Uptake of thallium from artificially contaminated soils by kale (*Brassica oleracea* L. var. *acephala*)

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ABSTRACT

A pot experiment focused on the study of factors influencing thallium transfer from contaminated soils into kale (green cabbage, *Brassica oleracea* L. var. *acephala*, variety Winterbor F1) was evaluated. Three different types of topsoils with naturally low content of thallium (heavy, medium and medium-light soil) were used for pot experiments. The soils were contaminated with thallium sulfate to achieve five levels of contamination (0, 0.52, 2.10, 4.20 and 5.88 mg/kg). There were six replicates for each combination (90 pots in the experiment). The first part of the experiment started in the year of contamination (2001) and continued in 2003. The soil samples and the samples of kale (leaves and stalks were sampled separately) were collected and analysed. Kale was found to be able to accumulate Tl without any influence on yield. The highest thallium concentration was found in the leaves of kale in the first year of the experiment and reached 326 mg/kg dry matter. Bioaccumulation factor (Biological Absorption Coefficient – BAC) was found to be over 80 during the first year of the experiment. In the third year the BAC was around 3 for the soil with the highest pH and the highest organic matter content but as high as 15 for an acid soil with the lowest content of organic matter and the lowest Cation Exchange Capacity (CEC) of soils. The content of thallium in the leaves of kale was found to be 7 to 10 times higher than in the stalks in the third year. In the first year this ratio was up to 18. From these findings it can be concluded that the ability of some plants of Brassicacea family, that are planted as common vegetables, to accumulate thallium is very high and can be a serious danger for food chains. Neutral soils high in CEC and organic matter are able to bind thallium more effectively than poor acid soils and the transfer of Tl into plants from these soils is substantially lower. The uptake of Tl from contaminated soils into kale can be very high and without any negative effect on the plant growth. The transfer of Tl into kale decreases with the time necessary to reach the equilibrium between the added Tl and the soil (ageing of a sample).

Keywords: thallium uptake; soil contamination; kale

Tl is toxic to all organisms in both mono- and trivalent forms. Its toxicity can be compared to cadmium and mercury. Ionic radius of Tl^{+} is similar to that of K^{+} thus Tl^{+} can mimic potassium in metabolic processes. Tl has also a strong attraction to sulfhydryl groups, thereby inhibiting and inactivating enzymes. Despite this fact Tl is only little studied element (Merian 1991, Tremel et al. 1997a). A demand for determination of thallium is expected to rise because of the high toxicity and bioavailability of this element.

Tl in soils is usually of the pedogeochemical origin (Sager 1986, Jones et al. 1990, Tremel and Mench 1998, Zbíral et al. 2000, 2002, Pavlíčková et al. 2003, 2005, 2006). There are only very few reports on the anthropogenic soil contamination. Ore smelting, cement production and fossil fuels are the main anthropogenic sources of this element (Jones et al. 1990, Kemper and Bertram 1991, Lin et al. 1999a, b, Lustigman et al. 2000, Wierzbicka et al. 2004). Thallium was found to be relatively mobile in soils. The physicochemical form, form

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of binding and the total content of Tl in soil seem to be the main factors influencing its uptake by plants (Pavlíčková et al. 2005).

The phytoavailability of Tl depends on plant species and plant parts and the status and content of Tl in soil. Some brassicaceous plants commonly grown as vegetables behave as hyperaccumulators of Tl. The uptake of thallium can vary not only among plant species but also among cultivars within a given plant species (Kemper and Bertram 1991, Tremel et al. 1997b, Martin and Kaplan 1998).

The content of Tl in edible parts of plants can differ substantially (nearly 20 times in maximum). The highest concentrations were observed mainly in the green parts of plants. Similarly, concentrations of Tl in rapeseeds were observed to be up to half of its total content in a particular non-contaminated soil. But in many cases the concentration in plants can substantially exceed the concentration in soils (Pavlíčková et al. 2003, 2005, 2006).

Food and feed are the most important source of Tl for humans. The normal human daily intake is estimated to be about 2 μg in non-polluted areas (Anonymous 1997, 1998). The uptake of Tl from soils with naturally high content of Tl can be high enough to seriously endanger food chains. Vegetables with the good availability of Tl grown on soils with naturally and/or pedogeochemically increased contents of Tl should be monitored (cabbage, turnip and rape) and those with high Tl accumulation should be excluded from growing for human or animal nutrition.

MATERIAL AND METHODS

Instruments. Plant samples were finely ground using a high-speed knife mill Grindomix GM 200 (Retsch, Haan, Germany) and digested by nitric acid in the closed high-pressure microwave system Ethos SEL (Milestone, Sorisole, Italy). Dry matter (DM) was determined using a MA 30 moisture analyser (Sartorius, Goetingen. Germany). A sixteen-position double heating block with digestion tubes and coolers MB 422 BH (Uni Elektro, Hradec Králové, Czech Republic) was applied for soil digestion. A sub-boiling distillation unit BSB-939-IR (Berghof, Eningen, Germany) was used for purification of nitric acid of p.a. purity. Inductively coupled plasma mass spectrometer ICP-MS ELAN 6000 with a cross flow nebulizer, Scott's type spray chamber and Gilson 212 peristaltic pump was used for determination of Tl. The MS part was regularly controlled by a calibrating solution (all Perkin-Elmer SCIEX, Norwalk, CT, USA). The operating parameters were identical with those given in the previous papers (Pavlíčková et al. 2003, 2005, 2006).

Reagents. All reagents and standard solutions were prepared using Milli Q deionized water (Millipore, Bedford, MA, USA). All chemicals of reagent grade purity were from Analytika (Prague, Czech Republic), Merck (Darmstadt, Germany) or Pliva-Lachema (Brno, Czech Republic). Stock standard solutions 1000 ± 2 mg/l Tl in 2% (v/v) nitric acid and 1000 ± 2 mg/l Lu in 2% (v/v) nitric acid (National Institute of Metrology, Prague, Czech Republic) were used for preparation of calibrating standard solutions. Thallium sulfate solutions (0.12, 0.50, 1.01, 1.41 g/l Tl) were used for an artificial contamination of soils in pot experiments.

Pot experiments. Three different soils were collected. Basic parameters of the soils are summarized in Table 1. The soils were air dried, sieved and all particles < 2 cm were homogenized and used for the pot experiments. All soils were fortified with the thallium sulfate solutions [50 ml of 0.12, 0.50, 1.01, 1.41 g/l Tl(I) per 12 kg of soil] to

Table 1. Basic characteristics of topsoils used for pot experiments

Parameter	Unit	Hrubčice	Míchov	Račín
Tl*	mg/kg	0.5	0.3	0.4
pH/$CaCl_2$		7.0	6.3	5.0
P**	mg/kg	138	178	58
K**	mg/kg	301	459	253
Mg**	mg/kg	463	233	49
Ca**	mg/kg	7090	3230	723
Fraction < 10 μm	%	63.7	37.9	28.1
Fraction < 1 μm	%	38.4	17.2	9.6
Fraction 1–10 μm	%	25.3	20.7	18.5
Fraction 10–50 μm	%	28.5	40.1	26.5
Fraction 50–250 μm	%	6.7	19.2	19.7
Fraction 0.25–2 mm	%	1.1	2.8	25.7
N(t)	%	0.22	0.14	0.09
C_{ox}	%	2.35	1.32	1.07
CEC	mM	430	270	150

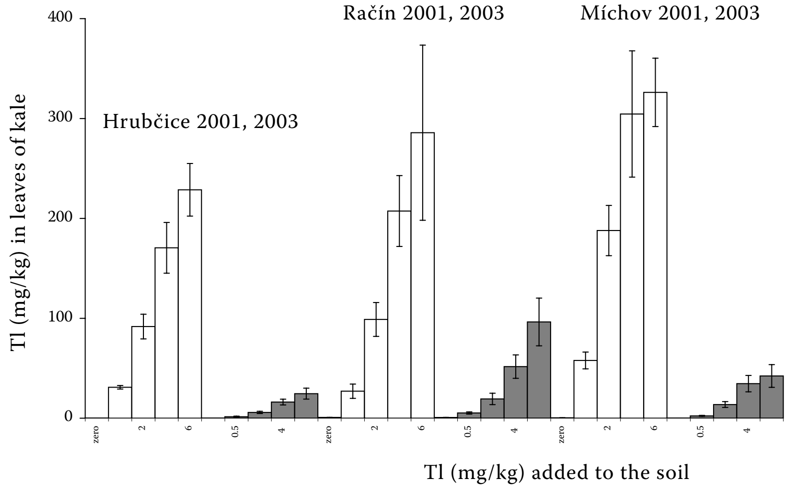
*content in H_2O_2 – HNO_3 extracts, **content in Mehlich 3 extracts achieve five levels of added thallium content (0, 0.52, 2.10, 4.20 and 5.88 mg/kg). Kale (*Brassica oleracea* L. var. *acephala*, cultivar Winterbor F1) was planted in the 15 l pots during the first and the third year after the fortification (rape *Brassica napus* L. var. *napus*, cultivar Golda was planted in the second year – Pavlíčková et al. 2003, 2005, 2006). The content of the pot was carefully cleaned and homogenized after the harvest and reused for the next experiments. Six replicates were used for each soil and each level of contamination (90 pots in the experiment). Natural content of Tl in the soil was 0.5 mg/kg (Hrubčice), 0.3 mg/kg (Míchov) and 0.4 mg/kg (Račín). The soils were fertilized with 83 mg/kg N (NH_4NO_3), 25 mg/kg P ($CaHPO_4.2\ H_2O$) and 56 mg/kg K (KCl) to provide a sufficient nutrient supply. Exactly 8 seeds of kale (*Brassica oleracea*, var. *acephala*, cultivar Winterbor F1) were sowed in each pot in April. Normally developed plants were singled out in May. Thus only two best-developed plants remained in a single pot. The pots were protected against rain during the whole period. Soil moisture was adjusted to 60% of the maximum water capacity by daily watering with deionized water. Fully mature plants were harvested in August, leaves and stalks were collected separately.

Sample preparation and digestion. Plant samples were cleaned immediately after the harvest by a quick washing with deionized water to remove dust particles, dried at laboratory temperature and finely ground. The samples (1 g) were digested by nitric acid (8 ml HNO_3 and 10 ml H_2O) in microwave digestion system at 145°C and 700 W for 5 min, 180°C and 600 W for another 5 min and finally at 180°C and 1000 W for the next 5 min (Medek et al. 2001). The digests were adjusted to the final volume of 50 ml with deionized water. The digests were further diluted 1–100 times by deionized water before the ICP/MS measurement of thallium content. Each series consisted of a suitable amount of samples given by the procedure, one internal reference standard (lutetium at a concentration 0.4 mg/l) and two blanks.

Determination of thallium by ICP/MS. Single element calibrating standard solutions were used

Table 2. Leaves and stalks of kale from the years 2001 and 2003; yield (g DM), content of thallium (mg/kg), total uptake of thallium (µg) from one pot, and translocation factor (TLF = leaves/stalk thallium concentration quotient); n = 6

Soil	Parameter	Tl added (in mg/kg)									
		0	0.5	2	4	6	0	0.5	2	4	6
		leaves 2001					leaves 2003				
Hrubčice	yield (g DM)	33.7(19)	36.7(10)	33.4(18)	27.7(30)	28.7(8)	35.6(12)	30.7(27)	29.5(21)	34.8(11)	29.0(18)
	uptake (µg)	5.23(31)	1131(6)	3093(29)	4680(28)	6584(18)	3.16(27)	45.7(42)	173(30)	558(15)	716(32)
	TLF	9.8(22)	14.1(28)	14.9(16)	15.0(15)	15.8(10)	6.8(29)	7.2(39)	6.8(19)	8.8(16)	9.7(19)
Míchov	yield (g DM)	27.2(7)	27.3(16)	25.1(13)	22.4(27)	27.8(16)	44.2(5)	39.1(3)	33.9(12)	28.9(26)	37.8(10)
	uptake (µg)	10.3(8)	1567(18)	4714(17)	6830(30)	8963(10)	6.57(14)	90.8(19)	463(23)	958(17)	1588(26)
	TLF	8.6(26)	18.4(31)	16.6(16)	14.3(23)	13.9(17)	7.7(26)	7.7(13)	7.3(14)	7.7(25)	7.3(26)
Račín	yield (g DM)	18.7(33)	17.5(28)	13.8(16)	19.0(18)	15.2(20)	16.7(25)	15.1(26)	18.3(17)	18.2(21)	15.3(20)
	uptake (µg)	14.7(34)	450(23)	1385(28)	4012(30)	4543(47)	11.4(34)	77.7(24)	349(27)	911(19)	1458(29)
	TLF	8.4(18)	10.8(43)	7.5(27)	8.3(18)	7.2(27)	7.6(22)	11.4(13)	9.5(20)	6.9(32)	9. 1(40)
		stalk 2001					stalk 2003				
Hrubčice	yield (g DM)	15.0(4)	13.1(8)	15.9(9)	14.7(6)	14.6(14)	4.1(35)	4.5(17)	5.4(26)	4.6(30)	5.1(9)
	uptake (µg)	0.247(32)	30.3(24)	98.8(15)	168(11)	210(15)	0.05(22)	1.04(39)	4.90(23)	9.94(26)	13.7(43)
Míchov	yield (g DM)	11.4(6)	11.6(11)	10.6(10)	9.4(16)	9.5(9)	5.0(11)	4.8(20)	5.0(22)	4.3(28)	4.1(29)
	uptake (µg)	0.539(29)	37.9(19)	121(12)	201(9)	226(10)	0.100(25)	1.44(28)	9.49(31)	19.0(26)	24.3(35)
Račín	yield (g DM)	7.2(7)	7.7(8)	7.3(17)	6.6(14)	6.9(9)	1.4(44)	1.6(46)	1.5(26)	1.8(21)	1.2(50)
	uptake (µg)	0.684(19)	21.4(34)	98(23)	173(32)	269(6)	0.118(26)	0.722(29)	2.99(26)	13.4(22)	14.8(71)



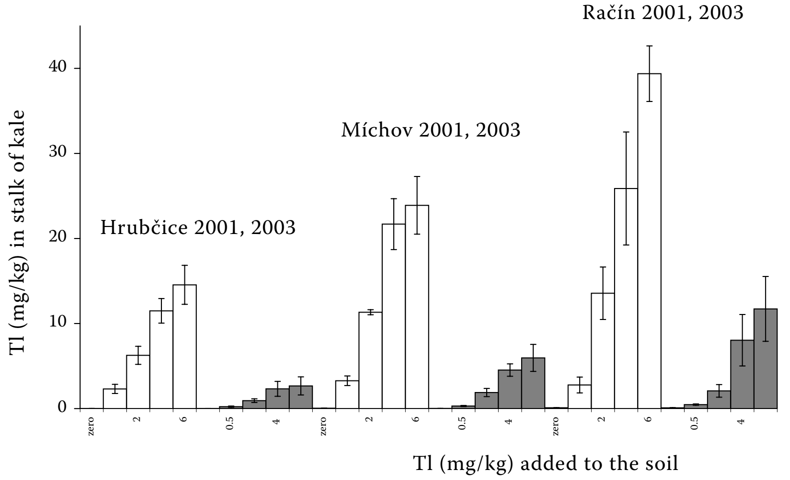


Figure 1. Relationship between the content of thallium (mg/kg) added to the soils from Hrubčice, Míchov, Račín and its content (mg/kg) in leaves and stalk of kale in the years 2001 and 2003

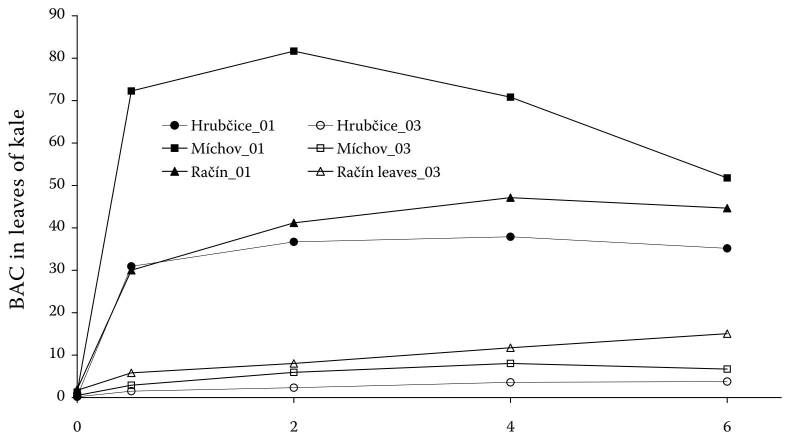
for calibration of the ICP/MS instrument at five different concentrations of Tl (0, 1, 5, 10 and 50 μg/l). Thallium content was determined from ^{205}Tl signal. Lutetium at the concentration 10 μg/l was used as an internal standard (^{175}Lu signals). The extraction agents, acids and lutetium concentrations in the standard calibrating solutions matched their concentrations in the sample solutions. The calibration curve was linear in the whole calibrating range ($r \geq 0.9999$). STATISTICA v. 6 (Statsoft, Tulsa, OK, USA) and EXCEL 97 (Microsoft, Redmond, WA, USA) software were used for statistical evaluation of the data.

RESULTS AND DISCUSSION

Addition of thallium to the soils did not significantly influence the yield of kale in both years of the pot experiment (Table 2). From the comparison of the year 2001 and 2003 the yield in 2003 was similar to the year 2001 for leaves of kale but the yield of stalk was substantially lower due to the consecutive planting of Brassicacea plants in the same soil.

The highest content of thallium in 2001 harvest was found for leaves of kale planted on the Míchov soil followed by the Račín soil and the lowest content was found on the Hrubčice soil. For the highest addition the content of Tl in leaves was more than 200 mg/kg. In 2003 the content of Tl in leaves was substantially lower. The highest content was found in the Račín soil (96 mg/kg in maximum) and the lowest in the Hrubčice soil (25 mg/kg in maximum).

It can be concluded that Tl is more plant-available from acid soils with low content of organic matter than from neutral soils with higher content



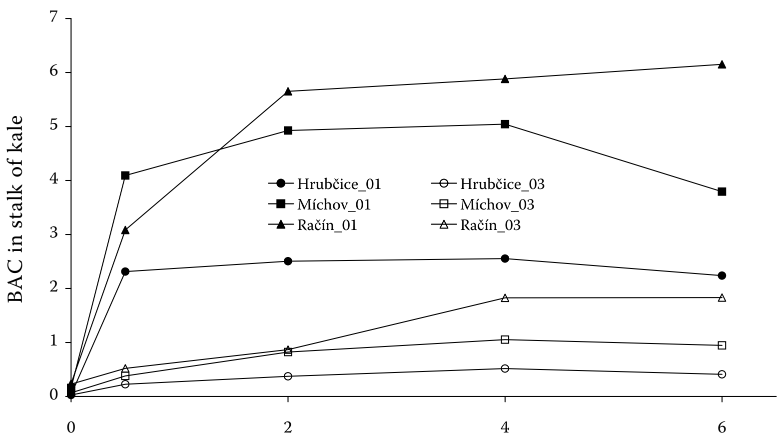


Figure 2. Relationship between the content of thallium (mg/kg) added to the soils from Hrubčice, Míchov and Račín and its BAC in leaves and stalk of kale in the years 2001 and 2003

of organic matter. The uptake of Tl can be different before reaching equilibrium between the soil and the added Tl (higher content of Tl in leaves planted on Míchov soil during the first year of the experiment). The results are given in Table 2 and in Figure 1.

Bioaccumulation factors for leaves (Biological Absorption Coefficient, the ratio of the plant Tl/soil Tl) in the first year were from 30 to 80. The highest value was observed for the Míchov soil in the case of 2 mg/kg added Tl. For stalk the values were between 2 and 6. In 2003 the BAC were substantially lower with the highest values for the Račín soil (6–15 according to the Tl addition) and the lowest for the Hrubčice soil (1.5 to 3.8). The results are summarized in Table 2 and Figure 2.

TLF – the translocation factor (ratio of Tl in leaves/Tl in stalk) was found to be from 8 to 9 for no Tl added to the soils. In the Račín soil this value remained practically constant for all Tl additions in both years. For the Hrubčice soil the value was up to 16 in 2001 but changed back to 8–9 in 2003. For the Míchov soil TLF was up to 18 in 2001 and changed back to 7–8 in 2003. From this observation we can conclude that the TLF between 7–9 can be taken as a constant value for kale if planted on the soil with Tl in equilibrium.

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