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Thallium uptake/tolerance in a model (hyper)accumulating plant: Effect of extreme contaminant loads

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Abstract: Thallium (Tl) is a toxic trace element with a highly negative effect on the environment. For phytoextraction purposes, it is important to know the limitations of plant growth. In this study, we conducted experiments with a model Tl-hyperaccumulating plant (*Sinapis alba* L., white mustard) to better understand the plant tolerance and/or associated detoxification mechanisms under extreme Tl doses (accumulative 0.7/1.4 mg Tl, in total). Both the hydroponic/semi-hydroponic (artificial soil) cultivation variants were studied in detail. The Tl bioaccumulation potential for the tested plant reached up to 1% of the total supplied Tl amount. Furthermore, it was revealed that the plants grown in the soil-like system did not tolerate Tl concentrations in nutrient solutions higher than ~1 mg/L, i.e., wilting symptoms were evident. Surprisingly, for the plants grown in hydroponic solutions, the tolerable Tl concentration was by contrast at least 2-times higher (≥ 2 mg Tl/L), presumably mimicking the K biochemistry. The obtained hydroponic/semi-hydroponic phytoextraction data can serve, in combination, as a model for plant-assisted remediation of soils or mining/processing wastes enriched in Tl, or possibly for environmental cycling of Tl in general.

Keywords: artificial soil; bioaccumulation; hydroponic; phytoextraction; Tl; uptake

Currently, the best-known plant species with the ability to accumulate thallium (Tl), being a highly toxic (global) pollutant, include *Biscutella laevigata*, *Iberis intermedia* and *Brassica oleracea acephala* L. (Al-Najar et al. 2003; Ning et al. 2015), *Brassica napus* L. (Pavlíčková et al. 2006; Madejón et al. 2007; Mestek et al. 2007; Liu et al. 2020) and *Sinapis alba* L. (Fargašová 2004; Krasnodębska-Ostręga et al. 2012; Vaněk et al. 2013; Grösslová et al. 2015; Mazur et al. 2016). World production of Tl is estimated at 10–15 t/year (Merian & Clarkson 1991). On the other hand, up to ~2 000–7 000 t of Tl per year is mobilized globally by human activities (Kabata-Pendias & Pendias 1992; Kabata-Pendias & Sadurski 2004) as solid/liquid emissions from coal combustion, ferrous/non-ferrous mining/smelting, or eventually cement production (Kazantzis 2000; Yang et al. 2009). Targets for the use of phytoextraction techniques

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are potentially linked with the process of wastewater treatments (Vácha et al. 2015; Gutiérrez et al. 2016). The Tl transfer mechanism from the waste materials into the plant tissue generally depends on the total bioavailable element fraction in soil (Harmsen 2007) and plant physiology (Sager 1994).

An advantage of phytoextraction processes using the hyperaccumulating plants could be the natural selection of trace elements (Čechmánková et al. 2011; Corzo Remigio et al. 2020), though, the Tl/element transfer under the laboratory trial has several limitations, such as the number of suitable plants with a high accumulation potential tolerance for specific soil/climatic conditions. The bioavailability of Tl in soil depends on the specific sorption, mainly onto specific Mn oxide, birnessite (δ-MnO_2) and illite [$(K,H_3O)Al_2(Al,Si)_4O_{10}(OH)_2$], being probably the most important Tl scavengers and the general stability of Tl-host phase (Al-Najar et al. 2003; Vaněk et al. 2013; Grösslová et al. 2015). Possibly incongruent leaching of Tl from the soil to the biological/plant material may refer to the Mn-Tl association (Vaněk et al. 2019). The physiological availability of Tl into the plant depends mostly on the crop species, storage organ structure, accessibility of plant exudates, state of enzymatic processes, and plant growing strategy (Merian & Clarkson 1991). It is supposed that the majority of the up-taken Tl run along nutrient paths into the cell cytosol and vacuole storage (Kwan & Smith 1991; Ning et al. 2015).

This paper aims to evaluate and to understand the limitations of white mustard, and possibly analogous *Brassicaceae* species, to absorb/accumulate Tl, i.e., as affected by extreme Tl loads. Two growth strategies, hydroponic and semi-hydroponic (artificial soil), were investigated in detail, both being in line with the Tl remediation planning of contaminated soils (wastes) in the future. Furthermore, the results are also important from the view of general knowledge of environmental Tl cycling.

MATERIAL AND METHODS

Plant growing. Sixteen plants (in total) of white mustard (*Sinapis alba* L.) (14 days pre-cultivated grown-up) were exposed to extreme concentrations of Tl (1 and 2 mg Tl/L) for 21 days. Two different plant growing systems were compared: A – hydroponic and B – semi-hydroponic (artificial soil) system (Figure 1). A total dose of Tl applied through lower concentration during 21 days was 0.7 mg Tl (A_1 and B_1) and 1.4 mg Tl for higher concentration (A_2 and B_2). Five growth phases (Figure 2) of the mustard plant with controlled Tl and nutrition dose exposure was monitored.

Nutrient solution control. Five (fresh) nutrient solutions with specific electrical conductivity (EC) (Figure 2) were prepared every week. The solution was Tl-contaminated with the dissolved Tl_2SO_4 (Fluka, Germany, p.a.) for the whole experiment. All the plants were cultivated in the Reid and York (1958) nutrient solution: 0.136 g/L KH_2PO_4, 0.373 g/L KCl, 0.555 g/L $CaCl_2$, 0.443 g/L $MgSO_4{\cdot}7H_2O$, 0.600 g/L NH_4NO_3, 0.049 g/L $FeCl_3{\cdot}6H_2O$, 0.066 g/L Na_2-EDTA, 0.200 mg/L $ZnSO_4{\cdot}7H_2O$, 0.611 mg/L H_3BO_3, 0.388 mg/L $MnCl_2{\cdot}4H_2O$, 0.100 mg/L $CuSO_4{\cdot}5H_2O$, 0.040 mg/L $Na_2MoO_4{\cdot}H_2O$, 0.055 mg/L $Co(NO_3)_2{\cdot}6H_2O$. The 100 ml of Tl and nutrient solution was dosed into the containers (volume approx. 600 ml) according to variants at a 2–3-day regime. For the hydroponic

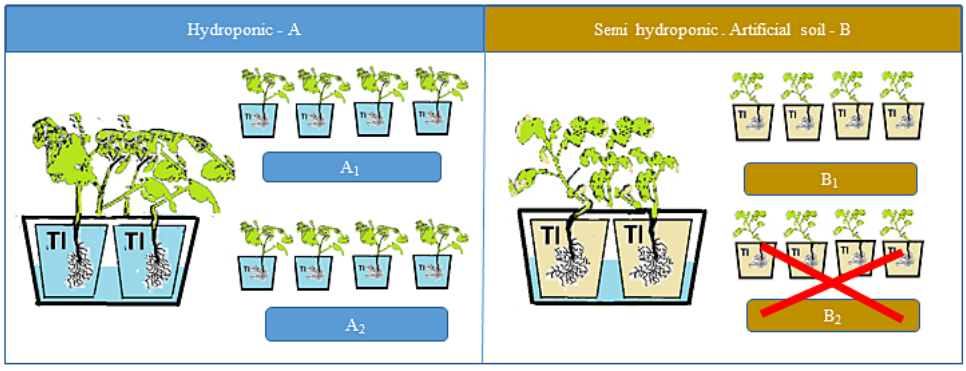

Figure 1. Experimental design of the 4-pots variants affected by (controlled) thallium (Tl) expositions; variant A – hydroponic cultivation; variant B – artificial soil cultivation; the Tl solution concentration was as follows: 1 mg Tl/L (A_1/B_1), 2 mg Tl/L (A_2/B_2); the total Tl dose for the 21-day-long experiment was 0.7 mg Tl (A_1/B_1) and 1.4 mg Tl ($A_2/_{B2}$), respectively; the B_2 variant was excluded, due to the significant wilting symptoms during the first 5 days

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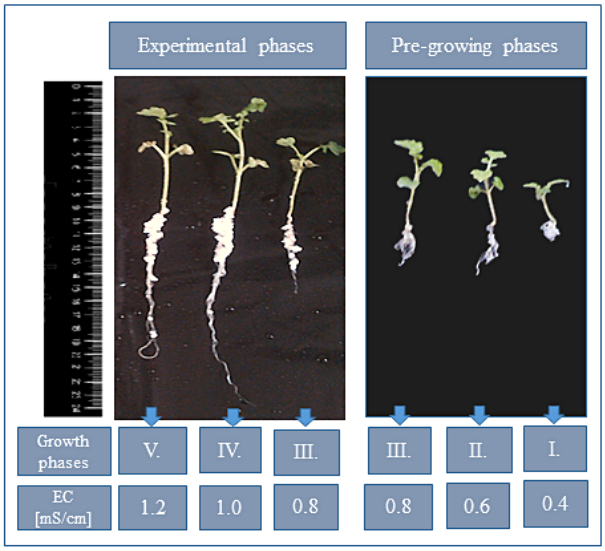

Figure 2. Growing phases of the tested plant (white mustard) and corresponding nutrient solution conductivity (EC)

system (A), a silica gel (white, Signus SG 5) grain size (2–5 mm) was used. The artificial soil variant (B) was prepared as 10% Sphagnum-peat, 70% quartz (SiO_2) sand (50–200 µm), and 20% of china clay (with ~30% kaolinite ($Al_2(OH)_4Si_2O_5$) content) were prepared according to the OECD (2009) standard.

Harvesting and digestion of plant materials. All the plants were harvested after a 21-day long experiment (on day 35 of their life); separated into leaves, stems, and roots; washed in deionized water and ethanol. Plant tissues were oven-dried at 60 °C for 24 h and homogenized. The biomass was decomposed using a mixture of concentrated (65%) HNO_3 (suprapure) with H_2O_2 (suprapure) (Merck, Germany) in a ratio of 4 : 1 which were added to the sample in a total volume of ~5 and 10 mL, then left in a closed 60-mL PTFE beaker (Savillex, USA) on a hot plate (150 °C) for 24 h.

Determination of Tl and element concentrations. The concentrations of the major/trace elements (Na, Mg, K, Ca, Mn, Fe, and Tl) in the total digests solution (of plant tissue) were determined using either inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP 6500, Thermo Scientific, UK) or quadrupole based inductively coupled plasma mass spectrometry (Q-ICP-MS, Xseries II, Thermo Scientific, Germany) under standard analytical conditions. The standard reference material INCT-TL-1 (tea leaves, Institute of Nuclear Chemistry and Technology, Poland) was used for QC of quantitative analyses of Tl/major elements; the detected relative Tl recovery was ≥ 90% of the certified concentration (n = 3).

Calculation of bioaccessibility index BAI. The bio-accessibility index (BAI) is thought to be based on publications (Zayed et al. 1998; Hladun et al. 2015; Kim et al. 2016) where the bioconcentration factor (BCF) is expressed. The calculation of BAI presents a different approach. While BFC is expressed as a proportion of the concentration of the metal in the plant's tissue (mg/kg)/metal concentration in the soil (mg/kg), the BAI was expressed as the sum of the element in a specific plant tissue (g) (X_{tissue})/ sum of the total element dose (g) applied into the pot system during the whole experiment (Y_{total}), according to the formula:

$$BAI = X_{tissue}/Y_{total} \times 100\ (\%)$$

The total bio-accessibility index (BAI_{tot}) is expressed as a sum of BAI (%). The distribution factor (DF) of the trace element is counted as BAI/BAI_{tot}.

RESULTS AND DISCUSSION

Thallium uptake and distribution in plant. The usual Tl concentration in the plant tissue is ≤ 0.05 mg/kg (Adriano 2001; Krasnodębska-Ostręga et al. 2012). The phytotoxic level of Tl for plants varies ~20 mg/kg (Kabata-Pendias & Pendias 1992). The authors estimated the threshold of Tl hyperaccumulation in a whole plant as either 100 mg/kg (Van Der Ent et al. 2013), 500 mg/kg (Leblanc et al. 1999), or up to 1 000 mg/kg (Krämer 2010). Our results slightly exceed the physiological limit of Tl intoxications, as determined by Kabata-Pendias and Pendias (1992). The plant concentrations of Tl varied from 30 to 60 mg/kg within our experiment (Table 1). It is a much lower concentration than ~340 mg Tl/kg recorded by Xiao et al. (2012) in the green cabbage in "Lanmuchang" Tl-polluted area.

We assume that more important than the total concentration of Tl in a plant itself is its actual Tl content in soil (or hydroponic) solution. The initial concentrations of 2 mg Tl/L were lethal for plants incubated in the artificial soil (variant B_2). All the plants growing under the semi-hydroponic conditions signalled from the 2nd week of their life wilting symptoms: blockade of the biogenic element (Merian & Clarkson 1991; Tremel et al. 1997) and pigment loss (Mazur et al. 2016). On the other hand, not even a high concentration of Tl under the hydroponic cultivation caused signs of wilting.

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Table 1. Total thallium (Tl) concentration and distribution factor (DF) of white mustard at different Tl doses and growing systems; data cumulate 4 plants replicate as a variant

Variant	Sample	Biomass (mg)	Tl level (mg/kg)	DF (%)
A_1	leaf	96	39.39	56.9
	stem	74	31.20	34.8
	root	47	11.76	8.3
	Σ whole plant	217	30.61	–
A_2	leaf	106	87.20	61.9
	stem	74	66.53	33.0
	root	43	17.57	5.1
	Σ whole plant	223	66.91	–
B_1	leaf	72	40.52	43.0
	stem	43	53.84	34.1
	root	55	28.32	22.9
	Σ whole plant	170	39.94	–

A – hydroponic system; B – semi-hydroponic (artificial soil) system; the whole plant level of Tl was calculated as weighted means of individual tissue concentrations; the DF was expressed as a specific plant tissue bio-accessibility index (BAI)/total plant (BAI_{tot}); the Tl concentration data are means with SD < 15%

When comparing the variants with the same Tl exposition (A_1 and B_1, Table 1), the preference of Tl bioconcentration into the root and stem in artificial soil variant is evident. At the same time, plants growing in the artificial soil (semi-hydroponics) demonstrate comparable (or slightly higher) Tl inputs into the whole plant. Despite that, the Tl bioconcentration in the soil-like system could be affected by the weak interaction with the clay/kaolinite as Grösslova et al. (2015) estimated as 0.6 ± 0.02 mg Tl/kg for the background phases with kaolinite. In hydroponic cultivation, Tl is concentrated predominantly in the shoots, negligibly in the root of the plants. This aspect, together with the higher tolerance of white mustard to Tl exposure in hydroponic systems could be crucial for the phytoremediation use.

In general, our experiment confirmed different Tl bioaccumulation into the specific plant tissues depending on the cultivation media which does correspond to our previous findings (Holubík et al. 2020). Most of the total received Tl was accumulated into the shoots of white mustard (regardless of the method of cultivation). The distribution factor (DF) of the bioaccumulated Tl into the foliar mass ranged from ~50 to 60% for the hydroponic cultivation (Table 1); the entry of Tl into root tissues is minimal in hydroponic cultivation (from ~5 to 8%). Under the semi-hydroponic (soil-like) cultivation almost 20% of Tl remained in the roots, 30% were shifted to the stem, and 40% to the leaf (Table 1). Higher root development in plants grown in the soil and higher development of green parts in hydroponic systems corresponds to a different plant growth strategy (Taiz & Zeiger 2003; Holubík et al. 2020).

Thallium bioaccumulation and nutrient control. It turns out that the Tl entrance into the plant was related to monovalent ion intake (mainly of K) within the hydroponic cultivation (Table 2). It is known, that under physiological conditions Tl tends to mimic K(I) in biochemical processes (Sager 1994; Galván-Arzate & Santamaría 1998). Our data clearly indicate reduced inputs of major/nutrient elements for plants grown in artificial soil. Despite minimal differences of BAI of main nutrients into the specific plant tissues (Table 3), we tried to relate which elements may accompany Tl on the way to the plant by linear regression relationship. Irrespective of cultivation and Tl spiked concentration the potassium follows the thallium into the plant due to: $BAI_{Tl} = 7.1\ BAI_K$ ($R^2 = 0.90$); similarly magnesium: $BAI_{Tl} = 8.0\ BAI_{Mg}$ ($R^2 = 0.96$), calcium as $BAI_{Tl} = 6.0\ BAI_{Ca}$ ($R^2 = 0.91$) and manganese as $BAI_{Tl} = 1.5\ BAI_{Mn}$ ($R^2 = 0.82$). The total plant Tl uptake (BAI_{tot}) was 0.99 ± 0.05% (Table 3). The hydroponic cultivation prefers Tl bioaccumulation in the shoot (0.9–1% of total Tl pool) and only a minimal proportion was accumulated

Table 2. The nutrient concentrations (g/kg) of the grow-up plants ($n = 4$)

Tissue	Variant	K	Mg	Ca	Fe
		(g/kg)			
Leaf	A_1	26.8	3.8	15.0	0.3
	A_2	12.1	2.9	11.7	0.3
	B_1	2.9	1.4	9.9	0.7
Stem	A_1	22.5	5.5	14.1	0.5
	A_2	19.6	4.1	10.3	0.5
	B_1	4.9	1.7	7.6	2.0
Root	A_1	20.8	3.9	14.1	0.4
	A_2	11.7	3.9	10.6	0.2
	B_1	1.8	1.0	3.8	0.7

A – hydroponic system; B – semi-hydroponic (artificial soil) system; the nutrient concentration data are means with SD < 20%

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Table 3. The bio-accessibility index (BAI) of the tested plant (white mustard) grown under different thallium (Tl) doses and growing systems; all the data cumulate the 4-plant replicates

Variant	Sample	BAI_{Tl}	BAI_{Na}	BAI_{Mg}	BAI_{K}	BAI_{Ca}	BAI_{Mn}	BAI_{Fe}
		(%)						
A_1	leaf	0.54	59	4.2	5.6	3.7	1.0	1.4
	stem	0.33	29	2.5	1.9	2.2	0.4	1.1
	root	0.08	5	0.8	0.3	1.2	0.3	1.7
	Σ whole plant	0.95	93	7.5	7.8	7.1	1.7	4.2
A_2	leaf	0.66	46	4.9	4.8	3.8	1.2	2.1
	stem	0.35	31	3.4	1.9	2.0	0.5	0.8
	root	0.05	3	0.5	0.2	0.4	0.2	1.6
	Σ whole plant	1.07	80	8.8	6.8	6.2	1.9	4.4
B_1	leaf	0.42	16	4.6	3.5	2.6	0.5	1.9
	stem	0.33	9	2.1	1.8	1.1	0.2	1.0
	root	0.22	3	1.1	0.6	1.1	0.2	5.6
	Σ whole plant	0.97	27	7.7	5.9	4.8	0.9	8.5

BAI was expressed as the sum of total element content in a specific plant tissue (g) (X_{tissue})/sum of the total element dose (g) applied during the whole experiment (Y_{total}), according to the formula: $BAI = X_{tissue}/Y_{total} \times 100$ (%); the total bio-accessibility index for the whole plant (BAI_{tot}) is expressed as a sum of specific tissue BAI (%)

into the roots (0.05–0.1 %). On the other hand, in the artificial soil system, a considerable portion of Tl (0.2%) was accumulated by root tissue. The different growth strategies (in soil/hydroponic) may affect individual nutrient uptake. Higher bioaccumulation of K and Na in the hydroponic system was observed (see Table 3), possibly due to the passive transport mechanism(s). For the semi-hydroponic cultivation, the Fe-Tl association was observed, which could have resulted from the active transport of Fe from the nutrient solution.

Environmental applications. Average levels in topsoils contaminated with Tl typically exceed 10 mg/kg (Xiao et al. 2004). Scheckel et al. (2004) demonstrated on Tl-spiked soils, that the Tl uptake mainly depends on the soil Tl concentration; the studied *Iberis intermedia* absorbed more than 13 mg Tl/g. Clearly, our results show a much lower absolute uptake of Tl into the white mustard plant tissue, reaching of ~0.05 mg/g (dry weight). Nevertheless, this behaviour could likely be compensated by a higher amount of biomass. We assume that the soil solution Tl concentration for phytoremediation cannot exceed 1 mg Tl/L. An important finding is that the concentration of 2 mg Tl/L is not limiting for hydroponic cultivation, being in contrast to Sager (1994) who reports for soybean plants (*Glycine max.*) ≤ 0.6 mg Tl/L (provoked leaf chlorosis), and ≤ 1 mg Tl/L (provoked leaf necrosis).

Our results demonstrate that the Tl plant uptake is primarily dependent on the concentration and speciation of Tl in the primary Tl pool, but for successful (efficient) phytoremediation use, it is also important to know the maximum available Tl load(s) and Tl distribution within respective plant organs/tissues. For practical use, it is still necessary to verify the experiment on an operational scale.

CONCLUSION

Based on our experiments, the tested mustard plant (*Sinapis alba* L.) could absorb up to 1% of the total supplied Tl amount, regardless of the method of cultivation. On the contrary, the distribution of Tl/element into specific tissue cytosol cell or vacuole storages depended on the cultivation strategy. For the hydroponic cultivation, up to 95% of the plant Tl was translocated into the shoots, and only ≤ 8% remained in the roots; the plants indicated by contrast a ~20% Tl root accumulation within the semi-hydroponics (soil-like system). In other words, we assume that Tl within the hydroponic system entered the plant tissues along the paths of the monovalent elements (mainly K), and contrarily to our/common expectations, for the semi-hydroponic system, the Tl introduction could have also been affected by the uptake of Fe. It should be noted that the higher Tl contamination in the soil/nutrient solution may

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decrease the uptake of biogenic elements. This aspect was evident for the soil-like cultivation at critical Tl doses (>1 mg Tl/L), where the plants suffered from wilting symptoms, which, by contrast, were negligible or absent for simple hydroponics and the same Tl load.

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