

The potential of *Lepidium sativum* L. for phytoextraction of Hg-contaminated soil assisted by thiosulphate

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Abstract

Purpose The possibility of using higher plants to extract mercury from contaminated sites is dependent on both the concentration of Hg and its bioavailability. To increase the solubility of Hg in soil, some chemical compounds can be used. The aim of this study was to evaluate the effectiveness of Hg soil cleaning with the use of *Lepidium sativum* L. and sodium thiosulphate, as well as the leach ability of Hg from soil after phytoextraction.

Materials and methods The experiment was conducted on soil artificially polluted by Hg, wherein sodium thiosulphate was tested as a phytoextraction promoter. The *L. sativum* L. plants were used for phytoextraction. The leaching of Hg was assessed by determination of Hg concentration in water extracts. All determinations of Hg in soil, plant and water extracts were analysed by CV-AAS method after acid mineralization.

Results and discussion The result of the study showed that *L. sativum* L. accumulated Hg from contaminated soil mostly in belowground tissues. Even less than 8 % of Hg was translocated to the shoots of *L. sativum* L. Application of thiosulphate increased the total Hg accumulation over 238–272 %, depending on both the Hg and thiosulphate concentrations in soil. After thiosulphate treatment, translocation of Hg to shoots of *L. sativum* L. increased even 10 times relative to unassisted process. Thiosulphate did not negatively affect

plant biomass; however, the increased leaching of Hg after thiosulphate treatment was observed.

Conclusions *Lepidium sativum* L. showed the potential of a non-hyperaccumulating plant that can be used during phytoextraction of Hg-contaminated soils in controlled conditions. Thiosulphate promoted the phytoextraction process by increasing the total Hg accumulation by whole plant and translocation of Hg to shoots of *L. sativum* L. Thiosulphate-mobilized Hg in soil, which increased the Hg leaching. This constitutes the limitation of applying the technique in the field due to risk of Hg transferring to deeper layers of soil or water. Applying the technique in the field should be preceded by further investigations.

Keywords Leaching · *Lepidium sativum* L · Mercury · Phytoextraction · Thiosulphate

1 Introduction

Soil contamination by mercury has become a serious problem in the world. Due to mercury toxicity and its physical and chemical properties, it constitutes a threat to the health of humans and wildlife, even in places which are not obviously contaminated. The risk is determined by both the likelihood of exposure and its chemical form.

The increasing concentration of mercury in the environment is mostly ascribed to human activity, which includes a variety of industrial processes, for example, coal burning, disposal of Hg-containing products, mining, smelting and solid waste combustion.

Mercury is often deposited in soils, where concentrations of this element can be large. In soil, mercury can be absorbed onto the solid-phase of organic matter or minerals (Evans 1989). However, a substantial fraction of Hg undergoes several transformations, including leaching,

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volatilisation, methylation or biological reduction (Moreno et al. 2005a). These processes increase the Hg mobility thus causing the spreading of the pollution. Soils contaminated by mercury constitute a danger for all living organisms because of the possibility of mercury absorption by microbes and plants, whilst at the same time, transferring Hg to the food chain (Kabata-Pendias and Pendias 1999). Therefore, the techniques of soil reclamation are still being searched.

In methods of soil remediation adequate for Hg-polluted soils, physical and chemical treatments should be mentioned. Nevertheless, these techniques are relatively expensive and generate wastes that should be utilised.

An alternative method, which is viewed as environmentally friendly is phytoremediation. Phytoremediation refers to the use of higher plants to decrease the toxicity of pollutants. One phytoremediation category is phytoextraction. This consists of the uptake of contaminants from soil or water by plant roots, their translocation and accumulation in plant shoots. The pollutants can then be removed by harvesting the above-ground tissues (Rafati et al. 2011; Ali et al. 2013; Wang et al. 2012).

The main problem that occurs during phytoextraction is a low solubility of mercury in soil solution, which results in decreasing bioavailability of the metal. This problem can be solved by addition of chemical compounds straight to the soil. These chemicals promote the solubility of metals by the formation of water-soluble complexes and as a result, increase the metal bioavailability (Wang et al. 2011).

The researchers have demonstrated that mercury can form water-soluble complexes with several compounds like potassium iodide KI (Wang and Greger 2006) and ammonium thiocyanate NH_4SCN (Moreno et al. 2005a). Moreover, investigations have also been conducted on the potential use of sodium thiosulphate $\text{Na}_2\text{S}_2\text{O}_3$ and ammonium thiosulphate $(\text{NH}_4)_2\text{S}_2\text{O}_3$ during induced phytoextraction of mercury-contaminated soils (Moreno et al. 2005a, b). The results of these studies showed that all of the aforementioned compounds increased the efficiency of phytoextraction compared to processes conducted without chemical enhancements. In cited studies, the potential of *Phaseolus vulgaris* L., *Brassica juncea* L., *Vicia villosa* L. and *Chenopodium gluacum* L. for Hg phytoextraction was evaluated. Nevertheless, there is still a lack of information about the leaching of mercury during the chemically induced phytoextraction.

This study presents (i) the possibility of using *Lepidium sativum* L. plants as Hg-extractors (ii) the influence of sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) on the mercury accumulation by *L. sativum* L. and (iii) leaching of Hg in neutral pH after phytoextraction assisted by sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$).

2 Materials and methods

2.1 Soil

The soil used in the experiment was collected from Lodz (Poland) at a depth of 0–30 cm. After being air-dried, the soil was passed through a 2-mm nylon mesh. Soil density was measured using a cylinder with a volume of 100 cm^3 . The soil moisture was determined after drying at $105\text{ }^\circ\text{C}$ for 48 h. Soil density was calculated as the ratio of soil dried weight to cylinder volume. The following soil properties were characterised as follows: pH was determined according to ISO 10 390:2005, organic matter according to ISO 14235:2003, total nitrogen according to ISO 11261:2002 and available phosphorous according to PN-P-04023:1996. Concentrations of chosen heavy metals in the soil (Pb, Cu, Zn) were determined by AAS graphite furnace after acid microwave digestion. Concentration of Hg in soil was determined by cold vapour atomic absorption spectrometry (CV-AAS) after acid microwave digestion. In all determinations, atomic absorption standards, J.T. Baker, were used.

2.2 Plant

Lepidium sativum L. plants were used as Hg accumulators. These plants are characterised by a low vegetation period and low nutrient requirements. The seeds used in the experiments came from Grono Company (Wroclaw, Poland). The optimal scale of sowing was $10\text{ g seeds kg}^{-1}$ of fresh soil. The cultivation was provided in day/night system at temperature $22/19\pm 1\text{ }^\circ\text{C}$, respectively, and a 14-h photoperiod. Cultivation pots were sprinkled with deionised water to keep the soil humidity at 35 %. The plant samples were collected 7 days after planting and were prepared for Hg determination. The samples were washed with deionised water to remove soil particles and weighed. Then, they were separated into roots and aboveground parts, dried at $35\text{ }^\circ\text{C}$ for 60 h and weighed once again. Plant samples were ground into powder and mineralized with 3 mL of 65 % HNO_3 (Suprapur, Merck) in a Teflon bomb at $160\text{ }^\circ\text{C}$ for 1 h (Cavallini et al. 1999). Concentration of mercury was determined by CV-AAS. Each of the cultures was cultivated in triplicate.

2.3 Phytoextraction

The phytoextraction process was conducted in soil artificially contaminated by mercury. The soil samples were put into plastic pots and supplemented by mercury (II) acetate $(\text{CH}_3\text{COO})_2\text{Hg}$ (Merck Company) in concentrations of 10 and 100 mg kg^{-1} soil dry weight. After 72 h of stabilization, sodium thiosulphate $\text{Na}_2\text{S}_2\text{O}_3$ (Merck Company) in concentrations of 100 or 500 mg kg^{-1} soil dry weight was added to pots supplemented earlier with mercury. The supplemented

soil samples were stabilized for another 72 h before the plant cultivation started. The controls were supplemented with mercury but did not contain sodium thiosulphate. The blank samples were cultivated in unpolluted soil, supplemented by thiosulphate to check its influence on plant biomass (Fig. 1). Each of the soil samples was homogenised. After the plant cultivation, Hg concentration in plant tissues was determined using the method described above (Section 2.2).

2.4 Leaching tests

The leaching tests were conducted after the plant cultivation at pots presented at Fig. 1 at pH=7 (distilled water). Fifteen grams of each soil sample was put in a round-bottomed flask and 150 mL of distilled water (pH=7) was added afterwards. The flasks were shaken for 1 h at 20 °C. After shaking, the soil samples were left for 24 h at room temperature and then filtered. The water extracts were acidified by concentrated nitric (V) acid (HNO₃) (Merck Company) and the concentration of Hg was determined after acid mineralization using CV-AAS method (Smolinska and Krol 2012). Each of the variants of analysis was carried out in triplicate, at regular time periods of 1, 5, 10, 15 and 20 days.

2.5 Calculation and statistical treatment

Accumulation factor (AF) was calculated according to Wilson and Pyatt (2007), bioconcentration factor (BCF) was calculated according to Zhuang et al. (2007) and translocation factor (TF) was calculated following Zacchini et al. (2009).

All analyses in this experiment were performed independently for each of the plant material and were repeated three times. The data obtained (in three replications) was statistically analysed using ANOVA test in Excel Data Analysis. Analysis of variance was performed to determine the significance of differences between the pairs of means. The differences were statistically significant when *p* value was less than 0.05. A multiple range test was performed to find out which means are significantly different from others. This test is based on Fisher's least significant difference (LSD) procedure.

3 Results

3.1 Physico-chemical properties of soil

The soil subjected to investigations was representative of an urban soil collected in the city of Lodz, Poland. The soil could be classified as a sandy loam with a soil density of $1.2 \pm 0.1 \text{ g cm}^{-3}$. pH value was 6.45 ± 0.01 . This result enabled to include the soil as slightly acidic according to the Soil Survey Division Staff (1993). The concentration of organic carbon, total nitrogen and available phosphorous (g kg^{-1} soil dry weight) was 5.47 ± 0.83 , 0.52 ± 0.10 and 0.38 ± 0.07 , respectively. Concentrations of these macronutrients were rather low, but sufficient for plant cultivation. The soil concentration of Pb, Cu and Zn (mg kg^{-1} soil dry weight) was as follows: 0.047 ± 0.005 ; 0.023 ± 0.003 and 0.039 ± 0.008 , respectively. Due to very low concentrations of Pb, Cu and Zn that made the natural soil background, the assumption was made that they have no influence on the experiment. The amount of the mercury in the soil was below the level of detection ($<0.005 \text{ } \mu\text{g kg}^{-1}$). With regards to the above, the soil was classified as uncontaminated.

3.2 Phytoextraction

Lepidium sativum L. plants were able to accumulate Hg. However, plants cultivated in polluted soil accumulated Hg mainly in their belowground parts (Fig. 2, controls). Less than 8 % of Hg was translocated to the shoots of *L. sativum* L. Thiosulphate treatment enhanced the total accumulation of Hg by *L. sativum* L. (Fig. 2). Hg concentration in thiosulphate-assisted process was increased by 238–272 % in whole plants of *L. sativum* L., in relation to controls. Concentrations of Hg in the roots of *L. sativum* L. maintained the same level regardless of both Hg and thiosulphate concentration in soil. It means that the accumulation of Hg increased mostly in aboveground parts of the plants. As can be seen in Fig. 2, Hg concentration in aerial parts of *L. sativum* L. increased after thiosulphate treatment. For 100 mg kg^{-1} of $\text{S}_2\text{O}_3^{2-}$ treatment,

Fig. 1 Experimental scheme

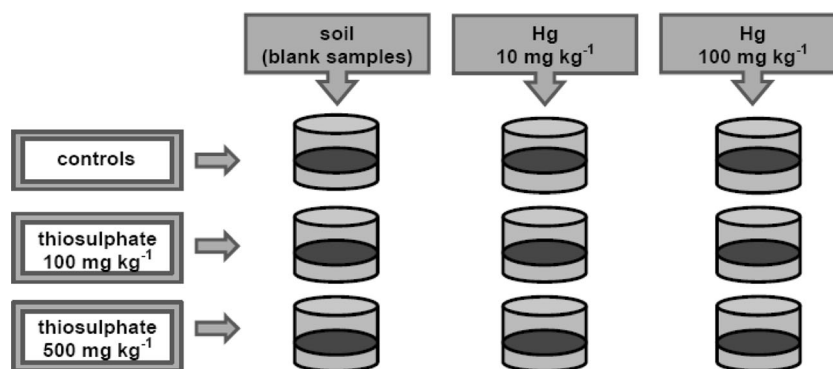
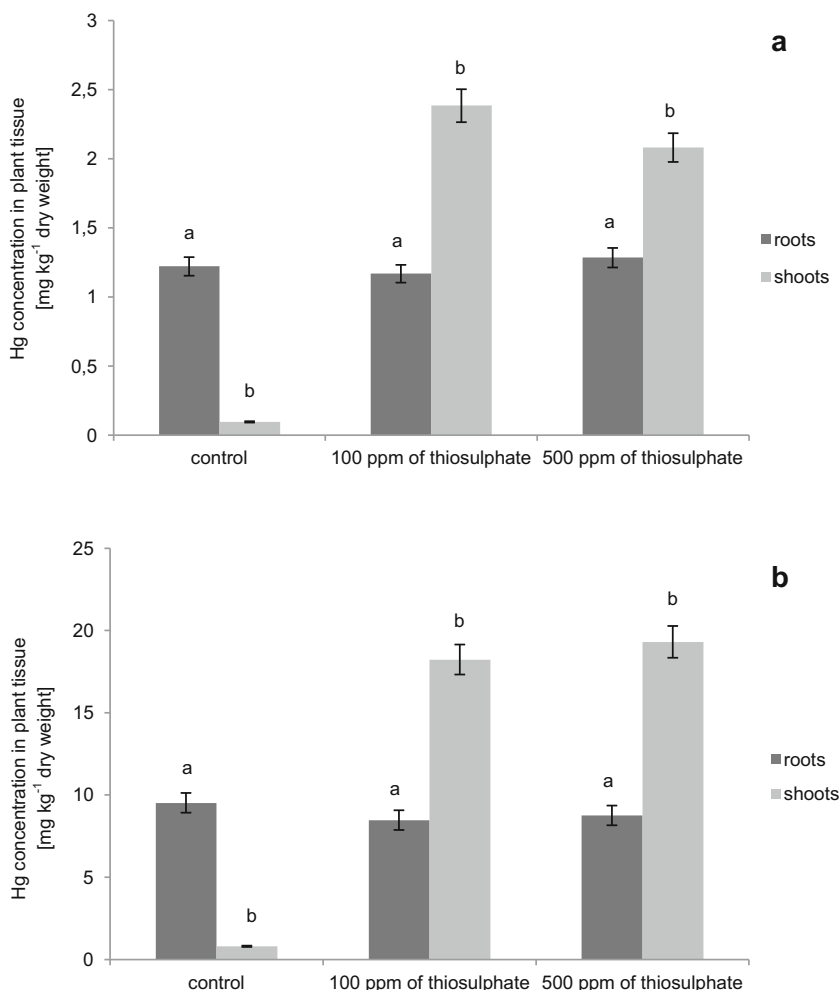


Fig. 2 Accumulation of Hg by *Lepidium sativum* L. for soil polluted by mercury: **a** in concentration 10 mg kg⁻¹ and **b** in concentration 100 mg kg⁻¹. Different letters above the bars indicate a significant difference at *p*<0.05



concentration of Hg in leaves and stems increased more than 2400 %.

One of the factors that determine the possibility of plant use in the field is its biomass. According to Table 1, biomass of *Lepidium sativum* L. was affected by Hg. The comparison analysis indicated that higher Hg concentration in soil

contributed to a decrease of plant biomass of about 35 % in relation to the control. No statistically different values were found in the values of roots biomass for both Hg concentrations in soil. However, the content of fresh mass in aerial parts decreased with increasing concentrations of Hg in soil. Thiosulphate treatment had no influence on plant biomass.

Table 1 Effect of treatments on plant of *Lepidium sativum* L. grown in different Hg concentrations

Hg concentration [mg kg ⁻¹ dry weight]	Concentration of Na ₂ S ₂ O ₃ [mg kg ⁻¹ dry weight]	Fresh weight [g]	
		Aerial parts	Roots
0	0	18.659±0.874a	11.400±0.474a
0	100	18.062±1.125a	11.703±0.745b
0	500	19.001±1.110b	10.210±0.496a
10	0	16.635±0.758a	6.508±0.699a
10	100	17.097±0.747b	7.028±0.459b
10	500	17.898±0.658b	6.895±0.688b
100	0	15.239±1.047a	4.046±0.469a
100	100	17.052±1.701b	5.127±0.698b
100	500	16.040±0.745b	4.936±0.558b

Significant differences among thiosulphate treatments are indicated by a and b letters (mean±SD, *n*=3; Fisher test *p*<0.05)

Plant growth was healthy and no visual differences appeared. After statistical analysis, the significant differences between biomass of plants cultivated on Hg-polluted soil and thiosulphate breeding were observed.

The values of bioconcentration factor (BCF), accumulation factor (AF) and translocation factor (TF) for phytoextraction promoted by thiosulphate are included in Table 2. Thiosulphate treatment significantly affects all of the described factors regardless of Hg concentration in soil. Accumulation factor increased over 2.5 times in all variants of investigations compared to controls. The results of the investigation showed that BCF for *L. sativum* L. was 0.182–0.238 for different treatments. Although the investigated plant cannot be classified as a hyperaccumulator, it has a potential for Hg accumulation due to translocation of Hg to the aerial parts. Calculation of TF gave very promising results. Thiosulphate treatment improved the translocation of Hg to aboveground parts of plants 10 times more compared to control.

3.3 Leaching of Hg

Determination of Hg leaching during thiosulphate treatment was provided by the evaluation of Hg extracted from soil after plant cultivation. The results are shown in Fig. 3. Leaching of Hg at neutral pH was dependant on both Hg concentration in soil and the time of analysis. For soil contaminated with 10 mg kg⁻¹ dry weight of Hg, the lowest amount of Hg in water leachates was observed for control samples. The Hg concentration ranged from 2.55 mg kg⁻¹ on the first day of investigation to 2.83 mg kg⁻¹ on the 20th day. Application of thiosulphate increased the Hg concentration in water extracts by 87–90 % for 100 mg kg⁻¹ of S₂O₃²⁻ to 109–115 % for thiosulphate treatment at a concentration of 500 mg kg⁻¹ compared to control. Moreover, the significant increase of Hg amount during the time of analysis was observed for the

100 mg kg⁻¹ dry weight of S₂O₃²⁻ treatment. For the higher thiosulphate concentration in soil, Hg concentration in water extracts stayed almost on the same level.

Slightly different results were observed for the process carried out in soil contaminated with 100 mg kg⁻¹ dry weight of Hg. In this part of the study, the increase of Hg concentration was noticed regardless of the thiosulphate concentration during the time of conducted experiment. For 500 mg kg⁻¹ of S₂O₃²⁻ treatment, Hg concentration in extracts increased by 29 % on the 1st day to 44 % on the 20th day, respectively, relative to control. After phytoextraction of Hg by *L. sativum* L. assisted by thiosulphate about 40–53 % of Hg stayed mobile in soils.

4 Discussion

Plant used in the investigations accumulated Hg mainly in roots. This result is a confirmation of investigations conducted by Perez-Sanz et al. (2012) for *Silene vulgaris* L. and Shiyab et al. (2009) for *Brassica juncea* L., who stated that increasing concentration of Hg in soil negatively affected plant accumulation. Slight transfer of Hg to aboveground parts of plants can be a result of plant response to stress conditions caused by mercury presence in soil. The typical answer is either formation of phytochelates in plant roots or binding the pollutants to the cell wall of root and as a consequence, deposition of the pollutant in that part of plant (Suszczynski and Shann 1995; Greger et al. 2005).

Application of thiosulphate promoted Hg phytoextraction. The results obtained for *L. sativum* L. are not as spectacular as those obtained in previous studies by Moreno et al. (2004) and Wang et al. (2011). These authors reported that the Hg concentration in the whole plants of *Brassica juncea* L. and *Chenopodium glaucum* L. exceeded 1800–4500 %, respectively, compared to controls. This phenomenon may be explained through the decreasing toxicity of Hg which was chelated with thiosulphate. Moreover, according to Wang et al. (2011) thiosulphate demonstrated properties of a good ligand for Hg in soil by forming water-soluble complexes, like Hg–S₂O₃. Formation of chelates with Hg increased its solubility thus increasing Hg uptake. Plant roots may be able to select Hg–S₂O₃ complex and transport it to shoots in preference to other mercury complexes in soil (Moreno et al. 2005b). The typical pathway of transporting metals uptaken by plants is the symplastic one. Nowack et al. (2006) reported that addition of chelates to the soil can change the primary route to apoplastic and in a consequence, increase the translocation of metals to aboveground parts of plants.

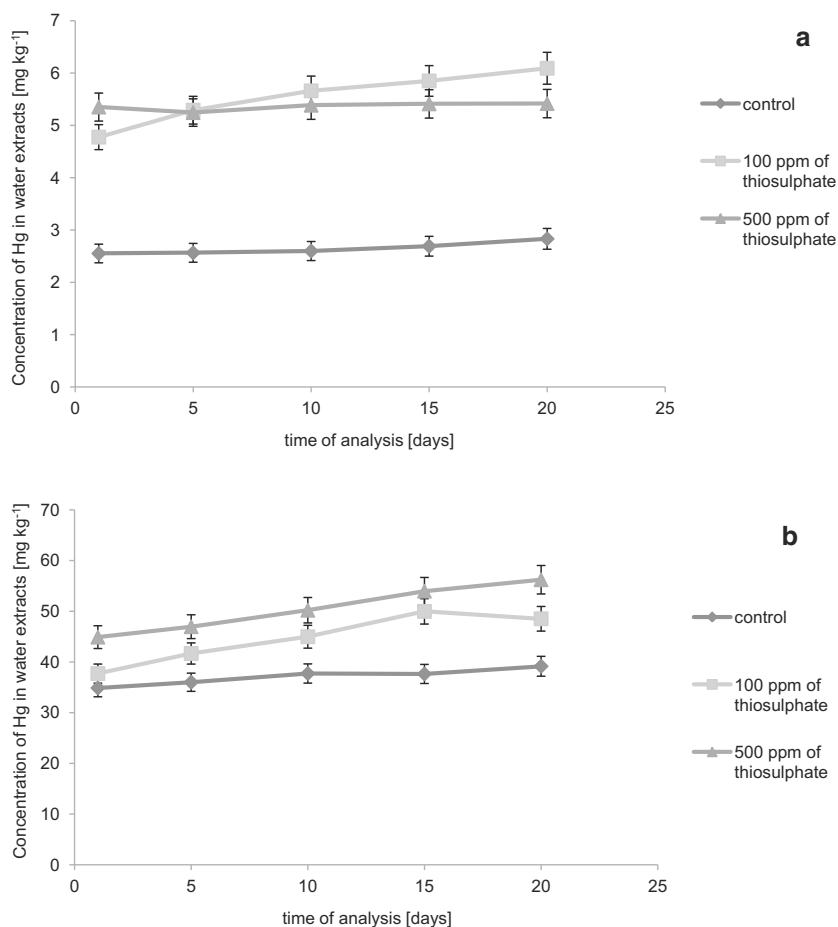
Hg is a highly toxic element that can negatively affect plant biomass. In previous studies, Patra and Sharma (2000) stated that dry matter production by higher plants such as *Brassica*

Table 2 Comparison of effective accumulation by whole plant (E_{WP}), shoots (E_S) and translocation (T) in different variants of the process

Factor	Hg concentration 10 [mg kg ⁻¹ soil dry weight]			Hg concentration 100 [mg kg ⁻¹ soil dry weight]		
	Concentration of Na ₂ S ₂ O ₃ [mg kg ⁻¹ soil dry weight]			Concentration of Na ₂ S ₂ O ₃ [mg kg ⁻¹ soil dry weight]		
	0	100	500	0	100	500
AF	0.141	0.355	0.337	0.103	0.267	0.281
BCF	0.010	0.238	0.208	0.008	0.182	0.193
TF	0.068	0.671	0.618	0.077	0.683	0.688

Accumulation factor (AF)=[Hg_{whole plant}/Hg_{soil}]; bioconcentration factor (BCF)=[Hg_{shoots}/Hg_{soil}]; translocation factor (TF)=[Hg_{shoots}/Hg_{roots}]

Fig. 3 Concentration of Hg extracted from soil after phytoextraction process for: **a** soil contamination by 10 mg kg^{-1} of Hg and **b** soil contamination by 100 mg kg^{-1} of Hg



oleracea L. var. *capitata*, Chinese cabbage, *Beta vulgaris* L. or *Pisum sativum* L. is inversely proportional to the concentration of Hg in soil. The similar tendency was observed for *L. sativum* L. The plant biomass production is one of the major key factors that determine the efficiency of phytoextraction. The other factors that describe the effectiveness of this process are accumulation, bioconcentration and translocation factors (McGrath and Zhao 2003). The accumulation factor (AF) identifies the ratio of metal accumulation by whole plant to its concentration in soil, whilst bioconcentration factor (BCF) is defined as the ratio of metal concentration in plant shoots to metal concentration in soil. The third component that can be useful during evaluation of phytoextraction efficiency is translocation factor (TF). The experiment showed that thiosulphate treatment increased all of the above-mentioned factors. According to Sakakibara et al. (2011), the bioconcentration factor is very important when considering the potential of a given plant species for phytoextraction. The ideal BCF of plants having the potential for phytoextraction is greater than 1 (for hyperaccumulator plants). However, the high concentrations of metals in soil can lead to decreasing BCF values (Ali et al. 2013; van der Ent et al. 2013).

Application of chelates to the soil during assisted phytoextraction can lead to increased solubility of metals thus

increasing their bioavailability. Nevertheless, the increasing solubility can result in transferring the pollutants through lower levels of pedosphere to groundwater, contributing to spreading the contamination. Therefore, the leaching tests under laboratory conditions should be provided before field-using method. There are many factors that can influence the leaching of Hg in natural soil environments. Leaching of Hg is dependent on the physical and chemical properties of soil (soil water, sorption, redox conditions, mechanical and chemical properties of soil), as well as the concentration of pollution and atmospheric precipitations (Chaney et al. 1997). Under these conditions, soluble forms of mercury can transform to insoluble and inversely, which changes leaching characteristics. The presented results showed that thiosulphate had a potential to mobilize the Hg in soil. The high concentration of Hg in water extracts suggested that after thiosulphate treatment, Hg can be transferred to below the root zone of plants and cause a danger to other ecosystems.

The results presented in this study are very promising, especially those that indicate the increasing Hg accumulation by *L. sativum* L. after thiosulphate amendment. Very high translocation rates create the possibility of trying to use the technique in the field conditions. Nevertheless, there are some limitations that have to be taken into consideration before the

field testing. The presented process was conducted in a laboratory under controlled conditions. Low soil humidity was kept to maintain the moisture just below field water capacity and at the same time, to ensure no leaching during plant cultivation and thus no vertical movement of Hg to below the root zone area of plants. In real conditions, the field water capacity should be analysed before the field testing technique. Therefore, in any field application of the presented technique, both the water use efficiency of the plants as well as field water capacity would need to be considered, mitigating any potential environmental risk.

Moreover, the leaching experiment showed that the application of thiosulphate-mobilized Hg, which means that Hg changed its form in the soil solution. Further investigations should be provided to verify the Hg form(s) after thiosulphate treatment. In general, the soluble exchangeable and specifically sorbed fractions of soil metal have higher bioavailability than other fractions (Wang et al. 2012). Therefore, Hg in these forms can be transported easier to deeper layers of soil or water spreading the pollution. Information about Hg forms after thiosulphate treatment would be a key factor that enables to verify the Hg behaviour in soil solution.

Van Nevel et al. (2007) reported that the use of some chelators could increase the risk of contaminant leaching, limiting at the same time, the use of suggested technique in the field. Based on the provided leaching experiments, thiosulphate can be included to these chelates. After thiosulphate treatment, almost half of the total Hg concentration stayed mobile in soil, which means that the risk of Hg leaching is highly probable. However, we believe that this risk may be mitigated through continuous planting of short vegetation period plant, like *L. sativum* L., to extract Hg from soil. The possibility of risk reduction also exists in lowering the dose of thiosulphate during assisted phytoextraction. However, some further investigations should be conducted to verify these proposals.

5 Conclusions

Lepidium sativum L. showed the potential of a non-hyperaccumulating plant that can be used during the phytoextraction of Hg-contaminated soils. This plant accumulated about 10–14 % of Hg depending on its concentration in soil. Thiosulphate promoted the phytoextraction process by increasing the total Hg accumulations by plant. Moreover, after thiosulphate treatment, Hg translocation to aerial parts of *L. sativum* L. increased greater than 10 times in relation to the unassisted process. No negative effect of thiosulphate on plant biomass was observed.

Although mercury uptake by *L. sativum* L. was promoted after thiosulphate treatment, the potential risk for the environment should be considered. The presented results showed that

thiosulphate-mobilized Hg in soil, which increased the Hg leaching. This constitutes the limitation of applying the technique in the field due to the risk of Hg transferring to deeper layers of soil or water. However, continuous phytoextraction and/or lowering dosage of thiosulphate may mitigate that problem.

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