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Thallium Uptake and Translocation in Barley and Sunflower Grown in Hydroponic Conditions

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ABSTRACT: Thallium (Tl) is highly toxic to humans compared with other heavy metals and metalloids, but the risk of its bioaccumulation has not been adequately considered. Thus, Tl uptake and translocation in barley and sunflower under hydroponics with the applications of diethylene triamine pentaacetic acid (DTPA) and citric acid were investigated. Thallium concentrations in roots of both the plants were higher than those in the shoots. Translocation index (TLI) of Tl in barley decreased from 14.9 to 10.0% with increasing Tl treatments in barley, but it increased from 5.9 to 7.5% in sunflower. The concentration of Tl in the barley tissues increased with DTPA applications, but it decreased with citric acid treatments. In contrast, Tl in the sunflower tissues decreased with DTPA treatments, while it increased with the applications of citric acid. However, Tl concentrations in the tissues of both plants were not significantly different between the two concentrations (5.0 and 30 mg/kg) of DTPA and citric acid applications.

Key words: Barley, Citric acid, DTPA, Sunflower, Thallium

INTRODUCTION

Thallium (Tl) is widely distributed in the environment with concentrations generally ranging between 0.1 and 2.0 mg/kg, but sometimes higher than 70 mg/kg (Zhou and Liu, 1985; Qi *et al.*, 1992). Thallium concentrations in the soils near cement plants in Korea have been reported up to approximately 13 mg/kg (Lee *et al*., 2015). In these specific areas, Tl could spread to agricultural lands and contaminate food crops. It is highly toxic to humans compared with other heavy metals and metalloids such as cadmium (Cd), copper (Cu), lead (Pb), and mercury (Hg). However, the risk of its bioaccumulation has not been adequately considered. Several food crops have been found to accumulate Tl at high concentrations including green cabbage, kale, radishes, rapeseed, turnips, watercress, and white mustard (Tremel *et al*., 1997; Kurz *et al*., 1999; LaCoste *et al*., 2001; Al-Najar *et al*., 2005; Pavlickova *et al*., 2005; Madejon *et al*., 2007; Scheckel *et al*., 2007; Vanek *et al*., 2010). Also, in some specific plants such as candytuft and buckler mustard, the Tl concentrations in their above-ground parts were as high as 1.94% and 0.4% of the total dry weight, respectively (LaCoste *et al*., 1999).

Barley is one of the main crops and sunflower is becoming more commonly grown as an important cash crop in Korea. These plants have been cultivated along with other crops near thallium contaminated areas. The concentrations of heavy metals including Tl in different parts of various plants varied (Allus *et al*., 1987; Asami *et al*., 1999; Al-Najar *et al*., 2003; Madejon *et al*., 2003; Sekara *et al*., 2005) because plants have specific mechanisms involved in the uptake, translocation, and storage of trace elements (DOE, 1994; Tangahu *et al*., 2011).

In plants, monovalent thallium ions (Tl^+) do not complex with other materials and are not transformed into trivalent ions (Tl^{3+}). Therefore, almost all thallium found in plants is the uncomplexed ionic Tl^+ species and is not detected as Tl^{3+} (Mestek *et al*., 2007). For this reason, plants are able to easily take up thallium as a monovalent cation (Tl^+).

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In terrestrial and aquatic ecosystems, the application of synthetic chelating agents, including DTPA, ethylene diamine tetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), generally enhances phytoextraction of trace metals (Souza *et al*., 2013). However, some chelates, especially DTPA, decrease or do not affect the uptake and translocation of certain trace metals in specific plants (Wallace, 1980; Tandy *et al*., 2006; Sherameti and Varma, 2011; Yeh and Pan, 2012). Also, low-molecular-weight organic acids, for examples acetic acid, citric acid, and malic acid, can positively or negatively affect the phytoextractability of metals/metalloids due to their complexing and chelating properties (Wu *et al*., 2003; Han *et al*., 2005; Kim *et al*., 2009; Sabir *et al*., 2014; Hassan *et al*., 2016).

Therefore, the objective of this study was to investigate the uptake and translocation of thallium in different plants, barley (*Hordeum vulgare var. hexastichon*) as a main crop cultivated in suspected areas of Tl contamination and sunflower (*Helianthus annus*) as a well-known hyperaccumulator of heavy metals, from thallium contaminated hydroponic systems as influenced by the application of diethylene triamine pentaacetic acid (DTPA) and citric acid.

MATERIALS & METHODS

The seeds of barley (*Hordeum vulgare* L.) and sunflower (*Helianthus annus*) were obtained from the Seed Center of Jeollabuk-do Agricultural Research and Extension Services, Korea.

The plants were grown in hydroponic culture because hydroponics can provide the ideal nutritional environment for optimum plant performance with or without different treatments. A nutrient solution consisting of KCl (150 mg/L), $MgSO_4$ (120 mg/L), $Ca(NO_3)_2$’”$4H_2O$ (946 mg/L), KH_2PO_4 (68 mg/L), $ZnSO_4$’”$7H_2O$ (0.06 mg/L), H_3BO_3 (0.69 mg/L), $CuCl_2$’”$2H_2O$ (0.017 mg/L), Na_2MoO_4’”$2H_2O$ (0.024 mg/L), $MnCl_2$’”$4H_2O$ (0.022 mg/L), and $FeCl_3$ (0.6 mg/L) was prepared (Garland *et al*., 1981; Lee *et al*., 2002). Thallium solutions with/without diethylene triamine pentaacetic acid (DTPA) and citric acid were also prepared. The initial concentrations of thallium in the nutrient solution were 0, 1, 5, and 10 mg/L of Tl applied as a TlCl solution. The concentrations of DTPA and citric acid added were 0, 5, and 30 mg/L. The nutrient solution pH with or without thallium, DTPA and/or citric acid treatments was adjusted to approximately 6.0 with either a 0.1 M NaOH or HCl solution. All reagents were of chemical grade from Sigma-Aldrich or Fisher Scientific.

Barley and sunflower seeds were soaked in distilled water for approximately 6.0 h and then spread between two damp filter papers in plastic Petri dishes. They were left overnight in the dark to sprout. Sprouted seeds were planted in seedbed material in pots. The seedlings were grown to approximately 10 cm in height and then removed from the pot. The roots of both barley and sunflower were washed with tap water and then rinsed with deionized water to remove attached seedbed materials. The plants were transferred to a set of polypropylene growth containers with 250 mL of nutrient solution containing 0, 1, 5, or 10 mg/L of Tl. Another set of growth containers was treated with a fixed concentration of 5 mg/L of Tl combined with 0, 5, or 30 mg/L of DTPA or citric acid. Barley and sunflower plant samples were placed under different conditions using an environmental growth chamber. The growth chamber temperature was maintained at 20±2 °C for barley and 25±2 °C for sunflower, both with a 12 h light cycle (66.5-79.8 μE/m^2/S) and 60-70% relative humidity. The plants were allowed to grow for 7 days, and nutrient solution was added every 24 h during the growing period to replace nutrients and water lost due to evapotranspiration.

After harvesting the plants, the plant roots were thoroughly washed and rinsed with deionized water, and moisture on the plants was removed using laboratory paper towels. The plants were separated into shoots and roots and dried for 24 h at 80 °C in a drying-oven. The separated plant tissues were digested in 69% HNO_3 and 30% H_2O_2 (Jones, 1991). The digests were evaporated to near dryness and transferred to a 20 mL polypropylene vial with 0.1 M HNO_3 until the final volume became 20 mL. Thallium and potassium in the solution were measured using inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 7300DV, Perkin Elmer, USA).

Bio-concentration factor (BCF) (Zayed *et al*., 1998; Lee *et al*., 2002; Hladun *et al*., 2015) of thallium was calculated as the ratio of Tl concentration in the shoot or root of the plant (dry mass basis) to Tl concentration in the nutrient solution: BCF = (Tl_{ST} or Tl_{RT})/Tl_{NS}, where Tl_{ST} and Tl_{RT} are the Tl concentrations in the shoot and root, respectively, and Tl_{NS} is the Tl concentration in the nutrient solution. Translocation index (TLI, %) was also calculated as the ratio of the Tl concentration in the shoot of the plant (dry mass basis) to the Tl concentration in the whole plant: TLI (%) = $[(Tl_{ST})/(Tl_{ST} + Tl_{RT})] \times 100$ (Lee *et al*., 2002; Hladun *et al*., 2015).

Statistical analyses were operated using SPSS (statistical package for the social science, ver. 18.0, SPSS Inc., Chicago, IL, USA) statistical software. Significance of parameters was calculated by one-way analyses of variance on ranks followed by Duncan’s Multiple Range Test. The correlation among The values of Tl concentrations in plant, BCF, and TLI was performed by correlation analysis. The data (Tl concentration, BCF, and TLI) presented from a normal distribution by Shapiro-Wilk test ($p < 0.05$), and thus correlation coefficient (r) was calculated by Pearson's Correlation Analysis.

RESULTS & DISCUSSION

The uptake and translocation of thallium (Tl) in barley and sunflower based on Tl treatment with different concentrations in the nutrient solution are presented in Table 1. Thallium uptake in the shoots and roots of barley and sunflower increased with increasing Tl concentration. The Tl concentration in the roots was much higher than in the shoots of the plants at all doses of Tl application in the nutrient solution. Previous studies reported similar results that Tl concentration was higher in the roots compared to the shoots of barley (Allus *et al*., 1987) and sunflower (Madejon *et al*., 2003). Thallium translocation in mature sunflower plants decreased in the order of roots > stems > seeds with a ratio of 35:10:1. In contrast, the Tl concentration was greater in the shoots than in the roots of some other plants including rapeseed, Japanese mustard spinach, soya bean, kale, and candytuft (Allus *et al*., 1987; Asami *et al*., 1999; Al-Najar *et al*., 2003). In general, metal in soil solution is selectively taken up by plants. The metal uptake is dependent upon specific/genetic carriers or channels in the plasma membrane (Lyubenova and Schroder, 2010).

Furthermore, the bio-concentration factor (BCF; BCF_{ST}, BCF for plant shoot; BCF_{RT}, BCF for plant root) and translocation index (TLI) of Tl in the plants showed different trends. The values of BCF_{ST} for barley were similar between 1.0 and 5.0 mg/L of Tl application but decreased with application of 10 mg/L of Tl in the solution. The values of BCF_{RT} for barley increased with Tl treatment even though the BCF_{RT} values were not statistically different between 5.0 and 10.0 mg/L of Tl. However, the values of both BCF_{ST} and BCF_{RT} for sunflower decreased with increasing Tl concentration in the nutrient solution. Meanwhile, the TLI value decreased with increasing concentrations of Tl treatment in barley, whereas it increased with increasing concentrations of Tl application in sunflower; it decreased from 14.9±0.1 to 10.0±0.2% in barley, but increased from 5.9±0.9 to 7.5±0.1% in sunflower with increasing Tl treatment concentrations from 1.0 to 10.0 mg/L. The results indicated that the Tl-TLI values in the two different

Table 1. Thallium uptake and translocation in barley and sunflower as affected by the different concentrations of Tl application

Plants	Amount of Tl applied (mg/L)	Tl concentration (mg/kg)		BCF_{ST}[a]	BCF_{RT}[b]	TLI[c] (%)
		Shoots	Roots			
Barley	0	nd[d]	nd	-	-	-
	1.0	30±2 c	174±10 c	30.4±1.9 a	174±10 b	14.9±0.1 a
	5.0	164±3 b	1062±61 b	32.8±0.5 a	212±12 a	13.4±0.5 b
	10.0	246±20 a	2211±128 a	24.6±2.0 b	221±13 a	10.0±0.2 c
Sunflower	0	nd	nd	-	-	-
	1.0	89±6 c	1424±131 c	89.3±6.1 a	1424±131 a	5.9±0.9 b
	5.0	398±6 b	5987±333 b	79.6±1.1 b	1197±67 b	6.2±0.2 b
	10.0	637±21 a	7863±238 a	63.7±2.1 c	786±24 c	7.5±0.1 a

[a] Bio-concentration factor for plant shoot (BCF_{ST}) = Tl concentration in the shoot of the plant / Tl concentration in the nutrient solution.
[b] Bio-concentration factor for plant root (BCF_{RT}) = Tl concentration in the root of the plant / Tl concentration in the nutrient solution.
[c] Translocation index (TLI, %) = [(TI concentration in the shoot) / (Tl concentration in whole plant)] x 100.
[d] nd, not detected. The results are presented as means ± SD (n=3).
Values were presented as means ± standard deviation (n=3) followed by the same letter within a row in same plant are not significantly different by Duncan's multiple range test at $p<0.05$.

plants tended to be dependent on the differences of the root Tl concentrations between the plants. Some indirect studies with metal distribution in plant tissues showed that a large amount of heavy metal taken up by plant root is immobilized within the root tissues. A considerable amount of metal in plant roots is detected from apparent free space (AFS) that associated with pectin and protein fractions of the cell wall. For example, Cd is also immobilized in root tissue after uptake, mostly as adsorbed Cd in AFS, where cation exchange and complex binding at the cell wall takes place (Verkleij and Schat, 1990).

Correlation coefficients (r) of Tl uptake and translocation in barley and sunflower based on Tl treatment concentration are shown in Table 2. The Tl uptake concentrations in the shoots of both plants were positively correlated (at $p < 0.01$) with the uptake concentration in the roots. The Tl concentrations in the shoots and roots of barley were positively correlated (at $p < 0.05$–0.01) with the BCF_{RT} value, but were not significantly correlated with the BCF_{ST} value for the plant. In sunflower, the concentrations of Tl uptake by both shoots and roots were negatively correlated (at $p < 0.05$–0.01) with the BCF_{ST} and BCF_{RT} values for the plant.

In particular, the BCF_{ST} values for sunflower were positively correlated (at $p < 0.05$) with the BCF_{RT} values for the plant, however the BCF values for both the aerial and terrestrial parts of barley were not significantly correlated. On the other hand, the Tl concentrations in both parts of barley were negatively correlated (at $p < 0.01$) with the TLI values, whereas the TLI value for the sunflower tissues was only positively correlated (at $p < 0.1$) with the Tl concentration in the shoot of the plant. Based on the overall results, the correlations among Tl concentrations of the different plant parts, BCF, and TLI values for sunflower tended to be higher than those for barley.

The effect of DTPA application on Tl uptake and translocation in barley and sunflower is shown in Table 3. Tl concentration in the shoots and roots of barley increased with DTPA application, whereas the Tl concentration in both tissues of sunflower decreased with DTPA treatments. However, the Tl concentrations in the tissues of both plants were not significantly different between DTPA treatment with concentrations of 5.0 and 30.0 mg/L. Also, the estimated values of BCF_{ST} and BCF_{RT} in both plants based on DTPA application showed similar trends to the Tl uptake in the plants. In particular, the TLI values for sunflower increased with decreasing Tl concentration in the plant tissues due to DTPA application, while the values for barley did not significantly change with increasing Tl concentration in the plant.

Table 2. The values of correlation coefficient (r) relating thallium uptake and transport in barley and sunflower as influenced by the applications of different Tl concentration

Plant	Description[a]	Tl_{RT}	BCF_{ST}	BCF_{RT}	TLI
Barley	Tl_{ST}	0.977***	–0.532	0.929***	–0.924***
	Tl_{RT}	-	–0.688	0.866**	–0.981***
	BCF_{ST}		-	–0.252	0.796*
	BCF_{RT}			-	–0.784*
Sunflower	Tl_{ST}	0.985***	–0.942***	–0.946***	0.803*
	Tl_{RT}	-	–0.901**	–0.885**	0.714
	BCF_{ST}		-	0.906**	–0.726
	BCF_{RT}			-	–0.943***

[a] Tl_{ST}, Tl concentration in the plant shoot; Tl_{RT}, Tl concentration in the plant root; BCF_{ST}, bio -concentration factor for the plant shoot; BCF_{RT}, bio -concentration factor for the plant root; TLI, translocation index of thallium.

Correlation coefficient (r) was determined by Pearson correlation analysis to evaluate the relationship between variables. Result were evaluated with 90 %, 95 % and 99 % confidence intervals: *, significant at $p<0.1$; **, significant at $p<0.05$; ***, significant at $p<0.01$ level.

Table 3. Thallium uptake and translocation in barley and sunflower as affected by DTPA application

Plants	Treatment (mg/L)		Tl concentration (mg/kg)		BCF_{ST}[a]	BCF_{RT}[b]	TLI[c] (%)
	Tl	DTPA	Shoots	Roots			
Barley	5.0	0	164±3 b	1062±61 b	32.8±0.5 b	212±12 b	13.4±0.5 a
	5.0	5.0	194±23 a	1227±46 a	38.9±4.5 a	245±9 a	13.6±0.9 a
	5.0	30.0	179±8 a	1209±46 a	35.7±1.6 a	242±9 a	12.9±0.1 a
Sunflower	5.0	0	398±6 a	5987±333 a	79.6±1.1 a	1197±67 a	6.2±0.2 b
	5.0	5.0	344±26 b	3962±296 b	68.9±5.2 b	792±59 b	8.0±1.1 a
	5.0	30.0	319±32 b	3692±268 b	63.8±6.3 b	738±54 b	7.9±0.2 a

[a] Bio-concentration factor for plant shoot (BCF_{ST}) = Tl concentration in the shoot of the plant / Tl concentration in the nutrient solution.
[b] Bio-concentration factor for plant root (BCF_{RT}) = Tl concentration in the root of the plant / Tl concentration in the nutrient solution.
[c] Translocation index (TLI, %) = [(TI concentration in the shoot) / (Tl concentration in whole plant)] x 100.
Values were presented as means ± standard deviation (n=3) followed by the same letter within a row in same plant are not significantly different by Duncan's multiple range test at $p<0.05$.

These results indicated that Tl uptake and translocation in the two plants were differently affected by DTPA application. The DTPA treatment positively influenced Tl movement in the aerial and terrestrial parts of barley, however it negatively affected Tl uptake in both parts of sunflower tissues. Previous research reported that synthetic chelating agents, including DTPA, EDTA, EGTA, ethylenediamine di-o-hydroxyphenylacetic acid (EDDHA), ethylenediamine-N,N'-disuccinic acid (EDDS), and nitrilotriacetic acid (NTA), usually increase uptake of most heavy metals by plants (Zhao *et al*., 2010; Souza *et al*., 2013). In contrast, some chelates might not affect uptake and translocation of certain metals in specific plants. The application of DTPA decreased Cu accumulation in *Elsholtzia splendens* and reduced Cu-induced toxicity (Sherameti and Varma, 2011). Wallace (1980) reported that Ni uptake by *Phaseolus vulgaris* did not improve with DTPA treatment. Also, treatment with EDTA, DTPA, and EDDS did not increase the concentrations of Cu and Zn in the roots or enhance translocation of these metals in the above ground parts of sunflower (Yeh and Pan, 2012). In the presence of EDDS, the concentrations of the essential metals Cu and Zn were reduced in the shoots of sunflower, whereas uptake of non-essential Pb was enhanced (Tandy *et al*., 2006). Therefore, these observations indicated that Tl-DTPA complexes may not be taken up by sunflower or could have inhibitory effects on Tl uptake and translocation in the plant.

Thallium uptake and translocation in barley and sunflower as influenced by citric acid application is shown in Table 4. Tl concentrations in the shoot and roots of barley decreased with citric acid application in the nutrient solution, while those in both the shoots and roots of sunflower increased with citric acid treatment. However, the Tl concentrations in the aerial and terrestrial parts of the plants with 5.0 mg/L of citric acid treatment were not significantly different as comparing with those with 30.0 mg/L application. Also, the values of BCF_{ST} and BCF_{RT} for barley and sunflower showed similar trends to the change in Tl concentrations in the plants, and thus the values of TLI for the plants in the presence of citric acid did not change. Various results related to the uptake and translocation of different metals in plants grown under hydroponics have been reported. The application of citric acid increased the concentrations of lanthanum (La) in the shoots and roots of barley under hydroponic conditions (Han *et al*., 2005). The concentrations of Cu and Zn in sunflower were also significantly enhanced with the addition of citric acid (Yeh and Pan, 2012). The uptake and translocation of Cd in the tomato plant increased after citric acid pre-incubation in a hydroponic system (Senden *et al*., 1995). In contrast, Hassan *et al*. (2016) reported that Cd uptake by jute mallow and its accumulation in the shoots and roots of the plant were reduced with different concentrations of citric acid applied in hydroponic conditions. The Cd uptake decreased with all concentrations of citric acid treatment, which is a similar result as the Tl uptake by barley in this study.

Table 4. Thallium uptake and translocation in barley and sunflower as affected by the applications of citric acid

Plants	Treatment (mg/L)		Tl concentration (mg/kg)		BCF_{ST}[a]	BCF_{RT}[b]	TLI[c] (%)
	Tl	Citric acid	Shoots	Roots			
Barley	5.0	0	164±3 a	1062±61 a	32.8±0.5 a	212±12 a	13.4±0.5 a
	5.0	5.0	135±15 b	921±25 b	27.1±2.9 b	184±5 b	12.8±0.9 a
	5.0	30.0	142±15 b	946±24 b	28.3±3.0 b	189±5 b	13.0±1.5 a
Sunflower	5.0	0	398±6 b	5987±333 b	79.6±1.1 b	1197±67 b	6.2±0.2 a
	5.0	5.0	488±29 a	8267±736 a	97.6±5.8 a	1653±147 a	5.6±0.8 a
	5.0	30.0	502±15 a	9207±240 a	100.3±3.0 a	1841±48 a	5.2±0.3 a

[a] Bio-concentration factor for plant shoot (BCF_{ST}) = Tl concentration in the shoot of the plant / Tl concentration in the nutrient solution.
[b] Bio-concentration factor for plant root (BCF_{RT}) = Tl concentration in the root of the plant / Tl concentration in the nutrient solution.
[c] Translocation index (TLI, %) = [(TI concentration in the shoot) / (Tl concentration in whole plant)] x 100.
Values were presented as means ± standard deviation (n=3) followed by the same letter within a row in same plant are not significantly different by Duncan 's multiple range test at p<0.05.

CONCLUSIONS

Thallium uptake in barley and sunflower increased with increasing Tl concentration in the nutrient solution. However, the Tl concentration in the roots was higher than in the shoots of the plants. The values of BCF_{RT} for barley increased only with increasing the Tl concentration, while the values of both BCF_{ST} and BCF_{RT} for sunflower decreased. The TLI value for barley decreased with increasing Tl in the nutrient solution, whereas the value for sunflower increased. In particular, Tl concentrations in both the barley tissues were negatively correlated with the TLI values, while the TLI values for the sunflower tissues were only positively correlated (at $p < 0.1$) with the shoot Tl concentration of the plant.

The Tl in barley tissues, both above and below the ground, increased with DTPA application, but the Tl in sunflower tissues decreased. The TLI values for sunflower increased with decreasing Tl concentration in the plant tissues due to DTPA application, while the values for barley did not significantly change. Therefore, the DTPA application differently affected Tl uptake and translocation in the two plants. Tl-DTPA complexes may not be taken up by sunflower or could have inhibitory effects. In contrast to the effect of DTPA application, with the application of citric acid, Tl concentrations in barley tissues decreased with citric acid application, while those in sunflower tissues increased.

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