercury and arsenic from a multi-contaminated industrial soil. The possibility of using only one additive, ammonium thiosulphate, to remove mercury and arsenic from co-contaminated soil simultaneously was also investigated. The thiosulfate addition greatly promoted the plant uptake of both contaminants, with an efficiency comparable to that of phosphate specifically used to mobilize specifically arsenic. Repeated additions of mobilizing agents increased metal availability in soil, promoted plant uptake and consequently increased the removal of contaminants in the studied soil.

Repeated treatments with thiosulfate increased the concentration of mercury and arsenic in the *Brassica juncea* aerial part, but due to toxic effects of mercury that reduce biomass production, the total accumulation of both metals in plants tended to decrease at each subsequent re-growth.

The use of a single additive to remove both contaminants simultaneously offers several new advantages to phytoextraction technology in terms of reducing cost and time.

Keywords: mercury; arsenic; assisted phytoextraction; contaminated soil; phytoremediation; repeated harvest

1. Introduction

Mercury (Hg) is a global environmental pollutant that is highly toxic and widely distributed throughout the environment, since it can be transported over long distances in the atmosphere [1]. In the soil, Hg chemistry is characterized by several processes such as adsorption and release from solid phases, oxidation and reduction, complexation with organic and inorganic ligands, and methylation. Of the various technologies used to remediate Hg-polluted soils [2], phytoextraction has been chosen in many cases, with contrasting results due to site-specific conditions [3-6].

The phytoextraction of metals and metalloids has received significant attention as a non-impact, environmentally safe remediation strategy for polluted soils. In the case of contamination derived from more than one metal, a thorough investigation of the soil properties is essential for determining the main components responsible for the mobility and bioavailability of metals and metalloids [7]. Arsenic (As) is often present along with Hg in many contaminated sites.

Hg and As are typically non-essential elements for plants, with different chemical characteristics and different behavior in relation to soil properties, such as pH and cation exchange capacity (CEC). Their removal often requires separate remediation strategies. For Hg-assisted phytoextraction, a thiosulfate salt is usually used [3,6], whereas a phosphate salt [8] is the most suitable additive for As. In presence of mixed heavy metal pollution, implementing of phytoextraction at field scale can require a long time to reduce metal concentrations to safe levels and to comply with environmental regulations. The use of the same mobilizing agent to increase the bioavailability and plant uptake of both contaminants would facilitate the phytoextraction process by greatly reducing both time and costs.

Phytoextraction has been confirmed as an efficient bioremediation technology for soil contaminated by heavy metals. Its approach is in agreement with the latest environmental sustainability criteria, making it an emerging eco-friendly technique for soil clean-up, enjoying good public acceptance [9]. Phytoextraction is related to the green technology of phytoremediation, which exploits the ability of plants to remove pollutants (especially metals) from soil or water via their roots and store them in the harvestable part of plants [10-12]. Phytoextraction efficiency is affected by several factors related to both the plants and the soil; particularly important is metal bioavailability, defined as the mobile and available contaminant fraction in soil for uptake by plants and soil organisms [7,13-15]. Indeed, the plants are able to uptake only the substances present in soil solution in bioavailable forms [16].

Phytoextraction is an effective and economical technology compared to conventional soil remediation techniques; moreover, it causes less soil disturbance, preserving the structure and fertility of soil [17]. In some cases this technology can also offer the possibility of bio-recovery of metals (bio-phytomining) [18,19]. The ability of phytoextraction to remove hazardous contaminants while simultaneously restoring the polluted site have led to this technique's wide acceptance among communities. Thus, assessing phytoextraction's success should consider not only the total metal amount removed from soil but also the positive environmental impacts obtained.

Recently, several studies have focused on researching strategies to improve the technique's efficiency. Among the latest developments related to improving the phytoextraction of metal, the use of PGPR bacteria (Plant Growth-Promoting Rhizobacteria) and genetic engineering have obtained positive results [20,21]. Assisted phytoextraction also appears very promising, since it exploits certain properties of fertilizers or chemical additives to promote the release of metals from the solid

phase of soil, increasing the bioavailable metal concentration in soil solution and consequently metal uptake by plants [16,22].

In the last decade, synthetic chelators such as the aminopolycarboxylic acids (APCAs), including EDTA (ethylenediaminetetraacetic acid), HEDTA (hydroxyethyl ethylenediamine triacetic acid), and DTPA (diethylenetriamine pentaacetic acid), have been the most commonly employed chemical agents in assisted phytoextraction [23-25]. However, their high mobilizing capacity and long persistence in soil may cause increased metal concentrations in soil solution. Indeed, the amount of metal released often exceeds the bioavailable quantity absorbable by plants [26-28], with the possibility of their leaching towards ground water [29-31]. For this reason, new biodegradable mobilizing agents, such as natural low-molecular-weight organic acids (NLMWOAs), EDDS (ethylene diamine disuccinate), NTA (nitrilotriacetate), and humic substances, which could limit the metals' leaching from soil with reduced additional negative effects on the surrounding environment, have been tested recently. However, they do not eliminate the risk of contaminant mobilization and the associated hazards [22,32-34].

Repeated phytoextraction cycles are often needed to reduce soil metal concentration to acceptable levels [35,36]. After the first harvesting, a certain amount of metal can remain in soil and further cycles of plant growth can reduce residual metals to bioavailable forms. When the bioavailable metal pool is exhausted, phytoextraction efficiency is progressively reduced [37] and the process can be regarded as completed. The residual metal fractions in soil can be considered harmless and permanently unavailable [16]. In order to verify the technology's success and the absence of extractable metals, both metal concentration in plants and the amount of metal extracted by mobilizing agents from soil must be examined.

This study aimed to evaluate the efficiency of repeated phytoextraction cycles to remove Hg and As by means of *Brassica juncea* (Indian mustard) from a multi-contaminated soil. Two mobilizing agents, ammonium thiosulfate, $(NH_4)_2S_2O_3$, and potassium dihydrogen phosphate, KH_2PO_4, were used to increase metal bioavailability in soil with the further goal of investigating the possibility of using only one additive to remove both Hg and As from soil simultaneously. Four growing cycles on the same soil sample were carried out, with or without consecutive addition of mobilizing agents. Results showed that ammonium thiosulfate notably increased the plant uptake not only of Hg but also of As, with an efficiency comparable to that obtained by phosphate used to mobilize As. Treatment with a single additive can offer advantageous new developments for phytoextraction technology, since both time and costs are reduced.

2. Materials and Methods

2.1. Soil collection and characterization

Contaminated soil used in this study was collected from an industrial site located in northern Italy. Soil samples were collected at a depth of 0–1 m, air-dried, and passed through a 2-mm sieve for laboratory analysis. The following soil physical properties were determined according to standard methods [38]: soil pH, using a 1:2.5 soil/water ratio, cation exchange capacity using barium acetate, and texture (sand, silt and clay) via the pipette method. Organic matter content was measured with RC-412 Multiphase Carbon Determinator and N content with FP-528 Nitrogen/Protein Analyzer For Organic Samples. Total concentrations of Hg and As were determined via acid digestion using the EPA Method 3051A [39].

2.1.1. Soil extraction

The bioavailable fractions of metals in soil samples were evaluated by specific chemical extraction techniques. The Hg bioavailable fractions were determined by two steps of a sequential extraction procedure [40] with 1M NH_4Cl (ammonium chloride) (soil/extractant ratio of 1:50 for 1 h) and 0.27M $(NH_4)_2S_2O_3$ (soil/extractant ratio of 1:20 for 2 h). The maximum amount of extractable As was quantified, adopting the first two steps of modified Wenzel's sequential extraction [41] in which 0.05M $NaNO_3$ (sodium nitrate) and 0.05M KH_2PO_4 (soil/extractant ratio of 1:25 for 2 h) were added sequentially. $NaNO_3$ was used instead of $(NH_4)_2SO_4$ (ammonium sulfate), since a weaker extractant better simulates readily available As in this soil. The supernatant was separated after centrifugation at 15.000 rpm for 15 min.

To evaluate the long-term release of potentially bioavailable Hg and As from soil solid phases, five consecutive extractions with 0.27M $(NH_4)_2S_2O_3$ [3,5] were conducted on the same soil sample. As extractability was also determined using 0.05M KH_2PO_4 [42]. Each extraction was performed by shaking soil and extractant (ratio of 1:25) for 2 h, using 50 mL polypropylene centrifuge tubes. The supernatant was separated after centrifugation at 15.000 rpm for 15 min, and a new extraction was carried out on the remaining amount of soil.

Extractions were run in triplicate and the extracts were analyzed for Hg and As content after filtration.

2.2. First growing cycle

To maintain the real situation of the polluted soil, the sampled soil was prepared by eliminating the coarser material without sieving to 2 mm and accurately homogenized. The trials were carried out in 250 mL microcosms. The pots were filled with 200 g of contaminated soil and sown with 0.30 g of *B. juncea* seeds. This species was selected because of its versatility, rapid growth and ease of cultivation, as exhibited in previous microcosm studies [42-44]. The first harvest was considered preliminary to the long-term phytoextraction test, based on a total of four growing cycles.

During the growing period, the plants were watered daily with deionized water without additional nutrients. Two weeks after sowing, soil treatments with mobilizing agents, 0.27M ammonium thiosulfate (T) and 0.05M potassium dihydrogen phosphate (P), were started following the procedure in previous works [42,45,46]. The solutions were added to soil by splitting the total dose, 10 mL for each additive, over 5 days of applications to avoid or at least minimize possible toxic effects on plants [8]. Control microcosms (C) with untreated soil were run simultaneously. The pots were arranged in a completely randomized design and three replicates for control and twelve replicates for each treatment were prepared, with a total of 27 microcosms.

At the end of treatment, plants were harvested, separating roots from shoots, and prepared for analysis.

2.3. Repeated growing cycles

After the first harvest three further growing cycles were performed. The experimental design including the first growing cycle is reported in Figure 1. At each re-growth, the same sowing and treatment procedure was adopted. In some microcosms the treatments were repeated, while in other

pots the treatments were stopped (nt, not treated). At the end of the fourth growing cycle, microcosms received the additive, T or P, only one time (T/nt/nt/nt or P/nt/nt/nt), two times (T/T/nt/nt or P/P/nt/nt), three times (T/T/T/nt or P/P/P/nt) and four times (T/T/T/T or P/P/P/P).

About 2 months passed between one harvest and another. The plants were watered daily or as necessary.

At each growing cycle, 10 days after the end of treatments, all plants were harvested and aerial parts were separated from roots. Vegetal samples were washed with deionized water and roots were further washed in an ultrasound bath (Branson Sonifier 250 ultrasonic processor; Branson, Danbury, CT, USA) for 10 min to eliminate the possible soil particles remaining on radical surfaces.

The dry mass of shoots and roots was gravimetrically determined after drying in a ventilated oven at 50 °C to a constant weight. Dry plant samples were ground into a fine powder and digested with acid for metal analysis. The plants were evaluated in terms of growth (biomass values), accumulation, translocation and uptake of Hg and As.

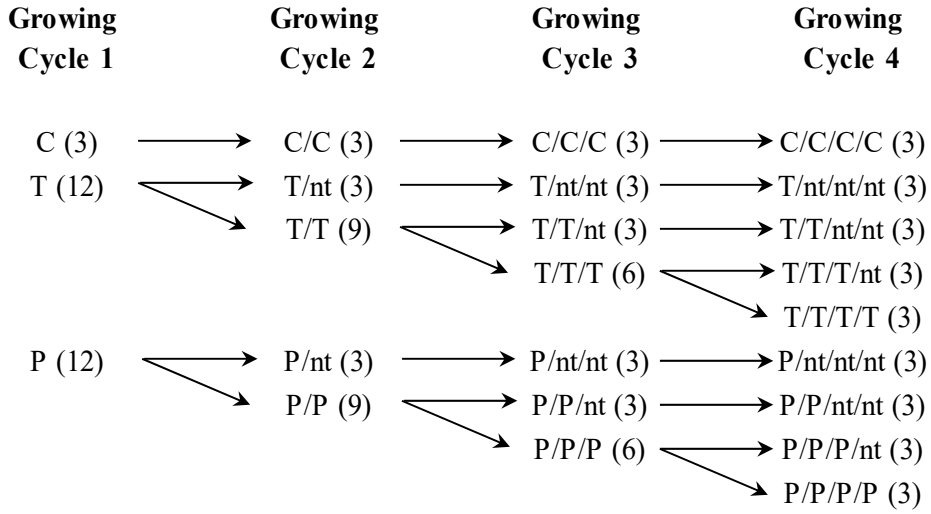


Figure 1. Experimental framework of the four repeated growing cycles. Note: when a mobilizing agent was added, the specific letter (T for thiosulphate and P for phosphate) is reported in the table. When the addition of the mobilizing agent was not repeated the symbol "nt" is used. In parentheses the number of microcosms used are reported.

2.4. Digestion and analysis

Metal content in soil and plant samples was determined in accordance with EPA Method 3051A [39] and EPA Method 3052 [47], respectively.

Samples were digested with HNO_3 (65%, v/v) and H_2O_2 (30%, v/v) mixture in a PTEF-TMF (polytetrafluoroethylene-tetra-fluoromethoxil) pressure digestion vessel using a microwave oven (FKV-ETHOS 900). Total Hg and As concentrations in digested samples and soil extracts were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) with Liberty AX Varian spectrometer.

Metal concentrations were expressed in milligrams per kilogram dry weight (mg kg^{-1}). All data reported are the average of three replicates.

2.5. Quality assurance and quality control

Quality assurance and quality control were performed by testing the standard solution every 10 samples. Certified reference material (BCR n°141) was used to control the quality of the analytical system. Detection limits were 2 μg L^{-1} for Hg and 5 μg L^{-1} for As, respectively. The recovery of spiked samples ranged from 93 to 101% with a RSD of 1.91 of the mean.

2.6. Statistical analysis

All statistical analysis was performed using STATISTICA version 6.0 (Statsoft, Inc., Tulsa, OK, USA). Treatments effects were analyzed using one-way analysis of variance (ANOVA). Differences among means were compared and a post-hoc analysis of variance was performed using the Tukey Honestly Significant Difference test ($p < 0.05$).

3. Results and Discussion

3.1. Soil analysis

3.1.1. Soil characterization

Soil was characterized by the following parameters: pH 8, clay 7.1%, silt 13.1%, sand 79.8%, CEC 15.6 $cmol_{(+)}kg^{-1}$, organic matter 1.48%, total N 0.08%. Metal concentrations were As 37.6 and Hg 67.0 mg kg^{-1} soil.

3.1.2. Soil extraction

Bioavailable metals are the fraction of the total amount of metals in soil available for plant uptake in a given time period [13,15,48]. Bioavailability evaluation is essential for phytoextraction tests, since only the amount present in soil solution can be taken up by plants [16,49].

The sequential extractions adopted provided useful information on mobility and long-term bioavailability of metals in soil (Table 1).

Since Hg was not extracted by NH_4Cl, most of it should be considered strongly bound to soil surfaces. Thiosulfate extracted about 16% of Hg total concentration. The action of thiosulfate may be ascribed to Hg affinity for thiol groups, with consequent formation of complexes with sulfur containing ligands [50]. Previous studies have shown the effectiveness of thiosulfate for increasing Hg bioavailability in soil [29,51-54]. Also, the As extractability test revealed a low solubility of this element in the soil used. Readily bioavailable As, extracted by $NaNO_3$, was negligible, whereas KH_2PO_4 extracted about 20% of the total concentration. This last extractant interacts with As fractions specifically adsorbed on soil surfaces. This solubilization can be explained by competition between phosphate and arsenate ions for soil sorption sites [55-57]. These ions have a high chemical similarity in that phosphate moves the As adsorbed on soil constituents through competitive exchange [58].

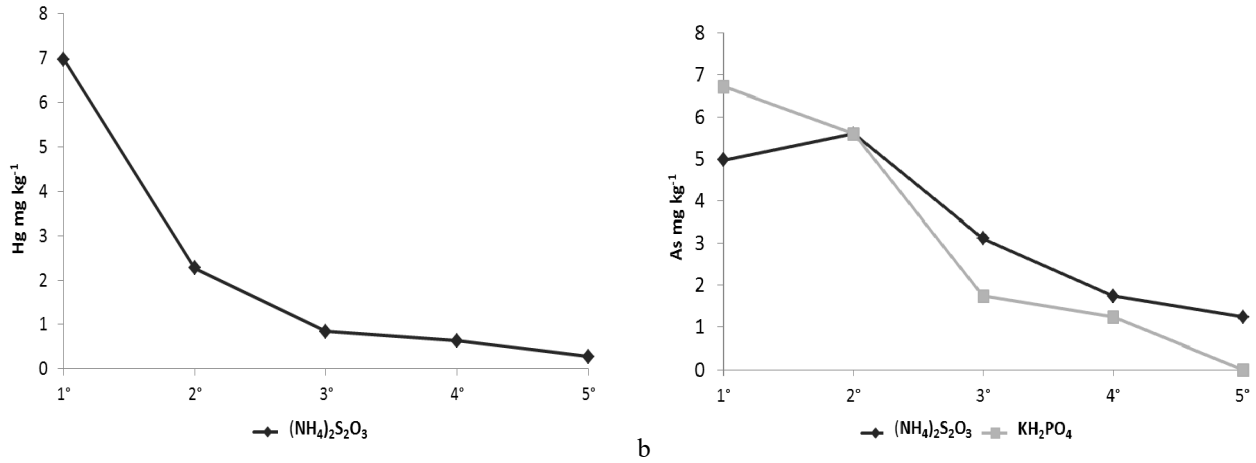
Table 1. Concentration (mg kg^{-1}) of Hg and As extracted by sequential procedure in soil samples. Data are means ± SD (n = 3)

Millán et al. [40]		
	NH_4Cl	$(NH_4)_2S_2O_3$
Hg	Bdl	10.4 ± 2.3
Wenzel et al. [41]		
	$NaNO_3$	KH_2PO_4
As	Bdl	7.2 ± 0.7

bdl: below detection limits.

These data indicate that it is only possible to solubilize sufficient amounts of the two metals by adding thiosulfate and phosphate. Since the aim of this study was to evaluate the results of consecutive phytoextraction cycles, repeated soil extractions were also performed. Thiosulfate was used as an extractant for Hg and As. For As, phosphate was also used.

As expected, when the extractions were repeated, the bioavailable amount of Hg and As in soil tended to decrease (Figure 2). After the second consecutive extraction, Hg concentration was significantly reduced by 67% and then tended to decrease to zero at the fifth extraction. Likewise, As extractability showed a progressive reduction, similar for both extractants used.



a b

Figure 2. Potential residual of Hg (a) and As (b) extracted using thiosulfate and phosphate. Data are means of three replicates and are expressed as mg kg^{-1} dry soil.

With a view to plant growth, results suggest that more treatments with these additives can effectively mobilize additional amounts of phytoavailable metals. The use of mobilizing agents can promote the phytoextraction process of Hg and As and enhance metal uptake in plants. However, after about five repeated extractions, further metal amounts cannot be released from soil. A residual fraction of metal remains irreversibly bound to soil surfaces. This fraction is nearly inert and not considered dangerous for human health and the environment [3].

3.2. Microcosm growing cycles

3.2.1. Biomass production

In phytoextraction tests, it is necessary to evaluate the re-growth capacity of plants in contaminated soil, since repeated harvests may be required to reduce metal amounts to acceptable levels in the soil [59,60]. *B. juncea* showed high tolerance for elevated concentrations of Hg and As, and throughout the course of the experiment (four growing cycles) no visual symptoms of phytotoxicity were detected, either in control or treated soils. *B. juncea* biomass production of each re-growth is reported in Table 2.

The decreased yield following thiosulfate addition can be ascribed to the increased Hg uptake by plants. As also observed by Pedron et al. [46], repeated thiosulfate treatments appear to increase the phytotoxic effect due to Hg, which can also adversely affect root development and consequently the growth of the entire plant [20,61]. Phosphate addition did not promote Hg uptake and the biomass production remained nearly the same. Radical yield was fairly similar among various re-growths, with a trend of reduction compared to biomass of the first growth.

Table 2. Effect of repeated growing cycles and mobilizing agents on shoot and root biomass of *B. juncea*. Data reported are the mean with standard deviations and are expressed as g dry weight.

	Growing Cycle 1		Growing Cycle 2		Growing Cycle 3		Growing Cycle 4	
	Treatment	Biomass	Treatment	Biomass	Treatment	Biomass	Treatment	Biomass
Shoot	C	0.43 ± 0.11	C/C	0.52 ± 0.12	C/C/C	0.41 ± 0.14	C/C/C/C	0.34 ± 0.18
	T	0.52 ± 0.15	T/nt	0.10 ± 0.04	T/nt/nt	0.38 ± 0.10	T/nt/nt/nt	0.33 ± 0.12
			T/T	0.30 ± 0.05	T/T/nt	0.29 ± 0.08	T/T/nt/nt	0.25 ± 0.08
					T/T/T	0.22 ± 0.12	T/T/T/nt	0.29 ± 0.10
							T/T/T/T	0.12 ± 0.04
	P	0.49 ± 0.08	P/nt	0.37 ± 0.08	P/nt/nt	0.43 ± 0.11	P/nt/nt/nt	0.25 ± 0.13
			P/P	0.68 ± 0.11	P/P/nt	0.50 ± 0.13	P/P/nt/nt	0.35 ± 0.09
					P/P/P	0.40 ± 0.09	P/P/P/nt	0.42 ± 0.11
							P/P/P/P	0.39 ± 0.08
Root	C	0.11 ± 0.08	C/C	0.04 ± 0.005	C/C/C	0.03 ± 0.02	C/C/C/C	0.03 ± 0.01
	T	0.10 ± 0.02	T/nt	0.03 ± 0.004	T/nt/nt	0.03 ± 0.01	T/nt/nt/nt	0.08 ± 0.02
			T/T	0.02 ± 0.005	T/T/nt	0.04 ± 0.01	T/T/nt/nt	0.06 ± 0.02
					T/T/T	0.02 ± 0.005	T/T/T/nt	0.07 ± 0.02
							T/T/T/T	0.02 ± 0.005
	P	0.11 ± 0.04	P/nt	0.05 ± 0.01	P/nt/nt	0.06 ± 0.02	P/nt/nt/nt	0.08 ± 0.01
			P/P	0.02 ± 0.005	P/P/nt	0.05 ± 0.03	P/P/nt/nt	0.07 ± 0.02
					P/P/P	0.06 ± 0.02	P/P/P/nt	0.08 ± 0.02
							P/P/P/P	0.09 ± 0.03

In this experiment, the potential toxic effect of Hg and As was probably opposed by phosphorus and sulfur fertilization, which facilitated the plants' growth. Splitting the total dose of mobilizing agents also helped minimize phytotoxic effects [62,63]. Thiosulfate and phosphate are

frequently used in phytoremediation at lab scale, since they can act both as mobilizing and detoxifying agents or nutrients, increasing sulfur, phosphorus and nitrogen availability in soil. In particular, sulfur seems to stimulate the plant's defensive systems through synthesis of sulfur-containing metabolites (glutathione and phytochelatins), allowing intracellular As detoxification processes [64-66]. Wu et al. [67] reported that in presence of high metal concentrations in soil, an elevated content of sulfur in *B. juncea* shoots was found, suggesting a possible physiological need of plants for metal tolerance mechanisms. In several studies [68-71], a biomass increase after phosphorus fertilization in different plant species grown in presence of As was observed. The influence of phosphorus nutrition on plant arsenate metabolism is probably due to interaction between both ions for biochemical processes in cell roots [72], causing a decrease in As phytotoxicity [73]. Tu and Ma [74] noted that As stimulated phosphorus uptake in *Pteris vittata*, producing a growth benefit and mitigating As phytotoxicity. However, considerable contradictory data remain regarding the influence of Hg and As on plant growth, so complete knowledge of tolerance mechanisms in plants is lacking. In phytoextraction field implementation, various agronomic strategies might be further considered to increase the vegetal biomass and reduce the phytotoxic effects of metals.

3.2.2. Hg and As in plants

Addition of mobilizing agents significantly increased the Hg and As concentration in *B. juncea* tissues. Phosphate and thiosulfate similarly influenced As absorption by plants. The As concentration increased greatly compared to control plants, in which the metal concentration was below the detection limit. Only the addition of thiosulfate significantly increased the Hg concentration; obviously phosphate had no influence on Hg uptake, so no data are reported.

In the repeated phytoextraction cycles, the effects of additives on metal concentrations in plants are reported in Tables 3 and 4.

Regarding Hg, thiosulfate addition increased the metal's mobility, thereby significantly increasing the concentration in shoots and roots of *B. juncea* with respect to controls. Data showed that without one further addition of thiosulfate (T/nt) the concentration of the Hg dropped from 121 to 52.8 mg kg^{-1} from the first to the second cycle. On the contrary, if the thiosulfate was added a second time, Hg concentration increased compared to that found in the first cycle, from 121 to 173 mg kg^{-1}. In the third growing cycle, Hg concentration decreased from 52.8 to 21.4 mg kg^{-1} if no more thiosulfate treatment was added to soil. The same trend also occurred in the microcosms treated with thiosulfate in the previous two cycles but not in the third (T/T/nt), with concentration values that decreased from 173 to 63.8 mg kg^{-1}. The third addition of thiosulfate (T/T/T) increased the concentration up to 104 mg kg^{-1}. However, this value was lower than that found as the maximum uptake in the second cycle. Finally, in the fourth growth cycle, in microcosms with one addition of thiosulfate (T/nt/nt/nt), the value fell to 16.9 mg kg^{-1}. A similar value, 15.2 mg kg^{-1} was also found after two thiosulfate additions (T/T/nt/nt). Where thiosulfate was added three times (T/T/T/nt), Hg concentration decreased from 104 to 61.4 mg kg^{-1} if compared to the previous cycle. After four treatments with thiosulfate (T/T/T/T), the plants tended to accumulate a certain amount of Hg with a mean value of 96.4 mg kg^{-1}. A similar trend is also found for the roots. For example, where only one treatment with thiosulfate (T) was performed, Hg concentration decreased from a value of 834 mg kg^{-1} in the first cycle to 56.0 mg kg^{-1} in the fourth cycle

(T/nt/nt/nt). Where thiosulfate was added four times (T/T/T/T), data show that the roots continued to absorb a certain amount of Hg.

Table 3. Effect of repeated treatments and growing cycles on concentration of Hg in *B. juncea* shoots and roots. Data reported are the mean with standard deviations and are expressed as mg kg^{-1} dry weight.

	Growing Cycle 1		Growing Cycle 2		Growing Cycle 3		Growing Cycle 4	
	Treatment	Concentration	Treatment	Concentration	Treatment	Concentration	Treatment	Concentration
Shoot	C	0.52 ± 0.1	C/C	1.47 ± 0.6	C/C/C	1.42 ± 0.5	C/C/C/C	0.80 ± 0.4
	T	121 ± 20	T/nt	52.8 ± 12	T/nt/nt	21.4 ± 4.8	T/nt/nt/nt	16.9 ± 2.3
			T/T	173 ± 26	T/T/nt	63.8 ± 9.8	T/T/nt/nt	15.2 ± 1.7
					T/T/T	104 ± 16	T/T/T/nt	61.4 ± 14
							T/T/T/T	96.4 ± 15
Roots	C	33.1 ± 12	C/C	18.6 ± 2.3	C/C/C	12.8 ± 1.8	C/C/C/C	4.23 ± 0.3
	T	834 ± 42	T/nt	161 ± 15	T/nt/nt	99.5 ± 10	T/nt/nt/nt	56.0 ± 2.8
			T/T	399 ± 28	T/T/nt	151 ± 16	T/T/nt/nt	104 ± 11
					T/T/T	558 ± 29	T/T/T/nt	452 ± 34
							T/T/T/T	561 ± 22

Treatment with phosphate increased the amount of As in *B. juncea* aerial parts. Without further addition of phosphate, this amount tended to decrease in the subsequent growth cycles, going from 7.35 mg kg^{-1} to the final value of 1.59 mg kg^{-1}. When the phosphate treatment was repeated (P/P in the second cycle), the plants absorbed an amount of As similar to that of the first cycle. This quantity remained constant even in the third cycle in the absence of any further addition of mobilizing agent (P/P/nt) and slightly decreased in the fourth cycle (P/P/nt/nt). In the third growth cycle, when the phosphate was added three times (P/P/P) the As concentration in the aerial part rose to 15.4 mg kg^{-1}. In the fourth cycle of growth, where phosphate solution was not added, As concentration decreased slightly, while further addition of phosphate (P/P/P/P) increased the value up to 18.6 mg kg^{-1}. It is well-known that phosphate ion is the specific mobilizing agent for As. The addition of thiosulfate caused an absorption trend of As similar to that resulting from the addition of phosphate, with an increase in the amount of As absorbed by *B. juncea* compared to controls. When the treatment was not repeated, the concentration of As in the aerial part tended to decrease from 7.32 mg kg^{-1} in the first cycle to 2.17 mg kg^{-1} in the fourth cycle. When treatment with thiosulfate was repeated, the amount of As absorbed by the plant increased up to 13.1 mg kg^{-1} in the fourth cycle (T/T/T/T). When the thiosulfate treatments were not repeated, there was a decreased As concentration in *B. juncea* plants in each cycle of growth, with a pattern similar to that described for microcosms treated with phosphate. Data regarding plant uptake showed the existence of a residual bioavailability of As in soil after the first treatments, and its increase with subsequent additions of both phosphate and thiosulfate in the following growing cycles.

Table 4. Effect of repeated treatments and growing cycles on concentration of As in *B. juncea* shoots and roots. Data reported are the mean with standard deviations and are expressed as mg kg^{-1} dry weight.

	Growing Cycle 1		Growing Cycle 2		Growing Cycle 3		Growing Cycle 4	
	Treatment	Concentration	Treatment	Concentration	Treatment	Concentration	Treatment	Concentration
Shoot	C	bdl	C/C	bdl	C/C/C	bdl	C/C/C/C	bdl
	T	7.32 ± 1.0	T/nt	5.53 ± 1.2	T/nt/nt	3.21 ± 0.8	T/nt/nt/nt	2.17 ± 0.8
			T/T	6.62 ± 0.9	T/T/nt	4.70 ± 1.2	T/T/nt/nt	3.12 ± 1.2
					T/T/T	9.68 ± 1.3	T/T/T/nt	7.5 ± 0.3
							T/T/T/T	13.1 ± 0.9
	P	7.35 ± 1.2	P/nt	4.75 ± 0.8	P/nt/nt	2.81 ± 0.4	P/nt/nt/nt	1.59 ± 0.6
			P/P	7.81 ± 1.4	P/P/nt	6.6 ± 0.9	P/P/nt/nt	4.8 ± 0.1
					P/P/P	15.4 ± 1.5	P/P/P/nt	12.1 ± 0.7
							P/P/P/P	18.6 ± 1.1
Roots	C	bdl	C/C	bdl	C/C/C	bdl	C/C/C/C	bdl
	T	45.9 ± 7.2	T/nt	32.7 ± 8.2	T/nt/nt	25.9 ± 6.5	T/nt/nt/nt	12.3 ± 1.6
			T/T	108 ± 15	T/T/nt	37.2 ± 5.4	T/T/nt/nt	14.2 ± 0.8
					T/T/T	85.1 ± 15	T/T/T/nt	25.9 ± 5.2
							T/T/T/T	60.5 ± 4.8
	P	97.5 ± 6.3	P/nt	86.1 ± 11	P/nt/nt	80.2 ± 12	P/nt/nt/nt	71.3 ± 14
			P/P	154 ± 19	P/P/nt	96.2 ± 9.8	P/P/nt/nt	66.4 ± 14
					P/P/P	105 ± 12	P/P/P/nt	59.2 ± 12
							P/P/P/P	68.8 ± 10

bdl: below detection limits.

In agreement with several findings [3,69,75,76], Hg and As concentration in shoot tissues was significantly lower than in root tissues, indicating low mobility of metals within the plants. Hg and As remained stored in radical cells due to the roots' defensive mechanism [42]. Both the plant's specific features and the soil component can influence the uptake and the translocation of metals [5]. To obtain an efficient phytoextraction process, the metal fraction mobilized with additives should not only be taken up into roots but also subsequently transported to the easily harvestable plant portion. The ratio of metal concentration in shoot to root, translocation factor (TF), describes a plant's ability to move the metal from the root system to aboveground tissues. Species with TF ≥ 1 are generally considered hyperaccumulators [11,77-79], while the species with TF values slightly less than 1 are considered potentially suitable for phytoextraction. In this experiment TF values were considerably lower than 1, although the effect of treatments was relevant. Although the TF values were found to be low, *B. juncea* has a well-known high phytoextraction potential [6,80,81]. In fact, it is considered a metal-tolerant plant. The lower metal concentrations in plant tissues, compared to hyperaccumulator species, are balanced by higher biomass and faster growth rates that allow a greater total extraction [82].

In each growing cycle, Hg and As were concentrated mainly in roots rather than in shoots; however, in the time span of each growth cycle (about 30 days), the plants partially moved the absorbed metals from roots to aerial parts. Thiosulfate improved the translocation of Hg in *B. juncea* aerial parts although TFs remained considerably lower than 1. Similar results [51] have been

reported for *Lepidium sativum*, in which Hg translocation increased tenfold after thiosulfate addition, compared to control plants. No significant effect of phosphate on TF of As for *Pityrogramma calomelanos* species has been reported [83]. However, metal translocation in plants is influenced by several factors such as plant species, soil properties and soil metal concentration [84,85].

The "total accumulation" was evaluated to obtain the total metal amount extracted by plants, calculating the product of metal concentration and the aerial biomass. This parameter provides an estimation of phytoextraction efficiency, since it includes both metal uptake and vegetal biomass production [86]. Data of total accumulation showed the mobilizing agents' effectiveness on phytoextraction of both metals more clearly (Figures 3 and 4).

The continued extractive action of plants during more than one cycle of growth increased the removal of the bioavailable fraction. Although the laboratory data do not provide definite conclusions regarding performance in the field, the results suggested a potential applicability of repeated assisted phytoextraction for this soil.

In the aerial part, the addition of thiosulfate drastically increased the total accumulation of Hg. By further additions of thiosulfate, interesting values of total accumulation of Hg are obtained. In the roots the trend was generally similar to that of the aerial part.

Concerning As, the addition of phosphate increased the total accumulation in the aerial part. At each re-growth, further increases in total accumulation occurred only when phosphate was added. The addition of thiosulfate increased the total accumulation of As compared to controls; however, this parameter tended to decrease at each subsequent re-growth. For both metals, the trend of total accumulation is ascribable to the toxic effect of Hg, which is absorbed by the plant due to the addition of thiosulphate, causing decreased biomass production.

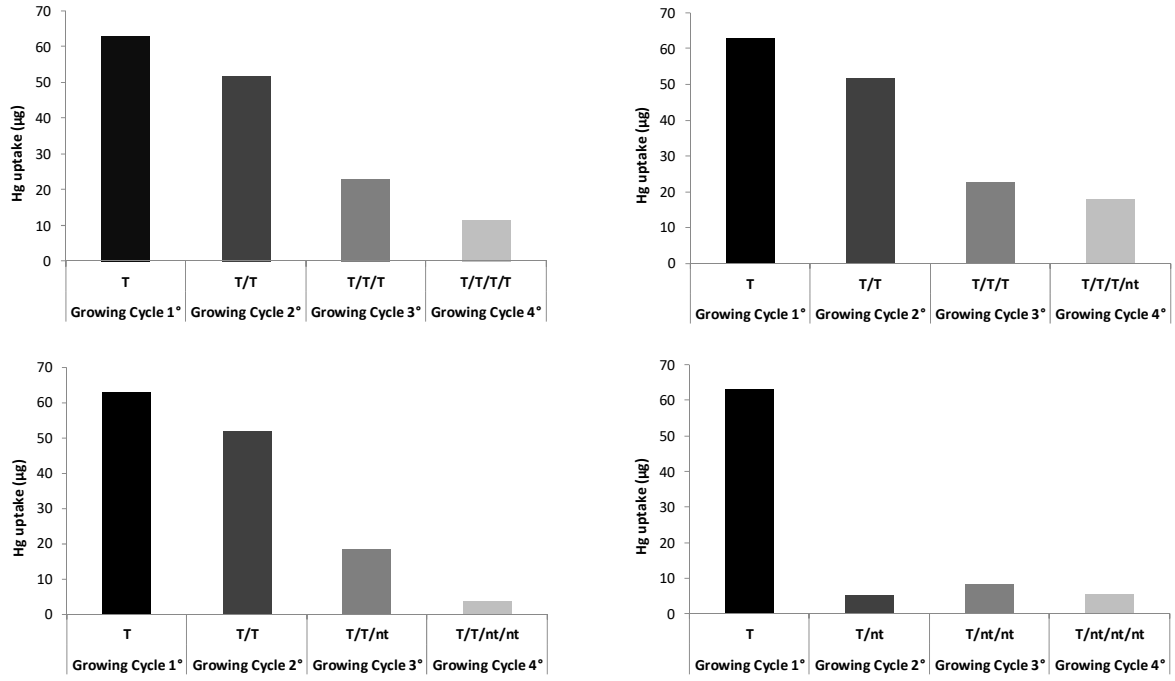


Figure 3. Effects of thiosulfate on total accumulation of Hg in *B. juncea* species. Data are expressed in µg.

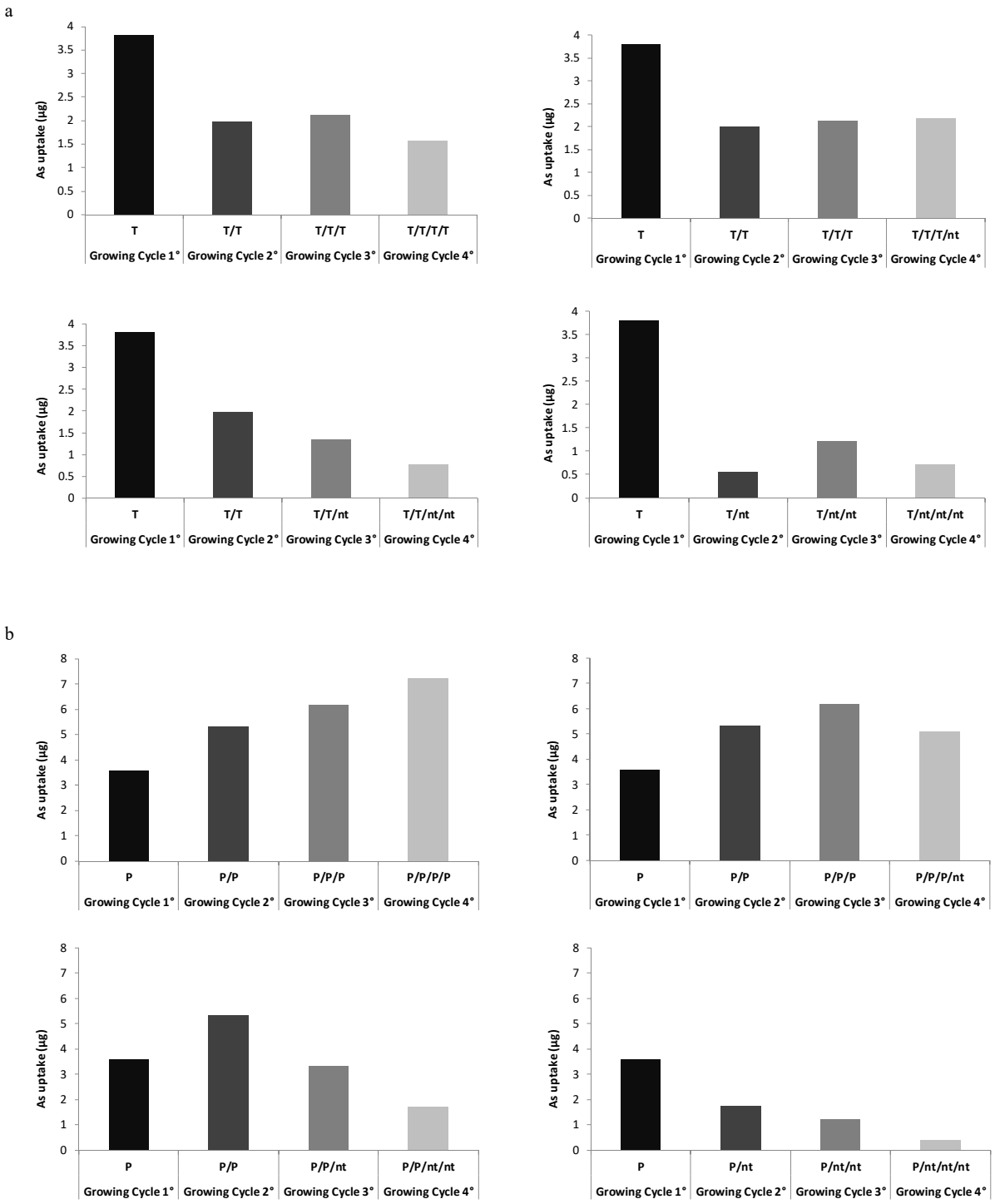


Figure 4. Effects of thiosulfate (a) and phosphate (b) on total accumulation of As in *B. juncea* species. Data are expressed in µg.

4. Conclusion

Both extractability tests and microcosm experiments show that more than one treatment with the additives used can effectively mobilize increasing amounts of metals, exploiting the bioavailable

pools. The ability of thiosulfate to induce Hg solubilization in soil and stimulate metal accumulation in plants is well-known [6,42,80]. Some researchers suggest that sulfur releases the Hg from soil sites to form stable complexes that are more easily absorbed by roots and preferentially transported to shoots [5,53,54,87].

The results of this experiment suggest that the thiosulfate also increased As phytoextraction. The increased phytoavailability in soil is probably due to competition between sulfate and arsenate ions for sorption sites on the same surface of oxides [88]. Thus, thiosulfate application in soils co-contaminated with Hg and As can prove an effective solution for simultaneous removal of both elements during assisted phytoextraction remediation, reducing both the time and cost of the phytoextraction process.

After only one harvest, bioavailable fractions of Hg and As still remained in soil. Further growing cycles decrease the bioavailable metal pools. Repeated additions of thiosulfate and phosphate further increased the metal's availability in soil, promoting plant uptake and increasing the metal's removal from contaminated soil.

Using assisted phytoextraction, mobilizing agents that have a short life-span in the soil should be used. The induced mobilization of contaminants must be effective only during the time needed to increase the uptake of the plants and then must disappear quickly enough to prevent leaching. This condition is generally not feasible and the residue contaminants remain in soluble form [89]. Thus, it is likely that contaminants may be leached along the soil profile and this risk must be accurately checked at field scale.

The promising laboratory results of this study need to be confirmed with further studies at field scale, where several agronomic strategies can be adopted to reduce the phytotoxic effects of metals and increase the vegetal biomass, enhancing the feasibility of phytoextraction technology. To obtain greater efficiency in the field, the best combination of numbers of repeated harvests and treatments with mobilizing agents should be evaluated according to the specific characteristics of soil and environmental factors that could influence plant growth at the contaminated site.

Acknowledgment

This study was supported by Eni S.p.A, Research & Technological Innovation Department, San Donato Milanese (Italy) and fully funded by Syndial S.p.A.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Wang D, Shi X, Wei S (2003) Accumulation and transformation of atmospheric mercury in soil. *Sci Total Environ* 304: 209-214.
2. Xu J, Bravo AG, Lagerkvist A, et al. (2015) Sources and remediation techniques for mercury contaminated soil. *Environ Int* 74: 42-53.
3. Pedron F, Petruzzelli G, Barbafieri M, et al. (2013) Remediation of a mercury-contaminated industrial soil using bioavailable contaminant stripping. *Pedosphere* 23: 104-110.

4. Su Y, Han FX, Chen J, et al. (2008) Phytoextraction and accumulation of mercury in three plant species: Indian mustard (*Brassica juncea*), beard grass (*Polypogon monospeliensis*), and Chinese brake fern (*Pteris vittata*). *Int J Phytoremediat* 10: 547-560.
5. Moreno FN, Anderson CW, Stewart RB, et al. (2005) Induced plant uptake and transport of mercury in the presence of sulphur-containing ligands and humic acid. *New Phytol* 166: 445-454.
6. Moreno FN, Anderson CW, Stewart RB, et al. (2004) Phytoremediation of mercury-contaminated mine tailings by induced plant-mercury accumulation. *Environ Pract* 6: 165-175.
7. Petruzzelli G, Pedron F, Gorini F, et al., Enhanced Bioavailable Contaminant Stripping (EBCS): metal bioavailability for evaluation of phytoextraction success; 2013; Roma. EDP Sciences.
8. Tassi E, Pedron F, Barbafieri M, et al. (2004) Phosphate-assisted phytoextraction in As-contaminated soil. *Eng Life Sci* 4: 341-346.
9. Rungwa S, Arpa G, Sakulas H, et al. (2013) Phytoremediation—an eco-friendly and sustainable method of heavy metal removal from closed mine environments in Papua New Guinea. *Procedia Earth Planet Sci* 6: 269-277.
10. Sas-Nowosielska A, Kucharski R, Pogrzeba M, et al. (2008) Phytoremediation technologies used to reduce environmental threat posed by metal-contaminated soils: Theory and reality. In: Barnes I, Kharytonov MM, editors. *Simulation and assessment of chemical processes in a multiphase environment*: Springer Netherlands. pp. 285-297.
11. McGrath SP, Zhao J, Lombi E (2002) Phytoremediation of metals, metalloids, and radionuclides. *Adv Agron* 75: 1-56.
12. Lasat MM (2000) Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *J Hazard Subst Res* 2: 1-25.
13. Parisien MA, Rutter A, Smith BM, et al. (2016) Ecological risk associated with phytoextraction of soil contaminants. *J Environ Chem Eng* 4: 651-656.
14. Bolan N, Kunhikrishnan A, Thangarajan R, et al. (2014) Remediation of heavy metal (loid) s contaminated soils–to mobilize or to immobilize? *J Hazard Mater* 266: 141-166.
15. Van Gestel CA (2008) Physico-chemical and biological parameters determine metal bioavailability in soils. *Sci Total Environ* 406: 385-395.
16. Petruzzelli G, Pedron F, Rosellini I, et al. (2015) The bioavailability processes as a key to evaluate phytoremediation efficiency. In: Ansari AA, Gill SS, Gill R, et al., editors. Phytoremediation: Springer International Publishing. pp. 31-43.
17. Wu G, Kang H, Zhang X, et al. (2010) A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. *J Hazard Mater* 174: 1-8.
18. Mahar A, Wang P, Ali A, et al. (2016) Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. *Ecotoxicol Environ Saf* 126: 111-121.
19. Ahmadpour P, Ahmadpour F, Mahmud TMM, et al. (2012) Phytoremediation of heavy metals: A green technology. *Afr J Biotechnol* 11: 14036-14043.
20. Franchi E, Rolli E, Marasco R, et al. (2016) Phytoremediation of a multi contaminated soil: mercury and arsenic phytoextraction assisted by mobilizing agent and plant growth promoting bacteria. *J Soils Sediments:* 1-13.

21. Eapen S, D'souza SF (2005) Prospects of genetic engineering of plants for phytoremediation of toxic metals. *Biotechnol Adv* 23: 97-114.
22. Evangelou MW, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere* 68: 989-1003.
23. Bhargava A, Carmona FF, Bhargava M, et al. (2012) Approaches for enhanced phytoextraction of heavy metals. *J Environ Manag* 105: 103-120.
24. Seth CS, Misra V, Singh RR, et al. (2011) EDTA-enhanced lead phytoremediation in sunflower (*Helianthus annuus* L.) hydroponic culture. *Plant Soil* 347: 231-242.
25. Wu LH, Luo YM, Xing XR, et al. (2004) EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. *Agric Ecosyst Environ* 102: 307-318.
26. Cao A, Carucci A, Lai T, et al. (2007) Effect of biodegradable chelating agents on heavy metals phytoextraction with *Mirabilis jalapa* and on its associated bacteria. *Eur J Soil Biol* 43: 200-206.
27. Santos FS, Hernández-Allica J, Becerril JM, et al. (2006) Chelate-induced phytoextraction of metal polluted soils with *Brachiaria decumbens*. *Chemosphere* 65: 43-50.
28. Luo C, Shen Z, Li X (2005) Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere* 59: 1-11.
29. Wang J, Xia J, Feng X (2016) Screening of chelating ligands to enhance mercury accumulation from historically mercury-contaminated soils for phytoextraction. *J Environ Manag*: In press.
30. Meers E, Tack FMG, Van Slycken S, et al. (2008) Chemically assisted phytoextraction: a review of potential soil amendments for increasing plant uptake of heavy metals. *Int J Phytoremediation* 10: 390-414.
31. Cooper EM, Sims JT, Cunningham SD, et al. (1999). Chelate-assisted phytoextraction of lead from contaminated soils. *J Environ Qual* 28: 1709-1719.
32. Doumett S, Fibbi D, Azzarello E, et al. (2010) Influence of the application renewal of glutamate and tartrate on Cd, Cu, Pb and Zn distribution between contaminated soil and *Paulownia tomentosa* in a pilot-scale assisted phytoremediation study. *Int J Phytoremediation* 13: 1-17.
33. Leštan D, Luo CL, Li XD (2008) The use of chelating agents in the remediation of metal-contaminated soils: a review. *Environ Pollut* 153: 3-13.
34. Karczewska A, Orlow K, Kabala C, et al. (2011) Effects of chelating compounds on mobilization and phytoextraction of copper and lead in contaminated soils. *Commun Soil Sci Plant Anal* 42: 1379-1389.
35. Epelde L, Becerril JM, Hernández-Allica J, et al. (2008) Functional diversity as indicator of the recovery of soil health derived from *Thlaspi caerulescens* growth and metal phytoextraction. *Appl Soil Ecol* 39: 299-310.
36. Raskin I, Kumar PN, Dushenkov S, et al. (1994) Bioconcentration of heavy metals by plants. *Curr Opin Biotechnol* 5: 285-290.
37. Shelmerdine PA, Black CR, McGrath SP, et al. (2009) Modelling phytoremediation by the hyperaccumulating fern, *Pteris vittata*, of soils historically contaminated with arsenic. *Environ Pollut* 157: 1589-1596.
38. Sparks DL (1998) Methods of soil analysis. Part 3. Chemical methods. Madison, USA: Soil Science Society of America.

39. EPA - U.S. Environmental Protection Agency (1995) Method 3051A, Microwave assisted acid digestion of sediments, sludges, soils and oils. In: Test Methods for Evaluating Solid Waste, 3rd Edition, 3rd Update, U.S. EPA, Washington D.C.
40. Millán R, Gamarra R, Schmid T, et al. (2006) Mercury content in vegetation and soils of the Almadén mining area (Spain). *Sci Total Environ* 368: 79-87.
41. Wenzel WW, Kirchbaumer N, Prohaska T, et al. (2001) Arsenic fractionation in soils using an improved sequential extraction procedure. *Anal Chim Acta* 436: 309-323.
42. Petruzzelli G, Pedron F, Tassi E, et al. (2014) The effect of thiosulphate on arsenic bioavailability in a multi contaminated soil. A novel contribution to phytoextraction. *Res J Environ Earth Sci* 6: 38-43.
43. Pedron F, Petruzzelli G, Barbafieri M, et al. (2011) Mercury mobilization in a contaminated industrial soil for phytoremediation. *Commun Soil Sci Plant Anal* 42: 2767-2777.
44. Pedron F, Petruzzelli G, Barbafieri M, et al. (2009) Strategies to use phytoextraction in very acidic soil contaminated by heavy metals. *Chemosphere* 75: 808-814.
45. Grifoni M, Schiavon M, Pezzarossa B, et al. (2015) Effects of phosphate and thiosulphate on arsenic accumulation in the species *Brassica juncea*. *Environ Sci Pollut Res Int* 22: 2423-2433.
46. Pedron F, Petruzzelli G, Rosellini I, et al. (2015) Ammonium thiosulphate assisted phytoextraction of mercury and arsenic in multi-polluted industrial soil. *Resour Environ* 5: 173-181.
47. EPA - U.S. Environmental Protection Agency (1995) Method 3052, microwave assisted acid digestion of siliceous and organically based matrices. In: Test Methods for Evaluating Solid Waste, 3rd Edition, 3rd Update, U.S. EPA, Washington D.C.
48. Peijnenburg WJGM, Jager T (2003) Monitoring approaches to assess bioaccessibility and bioavailability of metals: matrix issues. *Ecotoxicol Environ Saf* 56: 63-77.
49. Kamnev AA, Van Der Lelie D (2000) Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Biosci Rep* 20: 239-258.
50. Wallschläger D, Desai MV, Spengler M, et al. (1998) Mercury speciation in floodplain soils and sediments along a contaminated river transect. *J Environ Qual* 27: 1034-1044.
51. Smolinska B, Rowe S (2015) The potential of *Lepidium sativum* L. for phytoextraction of Hg-contaminated soil assisted by thiosulphate. *J Soils Sediments* 15: 393-400.
52. Muddarisna N, Krisnayanti BD, Utami SR, et al. (2013) Phytoremediation of mercury-contaminated soil using three wild plant species and its effect on maize growth. *Appl Ecol Environ Sci* 1: 27-32.
53. Wang J, Feng X, Anderson CW, et al. (2011) Ammonium thiosulphate enhanced phytoextraction from mercury contaminated soil—Results from a greenhouse study. *J Hazard Mater* 186: 119-127.
54. Moreno FN, Anderson CW, Stewart RB, et al. (2005). Effect of thioligands on plant-Hg accumulation and volatilisation from mercury-contaminated mine tailings. *Plant Soil* 275: 233-246.
55. Gao Y, Mucci A (2001) Acid base reactions, phosphate and arsenate complexation, and their competitive adsorption at the surface of goethite in 0.7 M NaCl solution. *Geochim Cosmochim Acta* 65: 2361-2378.
56. Liu F, De Cristofaro A, Violante A (2001) Effect of pH, phosphate and oxalate on the adsorption/desorption of arsenate on/from goethite. *Soil Sci* 166: 197-208.

57. Meharg AA, Macnair MR (1992) Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus lanatus* L. *J Exp Bot* 43: 519-524.
58. Smith E, Naidu R, Alston AM (2002) Chemistry of inorganic arsenic in soils. *J Environ Qual* 31: 557-563.
59. Raj A, Singh N (2015) Phytoremediation of arsenic contaminated soil by arsenic accumulators: a three year study. *Bull Environ Contam Toxicol* 94: 308-313.
60. Fayiga AO, Ma LQ (2006) Using phosphate rock to immobilize metals in soil and increase arsenic uptake by hyperaccumulator *Pteris vittata*. *Sci Total Environ* 359: 17-25.
61. Glick BR, Todorovic B, Czarny J, et al. (2007) Promotion of plant growth by bacterial ACC deaminase. *Crit Rev Plant Sci* 26: 227-242.
62. Shen ZG, Li XD, Wang CC, et al. (2002) Lead phytoextraction from contaminated soil with high-biomass plant species. *J Environ Qual* 31: 1893-1900.
63. Kayser A, Wenger K, Keller A, et al. (2000) Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. *Environ Sci Technol* 34: 1778-1783.
64. Duan G, Liu W, Chen X, et al. (2013) Association of arsenic with nutrient elements in rice plants. *Metallomics Integr Biomatel Sci* 5: 784-792.
65. Srivastava S, D'souza SF (2010) Effect of variable sulfur supply on arsenic tolerance and antioxidant responses in *Hydrilla verticillata* (Lf) Royle. *Ecotoxicol Environ Saf* 73: 1314-1322.
66. Mishra S, Srivastava S, Tripathi RD, et al. (2008) Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* L. *Aquat Toxicol* 86: 205-215.
67. Wu SC, Cheung KC, Luo YM, et al. (2006) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by *Brassica juncea*. *Environ Pollut* 140: 124-135.
68. Lou LQ, Ye ZH, Lin AJ, et al. (2010) Interaction of arsenic and phosphate on their uptake and accumulation in Chinese brake fern. *Int J Phytoremediation* 12: 487-502.
69. Pigna M, Cozzolino V, Violante A, et al. (2009) Influence of phosphate on the arsenic uptake by wheat (*Triticum durum* L.) irrigated with arsenic solutions at three different concentrations. *Water, Air, Soil Pollut* 197: 371-380.
70. Geng CN, Zhu YG, Hu Y, et al. (2006) Arsenate causes differential acute toxicity to two P-deprived genotypes of rice seedlings (*Oryza sativa* L.). *Plant Soil* 279: 297-306.
71. Vázquez Reina S, Esteban E, Goldsbrough P (2005) Arsenate-induced phytochelatins in white lupin: influence of phosphate status. *Physiol Plant* 124: 41-49.
72. Meharg AA (2005) Mechanisms of plant resistance to metal and metalloid ions and potential biotechnological applications. *Plant Soil* 274: 163-174.
73. Huang ZC, An ZZ, Chen TB, et al. (2007) Arsenic uptake and transport of *Pteris vittata* L. as influenced by phosphate and inorganic arsenic species under sand culture. *J Environ Sci* 19: 714-718.
74. Tu S, Ma LQ (2003) Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions. *Environ Exp Bot* 50: 243-251.
75. Zhong L, Hu C, Tan Q, et al. (2011) Effects of sulfur application on sulfur and arsenic absorption by rapeseed in arsenic-contaminated soil. *Plant Soil Environ* 57: 429-434.

76. Moreno FN, Anderson CW, Stewart RB, et al. (2005) Mercury volatilisation and phytoextraction from base-metal mine tailings. *Environ Pollut* 136: 341-352.
77. Lorestani B, Cheraghi M, Yousefi N (2011) Phytoremediation potential of native plants growing on a heavy metals contaminated soil of copper mine in Iran. *Proc World Acad Sci Eng Technol* 53: 377-382.
78. Yoon J, Cao X, Zhou Q, et al. (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci Total Environ* 368: 456-464.
79. Tu C, Ma LQ, Bondada B (2002) Arsenic accumulation in the hyperaccumulator Chinese brake and its utilization potential for phytoremediation. *J Environ Qual* 31: 1671-1675.
80. Cassina L, Tassi E, Pedron F, et al. (2012) Using a plant hormone and a thioligand to improve phytoremediation of Hg-contaminated soil from a petrochemical plant. *J Hazard Mater* 231: 36-42.
81. Rodriguez L, Rincón J, Asencio I, et al. (2007) Capability of selected crop plants for shoot mercury accumulation from polluted soils: phytoremediation perspectives. *Int J Phytoremediation* 9: 1-13.
82. Souza LA, Piotto FA, Nogueirol RC, et al. (2013) Use of non-hyperaccumulator plant species for the phytoextraction of heavy metals using chelating agents. *Sci Agricola* 70: 290-295.
83. Jankong P, Visoottiviseth P, Khokiattiwong S (2007) Enhanced phytoremediation of arsenic contaminated land. *Chemosphere* 68: 1906-1912.
84. Chaturvedi I (2006) Effects of arsenic concentrations and forms on growth and arsenic uptake and accumulation by Indian mustard (*Brassica juncea* L.) genotypes. *J Cent Eur Agric* 7: 31-40.
85. Matschullat J (2000) Arsenic in the geosphere-a review. *Sci Total Environ* 249: 297-312.
86. Jarrell WM, Beverly RB (1981) The dilution effect in plant nutrition studies. *Adv Agron* 34: 197-224.
87. Wang J, Feng X, Anderson CW, et al. (2012) Implications of mercury speciation in thiosulfate treated plants. *Environ Sci Technol* 46: 5361-5368.
88. Petruzzelli G, Pedron F, Rosellini I (2014) Effects of thiosulfate on the adsorption of arsenate on hematite with a view to phytoextraction. *Res J Environ Earth Sci* 6: 326-332.
89. Pedron F, Rosellini I, Petruzzelli G, et al. (2014) Chelant comparison for assisted phytoextraction of lead in two contaminated soils. *Resour Environ* 4: 209-214.

AIMS Press