

The Effect of Thiosulphate on Arsenic Bioavailability in a Multi Contaminated Soil. A Novel Contribution to Phytoextraction

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Abstract: In this study it was evaluated on a laboratory scale (microcosm), the possibility of using ammonium thiosulphate in assisted phytoextraction for the simultaneous removal of mercury and arsenic from multi-contaminated industrial soil. The species selected were *Brassica juncea* and *Lupinus albus* the addition of thiosulfate to the soil greatly promoted the uptake and translocation of both contaminants in the aerial parts of the plants. Mercury concentration in the aerial parts reached in *B. juncea* 120 mg/kg approximately 40 times the value of the control). The concentration of arsenic also increased significantly in the shoots of *B. juncea* (14.3 mg/kg), where the value in the control was negligible. Similar trends were obtained for *L. albus* The results confirmed the known positive effects of thiosulphate in increasing mercury bioavailability for plants, moreover they showed the ability of thiosulphate to mobilize arsenic and significantly to promote its uptake by plants. The increase of arsenic bioavailability, promoted by thiosulfate addition, could be attributed to the competition between arsenate and sulphate ions for the same active sites in the soil surfaces, with the release of arsenic in the soil solution. The use of thiosulfate appears to have great potential since it is a common fertilizer used to promote plant growth and is able to increase the uptake by plants of mercury and arsenic. The simultaneous increase of both contaminants uptake by plants, using a single additive, will provide new insights into the phytoextraction technology in terms of cost and time reduction.

Keywords: Assisted phytoremediation, chemical additive, metal mobilization, phytoavailability, plant uptake

INTRODUCTION

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, together with an accurate selection of the plant species. Only by carefully characterizing the soil, is it possible to determine the main components responsible for the mobility and bioavailability of the metals and metalloids (Petruzzelli *et al.*, 2013, 2012). This knowledge is the key to select the best mobilizing agents to increase phytoextraction in those cases where contaminant bioavailability is low.

Arsenic and mercury are typically non essential elements for plants, with different chemical characteristics and a different behaviour in relation to soil properties, such as the pH and cation exchange

capacity. In many contaminated sites, both metals are present simultaneously and their removal often requires separate remediation strategies. For Hg assisted phytoextraction, a thiosulphate salt is usually used (Moreno *et al.*, 2004; Pedron *et al.*, 2013), whereas for As, a phosphate salt (Tassi *et al.*, 2004) is the most suitable additive. The use of the same mobilizing agent to increase the bioavailability and plant uptake of both contaminants could be of great interest in the phytoextraction process by greatly reducing both time and costs.

The aim of this study was to investigate, on a laboratory scale (microcosm growing tests), the possibility of using assisted phytoextraction for the simultaneous removal of Hg and As from multi-contaminated industrial soil by using only ammonium thiosulphate. We believe that the simultaneous increase in contaminant bioavailability by a single additive, will provide new insights into the phytoextraction technology.

MATERIALS AND METHODS

Soil: The soil was sampled in a former industrial site that had been subjected to various chemical activities. The contamination from As and Hg had mean values of 41.1 and 67.0 mg/kg, respectively. Soil samples were air dried and sieved with a 2 mm sieve for soil analysis. Analysis of pH, cation exchange capacity, organic matter content and texture were performed according to soil analysis methods (Sparks, 1998).

Mercury and arsenic speciation: Speciation of mercury and arsenic in soil was carried out according to Millan *et al.* (2006) and Wenzel *et al.* (2001), respectively.

To evaluate the maximum amount of Hg and As extractable in soil with the additives employed in the assisted phytoextraction of these elements, the following procedure was adopted (Moreno *et al.*, 2004; Pedron *et al.*, 2013; Tassi *et al.*, 2004): soil samples were shaken with 0.27 M $(\text{NH}_4)_2\text{S}_2\text{O}_3$ and with 0.05 M KH_2PO_4 (soil:extractant 1:25 for 2 h) and the extracts analyzed after centrifugation and filtration. The As extractability in the soil was also evaluated using ammonium thiosulphate and ammonium sulphate in two different concentrations (0.14 M and 0.27 M). These extractions were performed by shaking the soil and extractant (ratio 1:25) for 2 h and As was analysed on the extracts after the centrifugation and filtration.

Microcosm experiments: The soil used in the microcosm experiments was prepared by eliminating the coarser materials, homogenized but without sieving to 2 mm, to obtain a more representative sample of the real field situation. Experiments were carried out in a growth chamber in controlled conditions: 14 h of light, with a temperature of 24°C and 10 h in the dark at 19°C. Relative humidity was maintained at 70%.

Microcosm experiments were carried out for 30 days. Two plant species were compared in terms of their growth in the studied soil and contaminant absorption and translocation to the aerial parts of the plants.

The plant species selected for the tests, *Brassica juncea* and *Lupinus albus*, were considered viable candidates for phytoremediation due to their ability to grow in a Mediterranean climate and their relative tolerance to heavy metal soil contamination.

Microcosm pots, filled with 400 g of contaminated soil were sown with 0.5 g per pot of *B. juncea* seeds or three seeds per pot of *L. albus* in five replicates for each species. After two weeks, the soil treatment started according to an experimental design already used in Pedron *et al.* (2013). A solution of ammonium

thiosulfate [0.27 M $(\text{NH}_4)_2\text{S}_2\text{O}_3$] was used as a single mobilizing agent for both contaminants and was added to the soil by splitting the total dose of 10 mL of solution in five day applications. The total dose was split in order to minimize the possible toxic effects on the plant species. Control microcosms consisted of replicates for each species grown in untreated soil in the same conditions and were run simultaneously. After harvesting, aerial parts and roots were separated and washed with deionized water. The roots were further washed in an ultrasound bath (Branson Sonifier 250 ultrasonic processor; Branson, Danbury, Conn.) for 10 min to eliminate any soil particles that might have remained on the root surfaces. Vegetal samples were dried in a ventilated oven at 40°C until a constant weight was achieved. The dry biomass of shoots and roots was gravimetrically determined.

Mercury and arsenic analysis: Mercury concentration in soil, plant samples and soil extracts was determined by atomic absorption spectroscopy with an Automatic Mercury Analyzer (AMA 254, FKV, Bergamo, Italy), according to the SW-846 method 7473 (USEPA, 1998). The arsenic concentration in the same samples was determined using ICP-OES (Varian AX Liberty) with a method for the generation of hydrides (Sparks, 1998).

Quality assurance and quality control: Quality assurance and quality control were performed by testing standard solution every 10 samples. Certified reference material (BCR n°141) was used to control the quality of analytical system. The detection limits were 2 µg/L for Hg and 0.05 mg/L for As, respectively.

The recovery of spiked samples ranged from 92 to 101% with a RSD of 1.90 of the mean.

Statistical analysis: All statistical analysis was performed using Statistica version 6.0 (Statsoft, Inc.). Effects of mobilizing agents were analyzed using one-way analysis of variance. Differences among means were compared and a post-hoc analysis of variance was performed using the Tukey Honestly Significant Differences test ($p < 0.05$).

RESULTS AND DISCUSSION

The main characteristics of the soil are reported in Table 1.

Following the extraction procedures for Hg (Millan *et al.*, 2006) and As (Wenzel *et al.*, 2001), the two contaminants in the soil solution were found to be negligible (for sake of simplicity data are not reported). The extractability of Hg and As using a thiosulphate solution is reported in Table 2.

Table 1: Main characteristics of the contaminated soil

pH	OM (%)	CEC (cmol/kg)	Sand (%)	Silt (%)	Clay (%)
8.06	1.5	15.6	78.9	13.1	8.0

Table 2: Extractability of As and Hg. Data are the means of three replicates and are expressed as mg kg⁻¹ dry soil

Extractant	As (mg/kg)	Hg (mg/kg)
0.05 M KH ₂ PO ₄	7.6 (±0.86)	nd
0.27 M (NH ₄) ₂ S ₂ O ₃	7.4 (±0.65)	12.9 (±0.88)

nd: below the detection limit. In brackets standard deviation

Table 3: Mean values and standard deviations (n = 5 replicates) of aerial part dry biomass of *B. juncea* and *L. albus* grown in control (CT) and thiosulphate treated soil (TS). Data are expressed as mg dry weight

Plants	CT	TS
<i>B. juncea</i>	430±15	302±22
<i>L. albus</i>	620±31	590±28

Table 4: As and Hg concentration in shoots and roots of the selected plants. Data are expressed as mean values mg kg⁻¹ dry weight and standard deviation of n = 5 replicates

	Shoots		Roots	
	As	Hg	As	Hg
CT B	nd	3.3±0.74	nd	16.9±4.9
TS B	14.3±4.4	120±7.5	54.3±4.4	1451±319
CT L	0.82±0.09	1.1±0.35	1.6±0.60	4.5±0.46
TS L	4.3±1.4	24.1±2.1	14.2±3.2	744±34.6

CT: Control; TS: Thiosulphate treatment; B: *B. juncea*; L: *L. albus*; nd: below the detection limit

The As extractability with the thiosulphate solution was compared with the phosphate extraction, which is the specific mobilizing agent for As in assisted phytoextraction. It is worth of note that the effectiveness of the thiosulphate in releasing As from soil surfaces was comparable to that of phosphate, which is the most important competing anion for arsenate frequently used in the assisted phytoextraction of this metalloid. These results strongly suggest that in multi element contaminated soil, assisted phytoextraction by adding a single mobilizing agent (thiosulphate) for both contaminants is highly effective, with significant savings in both time and costs.

Microcosm experiments were thus carried out to evaluate the effect of this additive on the bioavailability of As and Hg and their uptake by plants. It was observed that seed germination of both *B. juncea* and *L. albus* was not influenced by the presence of high concentrations of As and Hg. In addition, the application of thiosulphate only reduced the biomass production of the Brassica plants (Table 3), probably due to an increase in the concentrations of the two contaminants, in particular Hg, which significantly stressed the metabolism of the plant (Pedron *et al.*, 2013).

The Hg and As concentrations in the tissues of *B. juncea* and *L. albus* after 30 days of growing are reported in Table 4.

In the control microcosms (CT) the concentration of As was below the detection limit in both the shoots and roots of *B. juncea*, whereas *L. albus* plants showed

an average concentration of 0.82 and 1.6 mg/kg in shoots and roots, respectively. A low concentration was also found for Hg in CT plants with higher mean values of 3.3 mg/kg and 16.9 mg/kg in shoots and roots of *B. juncea*, respectively. The addition of the mobilizing agent (TS) led to a significant increase in Hg and As uptake by the plants with respect to the control microcosms.

In both CT and TS plants, the amount of As and Hg was higher in the roots than in the shoots. This indicates that the plants are able to uptake the metals and only partially translocate them to the aerial parts; in fact, as well known, roots act with defense mechanism against toxic elements.

The addition of thiosulfate to the soil greatly promoted the uptake and translocation of both Hg and As in the aerial parts of the plants. Mercury concentration in the aerial parts reached 120 mg/kg for *B. juncea* (approximately 40 times the value of the controls) and 24.0 mg/kg for *L. albus* (approximately 24 times the value of the controls). The concentration of arsenic also increased significantly in the shoots of *B. juncea* (14.3 mg/kg), where the value in the control was negligible. In *L. albus* shoots, after treatment, the concentration of As (4.3 mg/kg) increased by about five times compared to the control value.

Mercury is absorbed in greater quantities than As, particularly in the roots. This is not surprising since the additive used is specific for Hg however, the effect of thiosulfate on the bioavailability of As is of great interest.

The amount of contaminants extracted by the plants is a result of two dynamic processes, metal uptake and biomass production and can be expressed as "total accumulation" (Jarrell and Beverly, 1981). This is calculated as the product of the concentration of the metal in plant tissues and the respective dry biomass. The data are reported in Fig. 1.

Total accumulation provides an estimation of the amount of contaminants removed from the polluted soil and thus the phytoextraction efficiency. Results showed that the increase in metal bioavailability using (NH₄)₂S₂O₃ promoted higher Hg and As accumulation in plants and significantly increased the amount of metals extracted from the soil. In this experiment *B. juncea* showed a greater efficiency than *L. albus* and should be considered as the best candidate for further phytoextraction tests at the field scale.

The results confirmed the positive effects of thiosulphate in increasing Hg solubility and the potential bioavailability for plants and are in agreement with previous findings on the effects of thioligands on mercury bioavailability (Moreno *et al.*, 2004, 2005; Pedron *et al.*, 2011, 2013; Cassina *et al.*, 2012). Data on plant uptake confirm the results of the extractability tests, i.e., thiosulphate is also able to mobilize As in quantities that significantly promote uptake by both plant species.

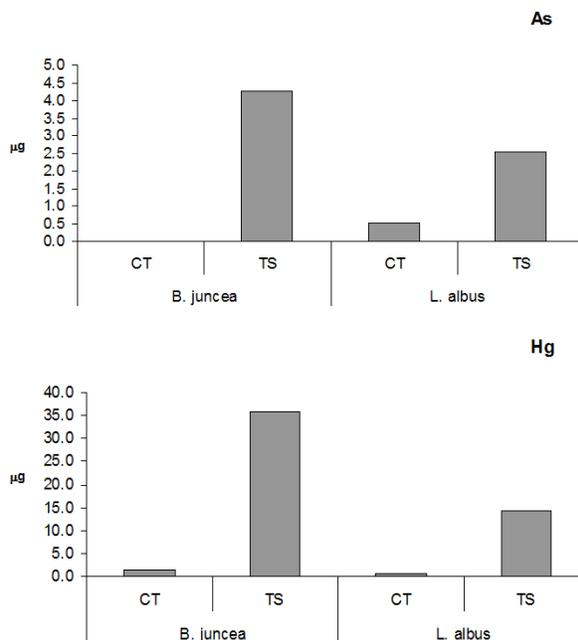


Fig. 1: Total accumulation of As and Hg in the selected plants. Data are expressed in µg

Table 5: Extractability of As by thiosulphate and sulphate. Data are the mean of three replicates, expressed in mg kg⁻¹ dry soil

Extractant concentration	(NH ₄) ₂ S ₂ O ₃	(NH ₄) ₂ SO ₄
0.14 M	3.83±0.05	3.87±0.08
0.27 M	6.98±0.08	6.06±0.03

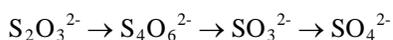
While the effect of thiosulphate on mercury bioavailability can be ascribed to the formation of the soluble complex mercury-thiosulphate Hg (S₂O₃)²⁻, which can be absorbed by plants (Moreno *et al.*, 2005), the thiosulfate-arsenic interaction in the soil-plant system has not yet been studied in terms of phytoextraction.

The increase in arsenic bioavailability promoted by thiosulfate addition could be attributed to the competition between arsenate and sulphate ions for the same active sites in the soil surfaces, with the release of arsenic in the liquid phase in potentially bioavailable forms.

In the soil, thiosulfate decomposes into sulphur and sulphate, where sulphur may give rise to precipitates while sulphate remains in solution. This explains the wide use of thiosulfate as a fertilizer; in fact sulphate has an immediate action on the crops, while the sulphur can be released over time.

Barbosa-Jefferson *et al.* (1998) reported data from thiosulfate incubation in different soils which revealed that the final concentration of sulphate deriving from thiosulfate transformation, is basically the same of original concentration of added thiosulfate.

In the soil, thiosulfate can be transformed into tetrathionate and subsequently to sulphate:



The reaction is either abiotic or biotic depending on the microbial community present. The kinetics of the oxidation depend on the characteristics of the soil. The first step is generally very rapid and only requires a few hours, after which the reaction is completed in 3-6 days (Barbosa-Jefferson *et al.*, 1998). The authors have also suggested that the presence of S₄O₆²⁻ in soil has a toxic effect on the growth of plants.

However in the microcosms conditions the presence of plants, increases the microbiological activity and the transformation of thiosulphate is likely to follow a different pattern without the production of S₄O₆²⁻ as intermediate:



This was confirmed by the absence, in our experiments, of negative effects on the growth of plant seedlings in this contaminated soil.

Thus, to test the hypothesis that thiosulphate added to soil (and its transformation into sulphate) could affect also As bioavailability, soil extraction with a sulphate solution was performed and compared with thiosulfate extraction using two different concentrations (Table 5).

Results showed a clear correlation between the amount of As extracted by sulphate and the amount extracted by thiosulfate. This confirms that sulphate-arsenate competition for the same soil absorption sites could be the basis for the release of arsenic in the soil solution in bioavailable forms to plants.

Trace elements which are present in soil in an anionic form are retained by sorption reactions at the reactive sites of variable charge minerals. Arsenate and sulphate form different surface complexes with a high affinity for iron oxides and hydroxides. Following the surface complexation model (Sposito, 1984), the competition between sulphate and arsenate for the same adsorption sites should depend on the sulphate concentration. At low concentrations, only outer sphere complexes are formed on soil surfaces, but at increasing concentrations (>0.1M SO₄²⁻) AsO₄³⁻ can also be substituted in the inner sphere complexes (Frau *et al.*, 2010).

The results from the sulphate concentration used in our experiments (0.27 M), suggests the quite strong competitive effect of sulphate, which probably involves the inner sphere complexation. Figure 2 shows the inner sphere complexes that could be formed with both arsenate and sulphate with soil Fe oxides-hydroxide surfaces.

In these specific soil conditions, we can assume that the excess of sulphate ions, deriving from thiosulfate addition, released the arsenate ions in the liquid phase of the soil in a potentially bioavailable

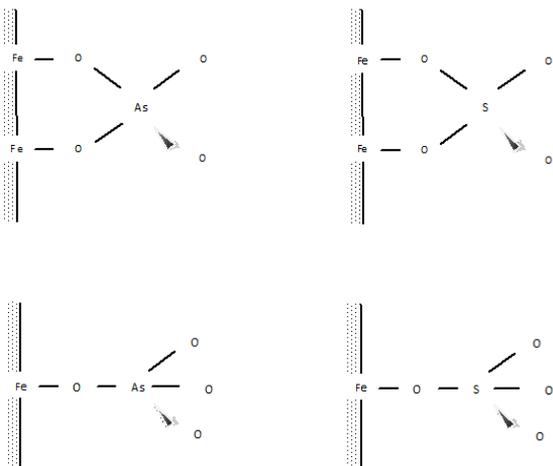


Fig. 2: Some examples of possible inner-sphere arsenate and sulphate complexes with soil surfaces

form for plant uptake. The competition between the two anions is higher in an alkaline environment (Myneni *et al.*, 1998), as was the case for our soil (pH = 8.06), thus promoting the substitution of arsenate by sulphate in inner sphere complexes.

However, further aspects need to be considered: the interactions between sulphur and As promote both the absorption of the contaminant and its transport to the aerial parts of the plants. The addition of S (as a thiosulphate fertilizer) to the soil promotes the efficiency of As phytoremediation as it plays an anti-stress role in reducing the toxicity of arsenic (Duan *et al.*, 2013). Thiosulphate can thus act both as a nutrient and detoxifying agent, due to the stimulation of the plant defense system influencing both the biomass production and As accumulation in plant tissues.

The increase in As accumulation both in the roots and shoots of the plants used in this research stimulates further investigations on the possibility to use thiosulfate as a viable alternative to promote arsenic phytoavailability. In particular in those cases of multiple contamination, when it is not possible to use phosphate as mobilizing agent, for example in the case of precipitation of insoluble phosphates.

Although we cannot draw any certain conclusions from laboratory data to predict performance in the field, the results highlight the potential of phytoextraction in reducing the concentration of contaminants to the target values. However, although phytoextraction is a non impacting and environmentally safe remediation procedure, it can take several years.

CONCLUSION

In conclusion, the presence of more than one metal or metalloid in soil is widespread in contaminated sites. Clean-up procedures often involve excavation and landfills, however there is an increasing demand for

alternative technologies based on sustainable concepts which balance financial feasibility with the conservation of natural resources and biodiversity (USEPA, 2008). In line with these new approaches, phytoremediation could be a viable alternative. Originally, phytoextraction of metals was based on the use of metal hyperaccumulator plants, which are able to take up huge amounts of heavy metals (or metalloids) in the aerial parts (Brooks, 1998). Hyperaccumulators however, are characterized by slow growth and a reduced production of biomass. In addition, they generally accumulate only one specific element, which makes them impractical in multi-contaminated soil. As an alternative to the use of hyperaccumulators, chemical additives can be used in combination with high biomass plants. Of the numerous additives reported in the literature for “assisted phytoextraction”, ethylenediaminetetraacetic acid (EDTA) is most commonly used, due to its high complexing capacity towards most metals, such as Pb, Cu, Cd and Zn, which generally leads to an increase in metal translocation from soil to plants (Luo *et al.*, 2005; Doumet *et al.*, 2008, 2011). However, given the persistence in the soil of chelating agents such as EDTA, there is a greater risk that mobilized metals will leach into the ground or surface water. In addition, for some contaminants, such as As and Hg, EDTA and similar complexing agents fail to exert an effective solubilizing action. Research is now aimed at new mobilizing agents that have no adverse effects on the environment while promoting bioavailability of contaminants. The use of thiosulfate thus appears to have great potential since it is a common fertilizer used to promote plant growth and is able to foster the uptake by plants of both Hg and As. Therefore applicability of phytoextraction technology in terms of cost and time can be greatly increased, in particular if the remedial targets will be based on bioavailable metal concentrations (Pedron *et al.*, 2013; Petruzzelli *et al.*, 2013).

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