

Evaluation of Extractants for Estimation of Tl Availability to Barley and Sunflower Grown in Tl-Contaminated Soils

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ABSTRACT

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Thallium (Tl) is a highly toxic heavy metal that poses a threat to various organisms, including human beings. In plants, Tl is mostly present as the uncomplexed Tl⁺ species, and its uptake by plants follows a similar trend to that of other monovalent ions such as NH₄⁺, Cs⁺, K⁺, Rb⁺, and others. In this study, we investigated the relationships between the Tl concentrations in barley and sunflower and the extractable-Tl concentrations in acidic, neutral, and alkaline soils. We used ammonium and potassium based-inorganic salt extractants, including 1 M NH₄Cl, 1 M (NH₄)₂SO₄, 1 M KCl, and 1 M KNO₃. The Tl concentration in the sunflower tissues harvested from the acidic soil with a treatment of 40 mg Tl kg⁻¹ was 1,910 mg kg⁻¹, indicating that sunflower could be considered a Tl-hyperaccumulator plant. Among the extractants, the highest concentration of extractable soil Tl was obtained using 1 M NH₄Cl solution, followed by 1 M (NH₄)₂SO₄, 1 M KCl, and 1 M KNO₃. The Tl concentrations in both plants were well related ($R^2 > 0.910$) to the Tl concentrations extractable by ammonium and potassium-based inorganic salts and showed the closest relationship ($R^2 = 0.936 - 0.980$) with that extractable by 1 M (NH₄)₂SO₄. Therefore, the ammonium and potassium based-inorganic chemicals can be considered applicable soil Tl extractants in relation to Tl uptake by plants.

Keywords: Ammonium and potassium based-inorganic salt extractants, Barley, Sunflower, Thallium

The coefficient of determination (R^2) values between the Tl concentrations in the plants and the chemical extractable-soil Tl concentrations.

Plant	Extractant	Coefficient of determination (R^2)		
		JJ [†]	IS	DY
Barley	1 M NH ₄ Cl	0.914	0.917	0.961
	1 M (NH ₄) ₂ SO ₄	0.962	0.936	0.963
	1 M KCl	0.935	0.910	0.948
	1 M KNO ₃	0.923	0.918	0.948
Sunflower	1 M NH ₄ Cl	0.945	0.981	0.973
	1 M (NH ₄) ₂ SO ₄	0.980	0.958	0.980
	1 M KCl	0.965	0.981	0.977
	1 M KNO ₃	0.946	0.973	0.984

†JJ, acidic Jeonju soil; IS, neutral Iksan soil; DY, alkaline Danyang soil.



Introduction

Thallium (Tl) is widely distributed in the Earth's crust with very low concentrations ranging from 0.1 to 1.7 mg kg⁻¹ (Smith and Carson, 1977; Peter and Viraraghavan, 2005). In Korea, soil Tl is predominantly found near cement plants, abandoned mines, and smelter areas, with concentrations ranging between 0.18 and 12.91 mg kg⁻¹ (Lee et al., 2015). Solid particles released from cement plants and ore mines usually contain higher Tl concentration (Gorbauch et al., 1984; Emers, 1988; Nriagu and Pacyna, 1988). The carbonate, nitrate, and sulfate forms of Tl are water-soluble, with Tl-sulfate being historically utilized as a pesticide (Lustigman et al., 2000).

Clay minerals play a crucial role as sorbents for heavy metals, including Tl⁺, in soils and sediments. The sorption behavior of Tl⁺ cation is similar to that of alkali metal cations such as potassium (K⁺), rubidium (Rb⁺), and cesium (Cs⁺), because the ionic radius and hydration enthalpy of Tl⁺ are similar to the alkali metals (Tremel et al., 1997a, 1997b; Wick et al., 2018). Moreover, the adsorption of Tl⁺ at the edges of clay minerals is significantly influenced by competition with K⁺ and NH₄⁺ ions. Consequently, the concentrations of Tl⁺ in soil solution increase as the adsorbed concentrations of K⁺ and NH₄⁺ at the edge surfaces of clay minerals increase (Wick et al., 2020).

Considering the similar properties of Tl and alkali metal cations, we assumed that the availability of Tl to plants in Tl-contaminated soil could be estimated by extracting Tl using inorganic salts. The objective of this study was to investigate the relationships between the Tl concentrations in barley (*Hordeum vulgare* L.) and Sunflower (*Helianthus annuus* L.) and the Tl concentrations in artificially Tl-contaminated different soils extracted using various inorganic salt extractants. Here, barley and sunflower plants were chosen for this study due to their significance as crop. Barley is the fourth-largest grain crop grown globally in temperate climates (FAO, 2022) and is one of primary feed grain crops in Korea. Sunflower, on the other hand, is one of the important oilseed crops cultivated worldwide (Khan et al., 2015; FAO, 2022) and is known for its capability to hyperaccumulate heavy metals (Ullah et al., 2011).

Materials and Methods

Soil and plant Surface 15 cm of three different Tl-free soils, acidic, neutral, and alkaline soils, were collected from Jeonju (JJ), Iksan (IS), Danyang (DY), Korea, respectively. The collected soil samples were air-dried, crushed and sieved using a 2-mm sieve. Selected chemical and physical properties of the soils are presented in Table 1 (Kim et al., 2016). Soil particle size distribution was evaluated by the wet-sieving and pipette method modified by Malo and Doolittle (2000). Soil pH was measured in a ratio of soil to distilled water (1:5 (w/v)) using a pH/EC meter (Seven Compact S220, Mettler Toledo). Soil organic matter (SOM) content was measured by the Tyurin titrimetric method, and exchangeable cations, Ca²⁺, Mg²⁺, K⁺, and Na⁺, were determined by 1 M ammonium acetate (CH₃COONH₄, pH 7.0) extraction method (NAAS, 2010). Total concentration of Tl in the soils was analyzed by the pseudo-total aqua regia (3 HCl:1 HNO₃) digestion procedure (ISO 11466, 1995). Barley (*Hordeum vulgare* L.) and sunflower (*Helianthus annuus* L.) seeds were brought from the Seed Center of Jeonbuk Agricultural Research and Extension Services, Korea.

Table 1. Selected physical and chemical properties of different soils studied.

Soil [†]	Particle size distribution (g kg ⁻¹)			pH (1:5)	Exchangeable cations (mg kg ⁻¹)				Total Tl (mg kg ⁻¹)	SOM [‡] (g kg ⁻¹)
	Sand	Silt	Clay		Ca	Mg	K	Na		
JJ	620	142	238	4.8	492	112	147	43	nd [§]	13
IS	534	200	266	7.2	1,434	254	305	47	nd	16
DY	377	342	281	8.7	4,145	339	322	49	nd	15

[†]JJ, acidic Jeonju soil; IS, neutral Iksan soil; DY, alkaline Danyang soil.

[‡]SOM, soil organic matter.

[§]nd, not detected.

Preparation of Tl-contaminated soil and growing procedure for plant Two sets of triplicate Tl-contaminated soil samples were prepared. Each 300 g (air-dry basis) soil sample was placed into polyethylene zipper bag. The soil sample was applied with 0, 5, 10, 20 and 40 mg Tl kg⁻¹ using TlCl solution. The Tl-contaminated soil in zipper bag was thoroughly mixed and incubated at 24 ± 2°C for 30 days at 70% of field capacity. After incubating, the Tl-contaminated soil sample was transferred into a 500 mL polypropylene wide-mouth bottle pot in preparation for planting. Another set of the soil sample was used for soil Tl analysis.

Barley and sunflower seeds were soaked in deionized water for approximately 5 h, spread between two damp filter papers, and left overnight in a dark condition to germinate. Sprouted seeds were planted in pots with an uncontaminated seedbed material, provided with deionized water, and allowed to grow to 10 - 15 cm in height. The young plants were transplanted into Tl-contaminated soil-culture pots. The plants were grown for 7 days in environmental growth chambers (Lee et al., 2002; Kim et al., 2016) because the 7-day growth period was suitable for them under the higher concentrations of soil Tl conditions that resulted from pre-experiment.

Determination of Tl in soil and plant Ammonium and potassium based-inorganic salt extractants, ammonium chloride (NH₄Cl), ammonium sulfate ((NH₄)₂SO₄), potassium chloride (KCl), and potassium nitrate (KNO₃), were used for proper assessment and determination of bioavailable Tl in the different soils. The single chemical extractants, namely 1 M NH₄Cl, 1 M (NH₄)₂SO₄, 1 M KCl, and 1 M KNO₃, were chosen because they were widely used for determining extractable and/or exchangeable metal fraction (Emmerich et al., 1982; Gianello and Amorim, 2015). Additionally, the ionic radii of NH₄⁺ (1.43 Å) and K⁺ (1.33 Å) are similar to that of Tl⁺ (1.49 Å) (Lide, 1989; Tremel et al. 1997a, 1997b), which suggests that competitive adsorption may occur between them in the soil-water system.

On the other hand, after harvesting the plants from the Tl-contaminated soil pots, the plants were washed and dried for 24 h at 80°C in a drying-oven after separated into shoot and root parts. The oven-dried plant tissues were digested in concentrated HNO₃ and 30% H₂O₂ (Jones Jr., 1991) to determine Tl concentration. The concentrations of Tl in the plants and extractable soil Tl were measured using an inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 7300DV, Perkin Elmer, USA).

Statistical analyses were operated using Statistical Package for the Social Sciences (SPSS) ver. 18.0 (SPSS Inc., Chicago, IL, USA). Significance of parameters was calculated by one-way analyses of variance on ranks followed by Duncan's Multiple Range Test.

Results and Discussion

The Tl concentrations in the shoots and roots of barley and sunflower increased with increasing Tl concentrations treated in the different soils. The Tl concentrations in both plants grown in the acidic JJ soil pot were higher than those in the neutral IS and alkaline DY soil pots. The root Tl concentrations of both plants were significantly higher than the shoot Tl concentrations of the plants grown in all the different soil pots. However, the shoot Tl concentrations of the barley were markedly higher than those of sunflower at all doses of Tl treatments in the three different soils. On the other hand, the root Tl concentrations of barley were significantly lower than those of sunflower grown in the soils with different Tl application levels. Nevertheless, the total concentrations of Tl uptake in the plants were much higher in sunflower than in barley (Table 2). In particular, the total Tl concentration in the sunflower tissues was 1,910 mg kg⁻¹, which was significantly higher than the concentration of 1,079 mg kg⁻¹ in the barley tissues harvested from the acidic JJ soil with a Tl application of 40 mg Tl kg⁻¹. Sunflower has high tolerance to various heavy metals and exhibits hyperaccumulation ability among metals (Saxena et al., 1990; Kamnev and van der Lelie, 2000; van der Lelie et al., 2001). Based on these results, sunflowers might be regarded as Tl-hyperaccumulator plant species.

Table 2. Thallium concentrations of selected plants grown in the artificially Tl contaminated soils.

Plant	Soil [†]	Tl concentration (mg kg ⁻¹)			
		5 [‡]	10	20	40
Barley	JJ	161.3 a [§]	309.3 a	704.5 a	1,078.6 a
	IS	44.8 c	64.5 c	190.0 b	244.5 c
	DY	54.1 b	94.3 b	164.6 c	278.1 b
Sunflower	JJ	288.4 a	409.9 a	1,137.2 a	1,909.8 a
	IS	54.3 c	86.4 c	210.0 c	544.4 c
	DY	72.6 b	98.0 b	266.9 b	681.6 b

[†]JJ, acidic Jeonju soil; IS, neutral Iksan soil; DY, alkaline Danyang soil.

[‡]Tl concentrations treated in the soils studied.

[§]Values (n = 8) were followed by the same letter within a column in same plant are not significantly different at $p < 0.05$ by Duncan's multiple range test.

The extractable soil Tl concentrations in the acidic JJ, neutral IS, and alkaline DY soils using ammonium and potassium based-inorganic salt extractants are presented in Table 3. The extractable soil Tl obtained by 1 M NH₄Cl solution was the highest concentration, while the soil Tl concentrations extracted by potassium based-salt chemicals were relatively lower in all the soils. Furthermore, the extractable soil Tl concentrations obtained using 1 M

Table 3. Thallium concentrations in the artificially contaminated acidic, neutral, and alkaline soils extracted by ammonium and potassium based-inorganic salt extractants.

Soil [†]	Extractant	Extractable Tl concentration (mg kg ⁻¹)			
		5 [‡]	10	20	40
JJ	1 M NH ₄ Cl	3.17 ± 0.02 a [§]	6.47 ± 0.31 a	12.15 ± 0.30 a	31.74 ± 0.11 a
	1 M (NH ₄) ₂ SO ₄	1.80 ± 0.02 b	4.65 ± 0.15 c	10.33 ± 0.54 b	21.37 ± 0.58 d
	1 M KCl	1.64 ± 0.07 c	3.64 ± 0.11 d	9.97 ± 0.24 b	22.53 ± 0.63 c
	1 M KNO ₃	0.88 ± 0.05 d	5.28 ± 0.16 b	10.00 ± 0.26 b	24.32 ± 0.78 b
IS	1 M NH ₄ Cl	1.92 ± 0.04 a	3.72 ± 0.89 a	8.53 ± 0.15 a	17.33 ± 1.22 a
	1 M (NH ₄) ₂ SO ₄	1.04 ± 0.08 c	2.83 ± 0.11 b	5.91 ± 0.29 d	11.06 ± 0.44 c
	1 M KCl	1.20 ± 0.09 b	3.12 ± 0.21 ab	6.71 ± 0.24 c	13.83 ± 0.19 b
	1 M KNO ₃	0.73 ± 0.04 d	2.98 ± 0.10 ab	7.12 ± 0.10 b	13.93 ± 0.65 b
DY	1 M NH ₄ Cl	1.90 ± 0.10 a	3.44 ± 0.12 a	9.33 ± 0.45 a	17.69 ± 0.35 a
	1 M (NH ₄) ₂ SO ₄	1.22 ± 0.06 b	2.40 ± 0.05 b	5.45 ± 0.24 c	11.12 ± 1.35 c
	1 M KCl	0.76 ± 0.04 d	2.53 ± 0.31 b	6.37 ± 0.34 b	12.68 ± 0.38 b
	1 M KNO ₃	0.91 ± 0.04 c	2.63 ± 0.10 b	6.08 ± 0.10 b	12.83 ± 0.75 b

[†]JJ, acidic Jeonju soil; IS, neutral Iksan soil; DY, alkaline Danyang soil.

[‡]Tl concentrations treated in the soils studied.

[§]Values were presented as means ± standard deviation (n = 3) followed by the same letter within a column in same soil are not significantly different at $p < 0.05$ by Duncan's multiple range test.

(NH₄)₂SO₄, 1 M KCl, and 1 M KNO₃ solutions were somewhat different depending on the soil types with Tl-contamination levels, but they tended not to be significantly different. The coefficient of determination (R²) values between the Tl concentrations in the plants and the chemical extractable-soil Tl concentrations were shown in Table 4. The R² values give us the percentage variation in y-axis (extractable soil Tl concentrations) explained by x-axis (plant uptake Tl concentrations). The R² values ranged from 0.914 to 0.980, from 0.910 to 0.981, and from 0.948 to 0.984 depending on the different soils, acid JJ, neutral IS, and alkaline DY soils, respectively. In particular, the Tl uptake concentrations in both plants were more closely related to 1 M (NH₄)₂SO₄-extractable soil Tl compared to other extractants, except in the neutral IS soil. However, it is worth noting that all the R² values were higher than 0.9, indicating that the concentrations of extractable soil Tl are well explained by the concentrations of Tl uptake in the plants, which might be attributed to the similarity in the ionic radii of K⁺ and NH₄⁺ ions to that of Tl⁺ ion. These results suggest that all the tested extractants would be applicable, and thus the ammonium and potassium based-inorganic chemicals might be appropriate soil Tl extractants in relation to Tl uptake by plants. For supplementary study, the Tl uptake concentrations in both plants were additionally examined along with the 0.005 M diethylene triamine penta-acetic acid (DTPA)-extractable soil Tl because 0.005 M DTPA extractant has been widely used for assessing the bioavailable fractions of various heavy metals such as cadmium (Cd), copper (Cu), Lead (Pb), nickel (Ni), zinc (Zn), etc. (Alloway, 1995; Oh et al., 2012; Gao et al., 2022). The concentrations of soil Tl extracted using a 0.005 M DTPA solution were exceptionally lower (0.06 - 0.83 mg kg⁻¹) compared to those (0.73 - 31.74 mg kg⁻¹) obtained by other inorganic salt extractants, depending on Tl contamination levels, even though the R² values ranged

between 0.854 - 0.995. These results indicate that the 0.005 M DTPA extractant might not be useful for assessing the soil Tl concentration associated with Tl bioavailability.

Table 4. The coefficient of determination (R^2) values between the Tl concentrations in the plants and the chemical extractable-soil Tl concentrations.

Plant	Extractant	Coefficient of determination (R^2)		
		JJ [†]	IS	DY
Barley	1 M NH ₄ Cl	0.914	0.917	0.961
	1 M (NH ₄) ₂ SO ₄	0.962	0.936	0.963
	1 M KCl	0.935	0.910	0.948
	1 M KNO ₃	0.923	0.918	0.948
Sunflower	1 M NH ₄ Cl	0.945	0.981	0.973
	1 M (NH ₄) ₂ SO ₄	0.980	0.958	0.980
	1 M KCl	0.965	0.981	0.977
	1 M KNO ₃	0.946	0.973	0.984

[†]JJ, acidic Jeonju soil; IS, neutral Iksan soil; DY, alkaline Danyang soil.

Conclusions

The concentrations of soil Tl were highest in the acidic JJ soil, and the 1 M NH₄Cl-extractable soil Tl was relatively higher compared to the Tl concentrations obtained using other inorganic salt extractants. In addition, the uptake of Tl by the plants was also higher in the acidic soil compared to the neutral IS and alkaline DY soils. Based on the R^2 values (0.910 - 0.984) in relation to the Tl uptake by the plants and the single chemical extractable soil Tl, the concentration of Tl uptake in the barley was highly related to 1 M (NH₄)₂SO₄-extractable soil Tl as comparing with other extractants. However, the Tl uptake by the sunflower was well related to most of the inorganic salt-extractable soil Tl studied. Thus, the ammonium and potassium based-inorganic chemicals might be suitable soil Tl extractants in relation to Tl uptake by plants. On the other hand, further researches are necessary for safe agricultural production because parts of agricultural fields are contaminated with Tl contaminants.

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