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Thallium speciation in plant tissues—Tl(III) found in *Sinapis alba* L. grown in soil polluted with tailing sediment containing thallium minerals

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ABSTRACT

Besides the dominant species in plants—Tl(I), noticeable amounts of Tl(III) (about 10% of total Tl content) were found in extracts of plants cultivated in the presence of tailing sediments, which are the main source of anthropogenic thallium already present in the environment. It is an important step of gaining knowledge about the detoxification mechanisms developed by *Sinapis alba*. This plant species is highly tolerant to Tl and it is able to cumulate high amounts of Tl and transport it into the above-ground organs. For more adequate estimation of accumulating abilities of *S. alba*, the elements' bioavailability was taken into consideration. The obtained bioconcentration factors of Cd (AF = 0.6) and Zn (AF = 1–2) were significantly lower than of Tl (AF = 100–200). The biomass production was similar to the biomass of control cultivation. The results were based on ICP MS measurements of total elements' content and HPLC ICP MS for speciation analysis. The quality of obtained results was evaluated based on the intermethod comparison with voltammetry as a reference method. Comparison of data obtained using ICP MS and electrochemical methods (after a proper chemical treatment) was also used for indication of Tl(III) presence and for proving that Tl(I) was not transferred into Tl(III) during analytical procedures.

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1. Introduction

Thallium belongs to the group of very toxic elements. Monovalent thallium had received much more attention than Tl(III), but both forms are toxic to humans, plants and animals and the redox state can markedly affect Tl toxicity, Tl(III) being a few thousand times more toxic than Tl(I) [1,2]. The mechanism of monovalent thallium ion toxicity is well-known and related to the interference with the vital potassium-dependent processes. Tl(I) can move across cell membranes and accumulate in cells [3]. Monovalent thallium is more toxic than many other elements such as Cu, Cd, Ni, Mn, Sn, As and Pb. With respect to degree of toxicity, evaluated for microorganisms: *Daphnia magna* [1] and *Chlorella* [2], trivalent thallo-compounds (nitrate, chloride and acetate) are approximately few thousand times more toxic than Tl(I), Cd(II), Cu(II) and Ni(II), and similar to Hg(II) [1,2]. Therefore, the determination of chemical species of thallium in the environment is important and the interest in it has increased considerably during the last few years. In comparison to Tl(III), monovalent thallium is thermodynamically more stable and less reactive, which makes it the dominating form of Tl, but not the only one existing in the environment. Another species found in environmental samples are: dimethylthallium in the Atlantic Ocean [4], Tl(III) in river waters [5] and some forms other than Tl(I) in plants [6].

Thallium is an element spread in the environment. It occurs in small amounts in sulfide ores (Fe, Zn, Cu, Pb) and selenite ores (Cu, Ag). Extensive sulfide ore mining, flotation treatment and smelting are the major sources of anthropogenic dispersion of tailing minerals of Cd, Pb, Zn and Tl, especially in the vicinity of the zinc–lead smelters. The areas of high content of that metal appear worldwide: in Poland [7], Spain [8] and China [9,10]. Fractionation studies considering the mobility of thallium in soil and sediment samples (tailing sediment used in this study) collected in a region strongly polluted with Tl indicated that this metal is not mobile. The extractability with acetic acid (mobile fraction) was about 10% of total content, only thallium bound to carbonate minerals can be easily leached after even small changes of the pH value [7]. In the vicinity of rivers, not only the dissolved fraction can be distributed, but also the mobile fraction of Tl can be moved downstream carried by sediment particles [10]. Elevated concentrations of thallium generally were found in plants growing in areas polluted with Tl and in soils of naturally high Tl content [11,12]. Some of these plants, especially species belonging to the *Brassicaceae* family—*Armoracia rusticana* [13], *Sinapis alba* [14] and *Iberis intermedia* [15] are able to cumulate extremely high amounts of thallium in their tissues. This ability, characterized by accumulation and translocation factors, was also observed for plants grown in

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hydroponics [6], in soil spiked with TlCl [16] and Tl_2SO_4 solution [17]. Studies of thallium speciation indicated that its dominating form in plant tissues is monovalent thallium [6,15,16], but *S. alba* cultivated in the presence of high thallium concentrations additionally contained another Tl species [6].

In case of hydroponics it is easy to control the conditions of plant growth but this kind of cultivation does not reflect the real situation that occurs in soil, where a part of the toxin will be immobilized by various soil phases (organic matter or manganese-iron oxides) [7,14,18]. The composition of soil as well as the source of thallium contamination strongly influence the immobilization and/or the uptake of thallium by plants. Generally, the addition of MnO_2 to the soil reduces Tl uptake by *S. alba* but in presence of carbonate minerals the effect is unequivocal [18].

The bioavailability of metal ions from soil is lower than from solution and that is why cultivation in solid medium should be the next step. Therefore, the work presented here focuses on defining of thallium speciation in *S. alba* plants grown in soil contaminated with sediments collected from an area strongly polluted with tailing minerals of zinc–lead ores flotation treatment—the real source of anthropogenic thallium contamination in that area. Data presented in this paper are a part of a project which main goals are to prove the hyper-accumulating potential of *S. alba* for extraction of thallium from polluted soil, define the tolerance level for thallium (the regular biomass production) and determine the accumulation factors (AF) of Tl. The results of speciation analysis should support the understanding of the mechanism of Tl detoxification by hyperaccumulators of this metal.

2. Materials and chemical analysis

2.1. Plant cultivation

Plant cultivation was carried out in a growth chamber at 20 °C/16 °C (14 h day/10 h night). Seeds of *S. alba* L. were sown into an artificial medium (glass balls) and seedlings in pots with soil. The following soils were used: (1) commercial potting soil; (2) soil spiked with thallium (I); (3) soil spiked with thallium (I) and cadmium (II); (4) soil mixed with tailing sediments collected from thallium polluted area (w/w 5:2). Plants were watered daily with distilled water. In variant (2) Tl (as a solution containing 500 μg L^{-1} Tl) was added gradually (3–4 mL per plant every 5 days) until reaching the total amount of 8.5 μg Tl per one plant. In variant (3) Tl and Cd (as a solution containing 500 μg L^{-1} Tl and 500 μg L^{-1} Cd) were added gradually until reaching the total amount of 8.5 μg Tl and 8.5 μg Cd per one plant. Sediments used in variant (4) were collected near "Bolesław" Mining and Smelting Works in Bukowno, from the outlet of the pond where the semi-liquid post-flotation wastes are deposited [7]. After 4 weeks of cultivation plants were harvested and divided into stems and leaves. Each plant material and each soil sample were air dried in an oven (55–60 °C, 10 h) and homogenized by milling in agate ball mill and sieving through a 0.125 mm nylon sieve.

2.2. Sample digestion

Plant samples (200 mg in 3 mL HNO_3) and soil samples (250 mg in 2 mL HNO_3 and 1 mL $HClO_4$) were placed in PTFE vessels and digested in a microwave system. In case of soils after the first step of mineralization in the second step 200 μL HF was added to the mixture. In all cases the mineralization was carried out in a closed system with automatic temperature control (ETHOS 1600; Milestone, Italy). Microwave energy of 1000 W was applied. The temperature control was as follows: 5 min, 90 °C; 10 min, 170 °C; 45 min, 200 °C – plant samples; 5 min, 80 °C; 10 min, 180 °C; 50 min, 200 °C – soil samples, first step; 5 min, 90 °C; 5 min, 170 °C; 15 min, 200 °C – soil samples, second step. After digestion the samples were diluted with DI water to the total volume of 25 mL.

2.3. Sample extraction

Fresh plant samples were snap frozen and immediately ground to a fine powder using a mortar and pestle in a liquid nitrogen bath. Then, the samples were extracted using 100 mmol L^{-1} ammonium acetate with 5 mmol L^{-1} DTPA, pH 6.2 (1 g in 8 mL of extractant shaken during 1 h at 37 °C) [6,19]. After extraction the suspensions were centrifuged (5000 rpm for 5 min).

Dried soil samples were extracted using 0.05 mol L^{-1} EDTA (1 g in 50 mL of extractant shaken during 1 h at room temperature). After extraction the suspensions were centrifuged and filtered [20].

2.4. Instrumental parameters—ICP MS

For total content determination all samples were diluted with water (1:10 and additionally 1:1000 for Zn determination in extracts of soils mixed with sediments) and analyzed by ICP MS (ELAN 6100 ICP; PE-SCIEX, Canada) equipped with Meihard-type nebulizer and Scott-type spray chamber. Measurements were performed applying the following experimental parameters: sweep: 5; number of replicates: 5; dwell time: 0.1 s; ICP RF power: 1100 W; lens voltage: 12 W; nebuliser gas flow: 0.95 L min^{-1}; plasma gas flow: 13.3 L min^{-1}; sample flow rate to the nebulizer: 1.0 mL min^{-1} and monitored isotopes: ^{103}Rh, ^{114}Cd, ^{64}Zn and ^{205}Tl. A solution of 10.0 ppb Rh was used as an internal standard. Quantitative analysis program was used to automatically correct the intensities of interfering isobaric and molecular ions. For quantitative determinations the calibration curve method was applied. Calibration points for Cd and Tl were 0, 1 and 10 ppb, and for Zn–0, 10 and 100 ppb. Samples were diluted to obtain analyte concentration within the range of the calibration curve. Correlation coefficient of each calibration curve was equal or above 0.9999. Standard solution (1 ppb Tl, 1 ppb Cd and 10 ppb Zn) was analyzed after every 5–10 samples to control instrumental drift.

For speciation analysis plant extracts were diluted 1:10 and separated using anion exchange HPLC (1200 HPLC pump; Agilent, USA and Model 7725 injection valve with a 100 mL injection loop; Rheodyne, Cotati, CA, USA) with Hamilton PRP-X100 column. Mobile phase was 100 mmol L^{-1} ammonium acetate with 5 mmol L^{-1} DTPA, pH 6.2. ^{203}Tl and ^{205}Tl were detected on-line with ICP MS [16].

2.5. Instrumental parameters—DPASV

Differential pulse anodic stripping voltammetry (DPASV) using hanging mercury drop electrode (μAutolab analyzer type II; ECO-Chemie BV, Netherlands with 663 VA Stand; Metrohme, Switzerland) with supporting electrolyte containing DTPA was applied as a reference method of thallium determination [21]. Specific character of electrochemical methods enables an indirect evaluation of speciation. In the presence of DTPA Tl(III) forms a non-electroactive complex. Therefore, in the presence of DTPA only Tl(I) ions are preconcentrated on the surface of the working electrode and for that reason the recorded signal corresponds only to Tl(I) concentration [21], whereas the results of ICP MS measurements correspond to total thallium content (both Tl(I) and Tl(III)). Therefore, the presence of Tl(III) will cause a discrepancy between the data obtained by ICP MS measurements and by DPASV measurements. ICP MS results higher than DPASV results allow to presume that part of Tl is present in the sample in a form of Tl(III).

For Tl determination the appropriate (known) volume of the sample was pipetted into a voltammetric cell containing 3.00 mL

of 0.15 mol L^{-1} DTPA solution and 3.50 mL of 0.2 mol L^{-1} acetate buffer (pH 5.5). The solutions were purged with argon for 10 min. Preconcentration was carried out for 120–420 s in a stirred solution at a potential of −0.75 V. After a resting time of 5 s, the voltammetric curves were recorded in the potential range from −0.75 to −0.20 V using differential pulse technique with a scan rate of 10.5 mV s^{-1} (potential step of 0.0221 V), modulation time of 0.05 s and amplitude of 50 mV. The double standard addition method was used for quantitative determination of Tl.

Comparison of the results obtained for extracts of soil and plant tissues using ICP MS and DPASV was done to confirm that Tl(I) is not transformed into Tl(III) in soil nor during extraction from mustard leaves. For this purpose Tl(I) ions were added to control sample (leaves of plants cultivated in soil without thallium) before extraction.

3. Results and discussion

Although the detailed mechanisms of uptake, detoxification and distribution of thallium among plant organs are not yet fully understood, the literature data suggest that it is related to the uptake of K^+ (nutrient ion) [4,14]. This could be confirmed by studies of the pathways of thallium transport, which are strongly correlated with the pathways of potassium transport [15]. Also, it was already stated that *S. alba* could probably be used as a phytoextractor of Tl, Pt and As [6,14,19,22]. Bioconcentration factors (also called accumulation factors and calculated as a ratio of the element's concentration in plant organ to its concentration in substrate) of all these elements, calculated for hydroponically cultivated *S. alba*, are all high, but the transport from roots into the above-ground organs, characterized by translocation factors (calculated as a ratio of the element's concentration in leaves to its concentration in roots), is effective only in case of Tl, which means higher final content of Tl in leaves and stems than in roots [6]. High Tl uptake was also found in *S. alba* grown in soil [14].

Speciation analysis is an important part of studies on detoxification mechanisms and hyperaccumulator's tolerance to xenobiotic. Therefore the analysis of Tl species in plant extracts was carried out simultaneously with bioaccumulation studies. The first step of the analytical procedure was extraction with DTPA in acetate buffer solution. Elemental detection after separation of chemical species was used to define Tl speciation in plant extracts. In order to assure the proper interpretation of obtained data, especially to confirm that Tl(I) is not transformed into Tl(III) during preparation procedures, some additional experiments were conducted—thallium (I) ions were added to control sample (leaves of plants cultivated in soil without thallium) before extraction. For such prepared samples both sets of data (ICP MS and DPASV) were statistically similar ($n = 3$, $P = 95\%$, $t_{exp} = 1.572 < t_{crit} = 2.776$). Therefore, if there was any Tl(III) in the samples, its content was below the LOQ (0.2 ng g^{-1}) of DPASV method. These results were confirmed by HPLC ICP MS measurements. In all plant extracts Tl(III) was not detected. The amounts of Tl in soil extracts (bioavailability test, extraction with EDTA) evaluated based on ICP MS and DPASV measurements did not differ statistically ($n = 3$, $P = 95\%$, $t_{exp} = 0.480 < t_{crit} = 2.776$), which is an indirect evidence that so-called "bioavailable" fraction of Tl in the soil is rather monovalent. However, it should be pointed that the trivalent Tl could be immobile in such conditions (bioavailability evaluation using solid–liquid extraction with EDTA solution), but available for plants (due to compounds released from roots into the rhizosphere).

Noticeable amounts of Tl(III) (detected as Tl(III)–DTPA) were found in extracts of plants grown in soil watered with thallium (I) nitrate solution, as well as in soil enriched with Tl in a form of tailing sediment containing thallium minerals (the mobile fraction of Tl is formed mainly with carbonate minerals) (Fig. 1). Each dry gram of plants grown in soil spiked with Tl(I) solution contained 70 ng of Tl(III), which is about 10% of extracted thallium (e.t.). Plants grown in soil spiked with Tl(I) and Cd solution and in soil mixed with sediments contained 8 ng (1.4% e.t.) and 170 ng (3.7% e.t.) of Tl(III) in 1 g d.m., respectively. The results were evaluated by comparing the peak areas of signals obtained for sample and standards. It is probable that plants are able to leach Tl(III) from its immobile compounds or oxidize small amounts of Tl(I) to Tl(III), which is more toxic but forms much more stable complexes with organic ligands. Literature data indicate that these complexes could be formed with cysteine or glutathione, which are thermodynamically stable [23]. Such a transformation seems to be probable, as examples of similar detoxification mechanisms can be found in the literature concerning As hyperaccumulators [24]. However, establishing where the oxidation process takes place (plant tissues or rhizosphere) and identification of compounds stabilizing Tl(III) in plant tissues, require further studies involving molecular detection techniques.

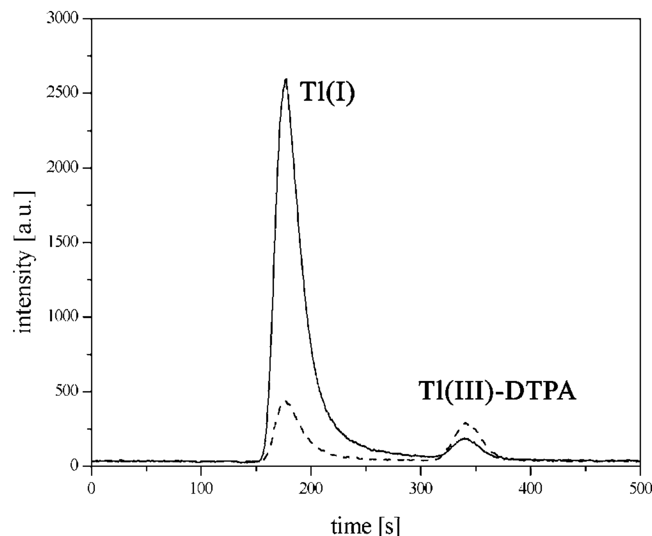

Fig. 1. IC chromatograms of extract of leaves of *Sinapis alba* grown in soil mixed with sediments, diluted 1:10 (solid line) and mixed 1 μg L^{-1} standards of Tl(I) and Tl(III) as Tl(III)–DTPA (dashed line), with on-line ICP MS detection of ^{205}Tl. The first signal recorded at $t_r = 190$ s and corresponding to Tl(I) represents 96% of total thallium content in the extract. The second signal recorded at $t_r = 370$ s was identified as Tl(III)–DTPA. This well measurable signal corresponds to Tl(III) concentration and represents 4% of total thallium content.

For the thorough evaluation of mustard plants' abilities to cumulate Tl, the biomass production and Tl bioavailability (Table 1) were controlled for all cultivations. It was found that the biomass of all cultivations run in soil with thallium additives was comparable to control cultivation (26–28 g) and the morphological structure of the plants was correct. The bioavailability of Tl, evaluated by leaching with EDTA solution, was used in calculations of the accumulation factors (AF), which were calculated for leaves and stems as a ratio of the element's concentration in plant organ to its concentration (only the bioavailable part) in solid medium used for cultivation (Table 2). For all variants of cultivation the accumulation factors of Tl were importantly higher than AFs of Cd and Zn. Moreover, Tl uptake is remarkably effective when plants are cultivated in the presence of sediments. AFs of Cd are higher when plants are watered with Cd(II) salt solution than when cultivated in the presence of sediments. AF of Zn, as well as its bioavailability, is highest in case of cultivation in pure potting soil. It was also found that the presence of sediments, which contain large amounts of tailing Zn ores (less mobile form), effects in a decrease of relative uptake (AF) of that metal, whereas the total content of Zn in plant organs

Table 1
Content [μg g^{-1} d.w.] of Tl, Cd and Zn in EDTA extracts of solid media used for cultivation and relative bioavailability of Tl, Cd and Zn from these media (presented in brackets as %). Data presented as mean ± SD ($n \geq 3$).

Sample	Cd	Zn	Tl
Pure sediment	7 ± 1 (23)	930 ± 140 (14)	0.08 ± 0.02 (0.6)
Before cultivation			
Soil + sediment	3.1 ± 0.3 (17)	474 ± 22 (12)	0.05 ± 0.01 (0.8)
Pure soil	0.12 ± 0.01 (64)	5.8 ± 0.4 (34)	0.06 ± 0.01 (97)
After cultivation			
Soil + sediment	3.0 ± 0.5 (14)	391 ± 67 (10)	0.05 ± 0.01 (0.7)
Soil + Tl solution	0.11 ± 0.02 (36)	17 ± 14[a] (34)	0.19 ± 0.02 (29)
Soil + Tl/Cd solution	0.35 ± 0.03 (46)	4.7 ± 0.6 (19)	0.07 ± 0.01 (11)

[a] High inhomogeneity.

Table 2
Total content [μg g^{-1} d.w.] of Tl, Cd and Zn in organs of *Sinapis alba* grown in solid media and in those media. Data are presented as mean ± SD ($n \geq 3$). Accumulation factors regarding bioavailability (AF-bioav) calculated for *S. alba* grown in solid media are presented in brackets as %.

Solid medium used for cultivation	Sample	Total content (AF-bioav)		
		Cd	Zn	Tl
Soil	Soil, before cultivation	0.18 ± 0.07	17 ± 5	0.06 ± 0.01
	Leaves	0.92 ± 0.06 (8)	93 ± 7 (16)	0.11 ± 0.01 (2)
	Stems	0.75 ± 0.05 (6)	100 ± 1 (17)	0.23 ± 0.01 (4)
	Soil, after cultivation	0.20 ± 0.03	22 ± 2	0.07 ± 0.01
Soil + sediment	Leaves	2.54 ± 0.07 (0.7)	709 ± 10 (1)	5.8 ± 0.1 (94)
	Stems	2.09 ± 0.04 (0.6)	933 ± 10 (2)	13.8 ± 0.4 (224)
	Soil, after cultivation	21 ± 3	4000[a]	8 ± 2
Soil + Tl solution	Leaves	0.7 ± 0.1 (6)	65 ± 15 (4)	1.9 ± 0.7 (10)
	Stems	0.6 ± 0.1 (6)	69 ± 3 (4)	2.17 ± 0.03 (11)
	Soil, after cultivation	0.32 ± 0.04	40[a]	0.7 ± 0.2
Soil + Tl/Cd solution	Leaves	1.15 ± 0.16 (3)	56 ± 2 (12)	1.0 ± 0.3 (14)
	Stems	0.75 ± 0.05 (2)	55 ± 1 (12)	1.60 ± 0.02 (22)
	Soil, after cultivation	0.76 ± 0.05	25 ± 4	0.7 ± 0.2

[a] High inhomogeneity.

increases 10 times. These data confirm that Tl is selectively (even specifically) transported from roots into the above-ground organs.

4. Conclusions

Speciation analysis conducted using HPLC ICP MS indicated that tissues of *S. alba* cultivated in the presence of tailing minerals from flotation treatment, contain noticeable amounts of Tl(III). Comparison of the results obtained by DPASV and ICP MS proved that procedures of sample preparation do not cause oxidization of Tl(I) to Tl(III). *S. alba* is capable of cumulating large amounts of Tl and transporting it effectively into the above-ground organs. In contrary, Cd, As, Pt and Zn are cumulated mostly in roots.

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