www.czasopisma.pan.pl www.journals.pan.pl
ACTA BIOLOGICA CRACOVIENSIA Series Botanica 58/2: 7–19, 2016
DOI: 10.1515/abcsb-2016-0012

THALLIUM HYPERACCUMULATION IN POLISH POPULATIONS OF *BISCUTELLA LAEVIGATA* (BRASSICACEAE)

MAŁGORZATA WIERZBICKA[1], MARIA PIELICHOWSKA[2], AGNIESZKA ABRATOWSKA[1], BOGUSŁAW WIŁKOMIRSKI[4], IRENA WYSOCKA[5], DOROTA PANUFNIK-MĘDRZYCKA[1*] AND EWA BULSKA[3]

[1]*Department of Ecotoxicology, Institute of Botany, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland,*

[2]*The Maria Grzegorzewska Academy of Special Education, Szczęśliwicka 40, 02-353 Warsaw, Poland,*

[3]*Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland,*

[4]*Department of Environmental Protection and Modeling, Faculty of Mathematics and Natural Science, The Jan Kochanowski University in Kielce, Świętokrzyska 15, 25-406 Kielce, Poland,*

[5]*Central Chemical Laboratory, The Polish Geological Institute, Rakowiecka 4, 00-975 Warsaw, Poland*

Received February 25, 2016; revision accepted July 4, 2016

Biscutella laevigata L. is known as a Tl hyperaccumulator. In Poland *Biscutella laevigata* occurs in the Tatra Mts (Western Carpathians) and on the calamine waste heap in Bolesław near Olkusz (Silesian Upland). The purpose of this work was to evaluate whether plants of both populations were able to accumulate an elevated amount of thallium in their tissues. The plants were cultivated in calamine soil in a glasshouse for a season and studied at different ages – from 2-week-old seedlings to 10-month-old adults. Additionally, the plants were grown for ten weeks in calamine soil with EDTA to enhance Tl bioavailability. The total content of Tl in plant tissues after digestion was determined by ICP-MS, whereas its distribution in leaves was studied by LA-ICP-MS. Of the total content of Tl in the soil in the range of (15.2–66.7) $mg \cdot kg^{-1}$d.m., only (1.1–2.1) $mg \cdot kg^{-1}$d.m. was present in a bioavailable form. The mean content in all the plants grown on the soil without EDTA was 98.5 $mg \cdot kg^{-1}$d.m. The largest content was found in leaves – 164.9 $mg \cdot kg^{-1}$d.m. (max. 588.2 $mg \cdot kg^{-1}$d.m.). In the case of plants grown on the soil enriched with EDTA, the mean content in plants increased to 108.9 $mg \cdot kg^{-1}$d.m., max. in leaves – 138.4 $mg \cdot kg^{-1}$d.m. (max. 1100 $mg \cdot kg^{-1}$d.m.). The translocation factor was 6.1 in the soil and 2.2 in the soil with EDTA; the bioconcentration factor amounted to 10.9 and 5.8, respectively. The plants from both populations did not contain a Tl amount clearly indicating hyperaccumulation (100–500 $mg \cdot kg^{-1}$d.m.), however, high (>1) translocation and bioconcentration factors suggest such an ability. It is a characteristic species-wide trait; *B. laevigata* L. is a facultative Tl hyperaccumulator. The largest Tl amount was located at the leaf base, the smallest at its top. Thallium also occurred in trichomes, which was presented for the first time; in this way plants detoxify Tl in the above-ground parts. Leaves were much more hairy in the Bolesław plants. This is an adaptation for growth in the extreme conditions of the zinc-lead waste heap with elevated Tl quantity.

Keywords: *Biscutella laevigata* L., hyperaccumulator, LA-ICP-MS, metal translocation, thallium, trichomes

* Corresponding author, email: dmedrzycka@uw.edu.pl

PL ISSN 0001-5296

INTRODUCTION

Thallium is known as a highly toxic metal. The biggest anthropogenic sources of thallium are carbon incineration, heavy metal ores containing thallium, non-ferrous metal smelting and refineries (Sager, 1994). Thallium is extremely toxic to people, animals and plants. It is similar to potassium and therefore disturbs the basic metabolism of cells (Scheckel et al., 2007).

Contamination of the environment with thallium in Poland has not been investigated for quite a long time. Over the last 15 years, only a few examinations have been performed (Dmowski et al., 1998; Dmowski, 2000; Dmowski and Badurek, 2001, 2002; Lis et al., 2003). In feathers of magpies (*Pica pica*), caught in the environs of Bolesław near Olkusz and the smelter in Bukowno (Bolesław Mining and Metallurgical Plant, ZGH Bolesław), the thallium content was a few hundred times higher than in feathers of magpies inhabiting areas in Poland that were non-industrialized and uncontaminated (Dmowski, 2000). The contamination of the Bukowno surroundings was caused by emission of chimney dust from the ZGH Bolesław plant and dust blown by wind from the surface of the tailing pond. A sample from the heap edge contained 149 $mg \cdot kg^{-1}$d.m. of Tl, whereas from the flotation tailings – 30–40 $mg \cdot kg^{-1}$d.m. (Dmowski and Badurek, 2002). For comparison, Tl content in uncontaminated soil amounts to 0.2-1 $mg \cdot kg^{-1}$d.m. (Cröβmann, 1984) or 0.02–2.8 $mg \cdot kg^{-1}$d.m. (Kabata-Pendias, 2011).

Plant species able to hyperaccumulate heavy metals in the above-ground parts are constantly sought because of their potential usefulness for soil remediation (phytoremediation) and isolation of precious metals from the soil (phytomining). Thallium is a very costly metal, similarly to gold (Scheckel et al., 2007; Sheoran et al., 2009) and this is why plant species hyperaccumulating it are worth a detailed study.

According to the literature, a plant species could be considered a hyperaccumulator when a content of at least 500 $mg \cdot kg^{-1}$d.m. is detected in the above-ground parts of plants (Anderson et al., 1999; Leblanc et al., 1999). A lower content of thallium hyperaccumulation in leaves, i.e., 100 $\mu g \cdot g^{-1}$d.m. (100 $mg \cdot kg^{-1}$d.m.) has been recently proposed (van der Ent et al., 2013).

Up to now, only a few plant species have been recognized as thallium hyperaccumulators, namely *Iberis intermedia*, *Biscutella laevigata* (Anderson et al., 1999; Leblanc et al., 1999; LaCoste et al., 1999, 2001; Scheckel et al., 2007; Sheoran et al., 2009; Pošćić et al., 2013, 2015) and *Silene latifolia* (Escarrè et al., 2011). In the study of the *Biscutella laevigata* plants growing in St. Laurent le Minier, southern France, the Tl content in the soil was 25 $mg \cdot kg^{-1}$d.m. (from 11 to 57 $mg \cdot kg^{-1}$d.m.), and in the plants 504 $mg \cdot kg^{-1}$d.m. (from 65 to 3920 $mg \cdot kg^{-1}$d.m.) (LaCoste et al., 1999).

In Poland *B. laevigata* occurs also in the metalliferous soil in the environs of Bolesław near Olkusz (Godzik, 2015). This is the utmost northern isolated location of this species. Also noted is *B. laevigata* subsp. *gracilis* Mach.-Laur. in the Tatra Mountains (southern Poland), where lies the northern limit of the continuous species range. The locations in Bolesław and the Tatra Mts. are 120 km away from each other (Szafer, 1927; Dobrzańska, 1955; Godzik, 1984, 1991; Grodzińska et al., 2000; Grodzińska and Szarek-Łukaszewska, 2002; Szarek-Łukaszewska and Niklińska, 2002; Wierzbicka and Pielichowska, 2004). *B. laevigata* is a montane species and, according to Szafer (1927), it can be found higher than 2128 m a.s.l., where it grows on calcareous rocks and screes; it occupies dry, warm and sunny habitats on the foreland of mountains and on lowlands. In the area of the calamine waste heap in Bolesław near Olkusz, *B. laevigata* grows in hollows and on so-called *warpie* (piles of calamine ores), the remains of mining outcrops, as well as freshly made piles (Wójcicki, 1913; Dobrzańska, 1955; Grodzińska et al., 2000).

The waste heap near Olkusz is located in the Silesian Upland, within the Garb Tarnogórski mesoregion. A part of this area forms a hummock built of Triassic limestones and ore-bearing dolomites. These formations contain zinc and lead ore bodies which are accompanied by iron ores. Lead ores also contain small amounts of silver, cadmium and thallium (Dobrzańska, 1955; Grodzińska et al., 2000). The opencast mining in this region began in the 13th century. In the surroundings of Bolesław intense mining works continued till the 1930s (Grodzińska et al., 2000; Grodzińska and Szarek-Łukaszewska 2002).

In order to examine the elemental composition of biological objects Inductively Coupled Plasma Mass Spectrometry (ICP-MS) becomes the method of choice due to its selectivity and sensitivity. It can be used for determination of the total content of elements of interest in the aliquots obtained after digestion or extraction. When coupled with laser ablation (LA), it can be used for examination of the element distribution directly in solids (Wysocka, 2004; Hanć et al., 2009; Wierzbicka et al., 2007).

The aim of this study was to evaluate whether plants of the two *B. laevigata* populations occurring in Poland accumulate such an amount of thallium in their tissues that they could be considered capable of hyperaccumulating this element.

MATERIALS AND METHODS

STUDIED PLANTS

Two isolated population of *Biscutella laevigata* were studied. The plants were grown in laboratory conditions from seeds collected in two locations in Poland:

- a zinc-lead (calamine) waste heap in Bolesław near Olkusz – the population is named in this work the "calamine population",
- the Western Tatra Mountains – the population from this location is named in this work the "montane population".

PLANT CULTIVATION IN CALAMINE SOIL

The *B. laevigata* plants of the calamine and montane populations were cultivated in calamine soil in laboratory conditions. The accumulation of thallium by plants of different ages, from 2-week-old seedlings to 10-month-old adults, was evaluated.

Seeds from both populations were incubated on wet blotting paper in Petri dishes for two weeks. Next, germination and cultivation of the plants was carried out in garden soil, at different times to obtain plants varied in age: 2-week-, 1-, 2- and 10-month-old ones. The plants of four groups were replanted partly in the calamine soil brought from the zinc-lead waste heap in Bolesław near Olkusz and partly in garden soil (control). The cultivation was continued for five months. It was done in a glasshouse, under the long-day conditions (16h/8h) and at the temperature of 29^{o}C /18^{o}C (day/night). The plants were watered with distilled water twice a week. Altogether 112 plants were cultivated.

PLANT CULTIVATION IN CALAMINE SOIL WITH ADDED EDTA

To enhance the bioavailability of metals, an EDTA solution (Na_2H_2 EDTA·$2H_2O$) was added to calamine soil. The salt solubility is equal to 11.1 g/100 ml H_2O at temp. 21^{o}C. With many di-, tri- and tetravalent cations EDTA forms persistent, easily water-soluble chelats, which is why this compound is quite often used for enhancing bioavailability of metals in soil (Szmal and Lipiec, 1996; Epstein et al., 1999; Heil et al., 1999; Liphadzi et al., 2003; Minczewski and Marczenko, 2004; Turgut et al., 2004).

The plants of the calamine and montane populations were grown in the following variants: calamine soil with 1.5 mmol·kg^{-1} EDTA; garden soil with 1.5 mmol·kg^{-1} EDTA; calamine soil without EDTA; and garden soil without EDTA.

Seeds of the plants from both populations were sown on wet blotting paper in Petri dishes. The seedlings were transferred to garden soil and next, they were cultivated for two months. Thereafter, the plants were moved from the garden soil, well washed and transplanted to pots with calamine soil as well as calamine soil with EDTA. The plants cultivated in the garden soil and the garden soil with EDTA were controls.

The calamine soil was prepared in the following way: the calamine substratum was sieved through a sieve with a mesh size of 2 mm and divided into two parts. One part was left without EDTA and the other was mixed with 1.5 mmol·kg^{-1} EDTA many times and left for four and a half weeks. The garden soil was prepared in the same way. Additionally, the calamine soil was triturated by hand, and the garden soil was mixed with 2-mm glass pellets, in the ratio 3:1.

During cultivation the plants were watered with distilled water twice a week. The cultivation was continued for two and a half months and was done in a glasshouse, under the long-day conditions (16h/8h) and at the temperature of 29^{o}C/18^{o}C (day/night). Altogether, 46 plants were grown.

ELEMENTAL COMPOSITION OF PLANTS AND SOIL AS WELL AS THALLIUM DISTRIBUTION IN LEAVES – INSTRUMENTATION

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS): an inductively coupled plasma mass spectrometer ELAN 6100 DRC (Perkin Elmer SCIEX, Canada; www.perkinelmer.com) was used for measurement of the total content of thallium in the soil and the plants after digestion as well as after extraction.

Laser Ablation Inductively Coupled Plasma Mass Spectroscopy (LA-ICP-MS): an inductively coupled plasma mass spectrometer ELAN DRC II (Perkin Elmer SCIEX, Canada; www.perkinelmer.com) equipped with the laser ablation system LSX-500 (CETAC, USA; www.cetac.com) was used. The LSX-500 combines a stable, environmentally sealed 266 nm UV laser (Nd-YAG, solid state, Q-switched) with a high sampling efficiency, variable 1 to 20 Hz pulse repetition rate and maximum energy up to 6 mJ/pulse. In order to evaluate the thallium distribution in leaves, 205Tl/13C was measured, and 13C was used as an internal standard.

All operating conditions of LA-ICP-MS and ICP-MS used in these studies are summarized in Tab. 1.

An ETHOS-PLUS (Milestone) microwave system was used for plant tissues and soil digestion. The mineralization time/power program was as follows: 5 min. at 100 W; 10 min. at 800 W; 15 min. of cooling.

In order to assure the accuracy of results, two standard reference materials were used: Pine

TABLE 1. Operating parameters of ICP-MS and LA-ICP-MS instrumentations.

Operation parameters	ICP-MS	LA-ICP-MS
RF power	1100 W	1050 W
Plasma Ar gas flow rate	13 l/min	15 l/min
Auxiliary Ar gas flow rate	1.1 l/min	1 l/min
Ar carrier gas flow rate	0.85 l/min	1 l/min
Sampler / skimmer cone (Ni)	1.1 mm/0.8 mm	
Sweeps/reading	30	1
Reading / replicates	1	300
Dwell time	50 ms	10 ms
Mode	Peak hopping (1 point per mass)	
Isotopes monitored	39K; 205Tl	
Laser operating parameters		
Wavelength		266 nm
Scan mode		Single line
Laser energy		5.4 mJ
Repetition rate		5 Hz
Scan rate		25 μm/s
Spot size		50 μm

Needles 1575 and Spinach 1570a from NIST as well as Soil NCS DC 73322 (GBW07404) from the China National Analysis Center for Iron & Steel.

THALLIUM AND POTASSIUM CONTENT IN PLANTS AND SOIL

After the cultivation had been ended, the plants were well washed and divided into roots and fresh and dry leaves, which were washed with distilled water (3 times), then dried on blotting paper. Next, the plant tissues that had been weighed and dried at 100°C to a constant mass were ground in an agate mortar. The plant tissues were digested with the use of 6 ml of the 69% mixture of HNO_3 : H_2O_2 (5:1). After digestion had been completed, thallium and potassium contents were determined by ICP-MS (Tab. 1). The soil from the calamine waste heap in Bolesław near Olkusz was dug from the growth place of plants (the soil associated with rhizosphere). The soil was sieved through a sieve with a mesh size of 1 mm, dried at 80°C, and ground in an agate ball mill. The mixture of 6 ml of HCl and 2 ml of HNO_3 was poured onto the 1 g sample weights. They were heated in an open system, cooled and infiltrated. Next, 50 ml of distilled water was added to the filtrate. The determination of thallium and potassium was performed by means of ICP-MS (Tab. 1).

In order to control the pH of the soil, around 50 ml of deionized water was added to a 5-g soil sample and stored for 24 hours. The pH of the solution above the soil sediment at 25°C was measured (Wąchalewski, 1999).

EXTRACTION AND DETERMINATION OF THALLIUM CONTENT IN SOIL

In order to determine the amount of thallium bound to various soil components (and thus differing in its bioavailability), single extraction with various media was applied. The assays were made for the vegetated calamine soil. The following extractants were used: 1) 0.01 mol/l $CaCl_2$; 2) 0.02 mol/l EDTA in acetic buffer, pH 4.65. A 5-g sample was extracted with 50 ml of the extractant at room temperature for 16 hours. The Tl content was determined by ICP-MS.

EVALUATION OF THALLIUM DISTRIBUTION IN LEAVES

Dried leaves of *B. laevigata* plants from the calamine and montane populations were used in this study. The plants were cultivated in: calamine soil with 3 mmol·kg^{-1} EDTA; and garden soil without EDTA.

In order to examine thallium distribution in leaves, an individual leaf was exposed to a laser beam along four lines (two lines in a petiole, one in the middle of a lamina, and one at the top of a lamina). In addition, trichomes along the lamina edge protruding outside it were studied.

Following the signals from 205Tl and 13C, obtained as a result of the laser beam scan along the lines, profiles of thallium distribution in both the lamina and trichomes were obtained.

VISUALISATION OF HEAVY METALS IN TRICHOMES

The heavy metal presence in trichomes of plants from both populations was detected by the non-specific histochemical method with the use of dithizone (diphenylthiocarbazone) (Seregin and Ivanov, 1997; Wierzbicka and Pielichowska, 2004; Olko et al., 2008). This compound forms chelats with metal cations, visible under the light microscope as variously shaped black deposits.

STATISTICAL ANALYSIS

The data were analysed statistically in PAST 2.17c software. All effects were tested by the non-parametric Kruskall-Wallis test with post-hoc pairwise comparisons using the Mann-Whitney U test.

RESULTS

THALLIUM AND POTASSIUM IN CALAMINE SOIL

The total content of thallium in the calamine soil was checked in two types of habitats. In the bare calamine soil, where plants (including *B. laevigata*) just begin to grow and in the already vegetated soil with humus, in the rhizosphere.

The thallium content amounted to 66.7 $mg \cdot kg^{-1}$d.m. and 15.2 $mg \cdot kg^{-1}$d.m., respectively (Tab. 2). The availability of thallium for plants estimated by the extraction in EDTA was relatively low (16.4 $mg \cdot kg^{-1}$d.m. and 3.1 $mg \cdot kg^{-1}$d.m., respectively), similarly in $CaCl_2$ (2.1 $mg \cdot kg^{-1}$d.m. and 1.1 $mg \cdot kg^{-1}$d.m., respectively). The calamine soil was reasonably rich in potassium (3004 $mg \cdot kg^{-1}$d.m.) in comparison with, e.g., the garden soil or the geochemical background in the area of Poland, and its pH was slightly alkaline (7.4–7.8) (Tab. 2).

THALLIUM CONTENT IN *B. LAEVIGATA* PLANTS OF CALAMINE AND MONTANE POPULATIONS

Plants of different ages (from 2-week-old seedlings to 40-week-old adults) were cultivated for five months in calamine soil. It was found that thallium was taken up by plants of both populations (Tab. 3). The mean Tl content in plants amounted to 98.5 $mg \cdot kg^{-1}$d.m. (max. 1100 $mg \cdot kg^{-1}$d.m.). The highest content of Tl was found in shoots, 164.9 $mg \cdot kg^{-1}$d.m. on average (from 59.8 to 588.2 $mg \cdot kg^{-1}$d.m.). In roots the Tl content was much lower, 32 $mg \cdot kg^{-1}$d.m. on average (from 11.1 to 93.6 $mg \cdot kg^{-1}$d.m.). It turned out that the Tl content in plants depended on their age at the beginning of cultivation. Thallium was accumulated mostly by 2-week-old seedlings (on average 219 $mg \cdot kg^{-1}$d.m.), and much less effectively by older plants (on average 58.2 $mg \cdot kg^{-1}$d.m.). Noteworthy is the fact that among the older plants (4-, 8-, 40-week-old ones) the Tl content decreased from 74.5 to 43.4 $mg \cdot kg^{-1}$d.m.

It was found that 16% of the total thallium remained in roots, whereas 84% was transported to the above-ground parts (Fig. 3). The translocation factor (shoot : root ratio; Anderson et al., 1999) amounted on average to 6.1, whereas the bioconcentration factor (shoot : soil ratio; Anderson et al., 1999) was 10.9.

TABLE 2. Thallium (Tl) and potassium (K) content [$mg \cdot kg^{-1}$d.m.] and pH values (n=3 soil samples) in different soil types: pure calamine, vegetated calamine, garden soil in comparison with the geochemical background (Kabata-Pendias, 2011).

Soil	Tl			K [$mg \cdot kg^{-1}$d.m.] *	pH N.S.
	Total content [$mg \cdot kg^{-1}$d.m.] *	Extracted by EDTA [$mg \cdot kg^{-1}$d.m.] *	Extracted by $CaCl_2$ [$mg \cdot kg^{-1}$d.m.] *		
Calamine	66.7±0.4 a	16.4±1.6 a	2.1±0.6 a	3004±104 a	7.81±0.06 a
Vegetated calamine	15.2±1.5 a	3.1±0.6 a	1.1±0.3 a	not studied	7.4±1.4 a
Garden	0.0 a	0.0 a	0.0 a	417±61 a	4.97±0.08 a
Geochemical background	0.02–2.8 a	no data	no data	130-20600 a	5–7 a

Asterisks (*) denote significant effects found in Kruskall-Wallis test; N.S. – non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Lower-case letters denote statistically significant differences in Mann-Whitney post-hoc pairwise comparisons, $p < 0.05$.

TABLE 3. Thallium content [mg·kg^{-1}d.m.] in differently-aged *B. laevigata* plants of calamine (C) and montane (M) populations, grown in calamine soil for five months; n=6 mixed plant sample weights; mean of means for roots, fresh and dry leaves.

Plant age [weeks]	C		M		Overall effect
	Roots *	Shoots *	Roots N.S.	Shoots ***	* Mean
2	93.6±63 a	126.5±173 a	68.2±176 a	588.2±488 a	219.1 a
4	19.0±7 a	155.4±54 b	17.4±6 a	106.0±9 b	74.5 ab
8	17.0±4,5 a	122.5±21 b	13.0±7 a	74.8±5 c	56.8 ab
40	11.1±4 a	59.8±24 a	17.0±4.5 a	85.9±31 bc	43.4 b

Asterisks (*) denote significant effects found in Kruskall-Wallis test; N.S. – non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Lower-case letters denote statistically significant differences in Mann-Whitney post-hoc pairwise comparisons, $p < 0.05$.

THALLIUM CONTENT IN *B. LAEVIGATA* PLANTS IN CALAMINE SOIL TREATED WITH EDTA

During the cultivation of the *B. laevigata* plants, either in the pure calamine soil or the calamine soil with EDTA, the growth of the plants was not inhibited. On the contrary, the plants often gained bigger biomass than in the control (cultivated in the garden soil). The presence of EDTA in the calamine soil enhanced bioavailability of thallium; its content in plant organs increased by around 15 mg·kg^{-1}d.m. on average (from 69.5 mg·kg^{-1}d.m. to 84.8 mg·kg^{-1}d.m.) (Tab. 4). The highest amount of Tl was found in fresh leaves (up to 306 mg·kg^{-1}d.m.), nearly a 50% lower amount in roots, and the lowest content in dry leaves (21.1–37.1 mg·kg^{-1}d.m.). As a consequence of the presence of EDTA in the calamine soil, the content of Tl in the entire plants (roots and shoot) was on average equal to 109 mg·kg^{-1}d.m.

In the control plants cultivated in the garden soil, the Tl content was very low and amounted to 0.05 mg·kg^{-1}d.m. to max. 0.30 mg·kg^{-1}d.m. The Tl content in plants of the calamine and montane populations did not differ, therefore the means for both populations were given together.

The Tl translocation factor for plants cultivated in the calamine soil with EDTA amounted on average to 2.2, whereas the Tl bioconcentration factor had the mean value of 5.8.

POTASSIUM CONTENT IN *B. LAEVIGATA* PLANTS

Thallium activity is primarily based on competition with potassium cations (Wenzel and Jockwer, 1999), therefore potassium content in the *B. laevigata* plants was also examined. The plants were cultivated in calamine soil without (control) and with the addition of EDTA as well as in garden soil (control) and garden soil with EDTA (control) (Tab. 5).

It was found that generally the potassium content in the plants cultivated in the calamine soil (with or without the addition of EDTA) was much lower (by more than 10 times) in comparison with the plants cultivated in the garden soil (with or without EDTA) – 3761 mg·kg^{-1} d.m. and 42856 mg·kg^{-1}d.m. on average, respectively. As the potassium content in both populations did not differ, the means for both populations were given together.

THALLIUM DISTRIBUTION IN LEAVES (LA-ICP-MS)

The distribution of thallium in the leaves of *B. laevigata* cultivated in the calamine soil with the addition of EDTA (plants cultivated in the garden soil without EDTA were controls) was examined by LA-ICP-MS.

As a result of the laser beam scan over the leaf lamina along indicated lines (Figs. 1 and 2), the profiles of the thallium distribution were obtained (Fig. 4). Next, peaks from consecutive profiles for

TABLE 4. Thallium content [mg·kg^{-1}d.m.] in *B. laevigata* plants grown for ten weeks in pure calamine soil and enriched with 1.5 mmol·kg^{-1} EDTA; n=12 mixed plant sample weights; mean of means for roots, fresh and dry leaves.

Soil	Roots	Fresh leaves	Dry leaves	Mean
Calamine	51.5±23 a	135.7±45 a	21.2±2.5 a	69.5 a
Calamine with EDTA	78.7±33.5 a	138.4±70.2 a	37.1±13.3 b	84.8 a

Lower-case letters denote statistically significant ($p < 0.05$) differences in Mann-Whitney two sample U test.

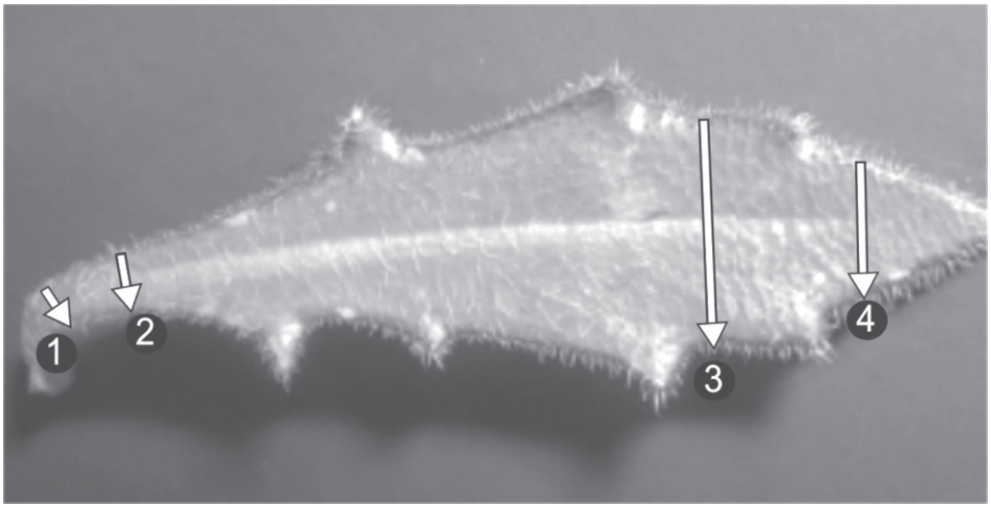

Fig. 1. Photo of a leaf examined by LA-ICP-MS. The arrows indicate the direction of the laser ablation line scan: mesophyll → vascular bundle → mesophyll consecutively in exposition lines from 1 to 4.

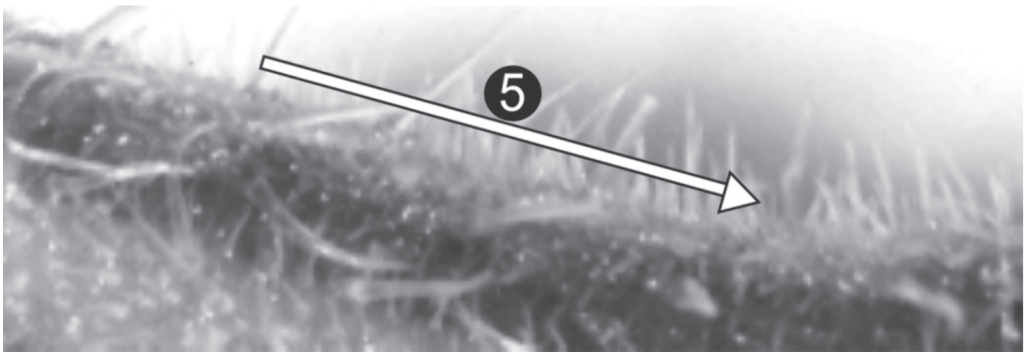

Fig. 2. Photo of trichomes on the leaf edge exposed to laser ablation via the line indicated by the arrow.

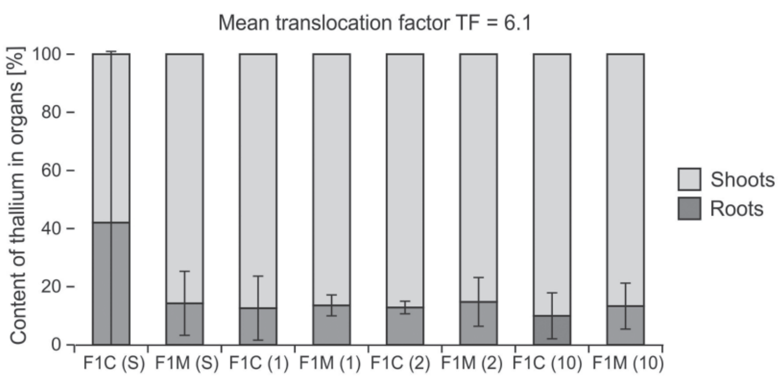

Fig. 3. Mean proportional thallium content [] in roots and shoots of *B. laevigata* plants from the calamine (C) and montane (M) population, cultivated from seeds (F1) in the calamine soil for five months. The age groups of plants at the beginning of cultivation: 2-week-old seedlings (S), 1-month-old (1), 2-month-old (2), 10-month-old (10) plants. Percentages were calculated as the thallium content in a shoot or a root [mg/kg d.m.], divided by the sum of the shoot and root Tl contents multiplied by 100%. The translocation factor (TF): the ratio of the thallium content in shoots and roots; n=6 mixed plant sample weights for each age group.

TABLE 5. Potassium content [mg·kg^{-1}d.m.] in *B. laevigata* plants grown for ten weeks in: pure calamine soil, enriched with 1.5 mmol·kg^{-1} EDTA, pure garden soil and enriched with 1.5 mmol·kg^{-1} EDTA; n=12 mixed plant sample weights; mean of means for roots, fresh and dry leaves.

Soil	Roots***	Fresh leaves***	Dry leaves***	Overall effect*** Mean
Calamine	2005±306 a	8063±4866 a	4149±2657 a	4739 a
Calamine with EDTA	1706±511 a	3608±434 a	3037±548 b	2783 a
Garden	10811±4008 b	66164±28471 b	43433±7841 c	40136 b
Garden with EDTA	7956±1670 b	78854±18325 b	49922±12600 c	45577 b

Asterisks (*) denote significant effects found in Kruskall-Wallis test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Lower-case letters denote statistically significant differences in Mann-Whitney post-hoc pairwise comparisons, $p < 0.1$.

leaves of the experimental plants were added and averaged.

In all the plants growing in the calamine soil with EDTA, the largest amount of thallium occurred in the basal part of a leaf (Figs. 1 and 5, lines 1, 2), and the smallest in its top part (Figs. 1 and 5, line 4). The thallium content in the leaves studied by ICP-MS amounted to 126 mg·kg^{-1}d.m. This indicates high sensitivity of the method used, well suited for biological material.

THALLIUM IN LEAF TRICHOMES (LA-ICP-MS)

Trichomes on *B. leavigata* leaves are alive and single-celled. At the base of a leaf there is a large vacuole. Between the trichome cell and mesophyll cells of a leaf there exists a connection through plosmodesmata (data not presented).

The investigation of the thallium presence in trichomes on the *B. laevigata* leaves was conducted by LA-ICP-MS. The laser beam was led along the leaf edge as shown in Fig. 2. In the leaf trichomes of both populations the presence of thallium was detected. The profiles obtained for different leaves enabled a relative assessment of the Tl amount accumulated there. In the leaves of plants cultivated in the calamine soil with EDTA, the amount of this element increased in comparison with the control (on the grounds of the profile reading): 300 times (calamine population) and 100 times (montane population). An exemplary profile for Tl in trichomes is shown in Fig. 6. After staining in dithizone there were observed deposits in the middle part of the trichome, in the central cell vacuole (Figs. 7 and 8).

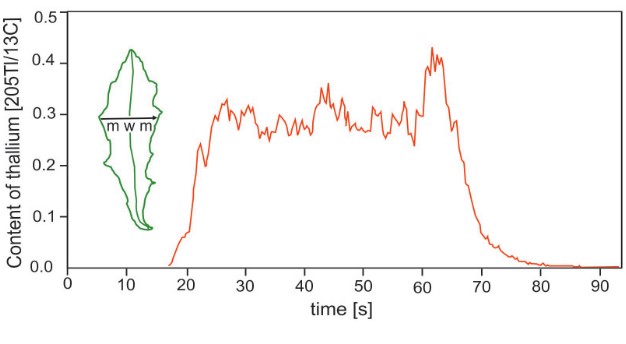

Fig. 4. LA-ICP-MS profile of thallium distribution in the leaf lamina of the montane plant (M) cultivated in the calamine soil with 3 mmol·kg^{-1} EDTA. The arrow: direction of the laser beam scan: mesophyll (m) → vascular bundle (w) → mesophyll (m). 205Tl/13C was measured, and 13C was used as an internal standard.

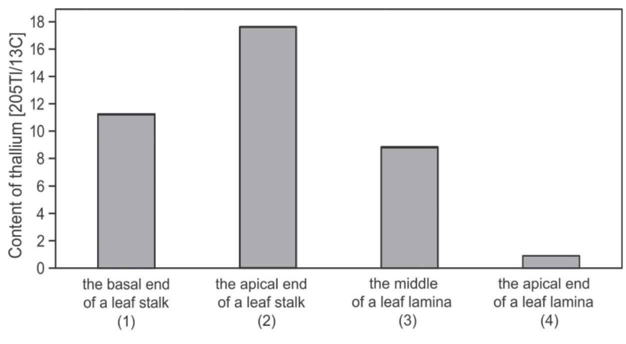

Fig. 5. The mean thallium distribution along the leaf midrib in the calamine plants (C) cultivated in the calamine soil with the addition of 3 mmol·kg^{-1} EDTA; n=3 leaves. 205Tl/13C was measured, and 13C was used as an internal standard.

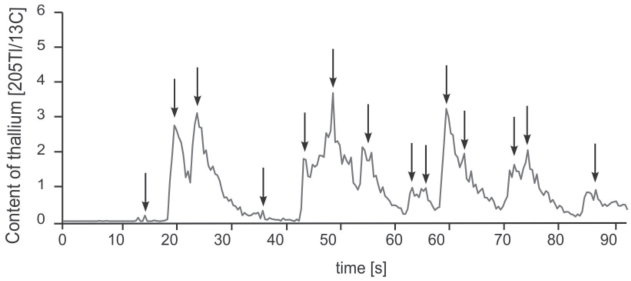

Fig. 6. Profile of Tl distribution, obtained by LA-ICP-MS method, in the leaf trichomes of the calamine plant (C) cultivated in the calamine soil with 3 mmol·kg^{-1} EDTA. Every peak in the line marked with an arrow indicates a single trichome on the leaf. 205Tl/13C was measured, and 13C was used as an internal standard.

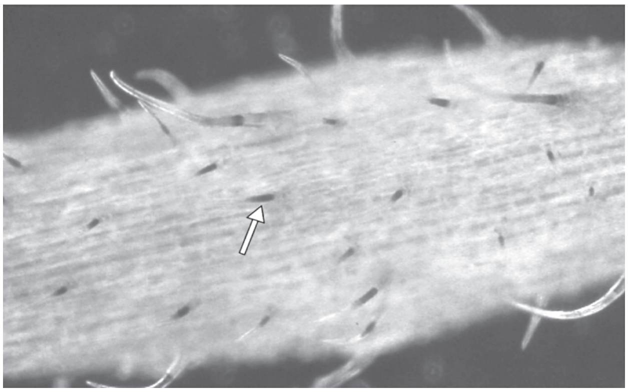

Fig. 7. Visualization of heavy metals in the trichomes (arrows) after the histochemical reaction. The plant cultivated in the calamine soil with 3 mmol·kg^{-1} EDTA.

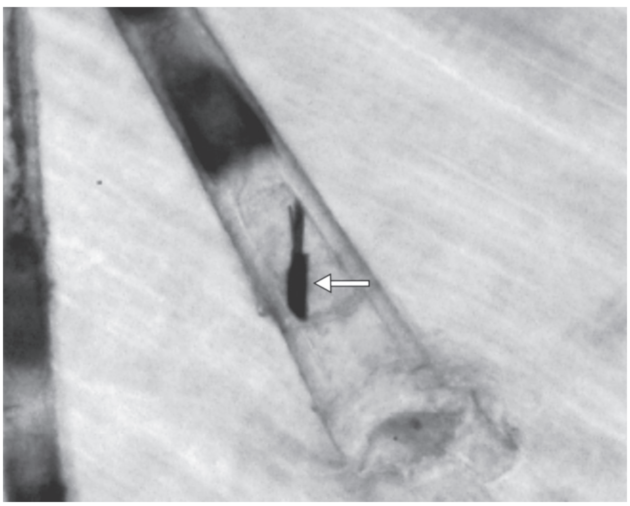

Fig. 8. Visualization of heavy metals in the trichome (arrow) after the histochemical reaction. The plant cultivated in the calamine soil with 3 mmol·kg^{-1} EDTA.

DISCUSSION

THALLIUM IN PLANTS

In Poland in the metalliferous post-industrial areas of Olkusz, the amount of thallium in soils amounts to 29 to 44 mg·kg^{-1}d.m., whereas in green plants, from 7.9 to 25.5 mg·kg^{-1}d.m. (Kicińska, 2009). It is noteworthy that in green plants the Tl content should be 0.05 mg·kg^{-1}d.m. (Kabata-Pendias, 2011). Our previous study (Wierzbicka et al., 2004) done in field conditions showed that metallophytes growing on the calamine waste heap in Bolesław near Olkusz contained thallium in shoots in the following contents: *Plantago lanceolata* – 54 mg·kg^{-1}d.m., *Silene vulgaris* – 7 mg·kg^{-1}d.m., *Dianthus carthusianorum* – 7 mg·kg^{-1}d.m. That study did not show a significant amount of thallium in *B. laevigata* plants (Wierzbicka et al., 2004), although the species is included among Tl hyperaccumulators (Anderson et al., 1999; LaCoste et al., 1999; Leblanc et al., 1999; Pošćić et al., 2013, 2015).

In view of the foregoing, we decided to check in detail whether the *B. laevigata* plants occurring in Poland have the ability to hyperaccumulate thallium. Field studies always have some limitations. One of these is the fact that thallium in plants can be accumulated from two sources: it can be taken up by roots and transported to shoots, but it can also be found on the leaf surface due to dust fall containing Tl. Especially in plants equipped with very numerous trichomes on their leaves the contribution of this Tl pool might be crucial.

It seems that other authors did not pay particular attention to this problem in their studies (Anderson et al., 1999; LaCoste et al., 1999; Leblanc et al., 1999), and thus the results regarding Tl accumulation in *B. laevigata* plants may have come from both sources. It is worth stressing that only thallium absorbed by roots and transported to shoots can testify to its hyperaccumulation.

Extremely varied chemical composition of the soil is another limitation of field studies in post-industrial regions. In the area of a few square metres there may occur a highly toxic substratum next to a pure one. As was shown by Vaněk et al. (2010), the Tl accumulation by plants depends on the soil type; a two- to threefold difference was found.

A diversified age of plants is the next limiting factor. *B. laevigata* is a perennial, and in the field there is no possibility of distinguishing older plants from younger ones. In studies on *Sinapis alba* it was shown that the Tl content in adult plants was three times lower than in the young ones (Vaněk et al., 2010).

Therefore, the detailed study of the ability of *B. laevigata* plants to accumulate thallium was done in controlled conditions, based on cultivation of plants in a glasshouse in the calamine soil containing Tl.

BISCUTELLA LAEVIGATA – THALLIUM HYPERACCUMULATOR

Thallium content in the soil of the calamine waste heap is varied. In this study it was shown that a threefold difference occurred in the Tl content between the bare soil without humus and places already possessing a thin litter layer and covered by plants (66.7 mg·kg^{-1}d.m. and 15.2 mg·kg^{-1}d.m., respectively).

Thallium distribution in plants depends on their age (Nriagu, 1988; Frattini, 2005). Usually, the highest content is recorded in seedlings, and with age of plants it gradually decreases. The *Sinapis alba* seedlings contained a few times more thallium than adult plants (Frattini, 2005).

In this respect the *B. laevigata* plants were studied in detail. Regardless of the population under study, the youngest (2-week-old) plants accumulated the largest thallium amount over five months. The Tl content was four times higher in seedlings than in the oldest plants (219 mg·kg^{-1}d.m. and 43 mg·kg^{-1}d.m. on average, respectively). Noteworthy is the fact that among the older plants, at the age of one to ten months, the amount of accumulated thallium (during the 5-month cultivation) decreased (from 74.5 mg·kg^{-1}d.m. to 43.4 mg·kg^{-1}d.m.). In this way it was proved that the Tl content in the *B. laevigata* plants depended on their age. However, over one growing season (5 months of cultivation) the *B. laevigata* plants contained altogether 98.5 mg·kg^{-1}d.m. of Tl on average, from which the mean Tl content in the above-ground parts (in this species mainly leaves – a rosette) amounted to 164.9 mg·kg^{-1}d.m. (from 59.8 to 588.2 mg·kg^{-1}d.m.). This is a very high Tl content to be accumulated during only one growing season.

In further study Tl bioavailability was enhanced by the addition of EDTA to the calamine soil. In the *B. laevigata* plants cultivated in these conditions the Tl amount increased to such a level that whole plants contained on average 108.9 mg·kg^{-1}d.m., of which 138.4 mg·kg^{-1}d.m. (max. 260 mg·kg^{-1}d.m.) was in fresh leaves.

These results should be compared with those of the field studies, on the grounds that *B. laevigata* was accepted as a Tl hyperaccumulator. The Tl content detected in leaves was then 504 (from 65 to 3920) mg·kg^{-1}d.m. and its content in the soil amounted to 25 (from 11 to 57) mg·kg^{-1}d.m. (LaCoste et al., 1999). The Tl amount in the *B. laevigata* plants discovered in this study does not reach the boundary value of 500 mg·kg^{-1}d.m. (Anderson et al., 1999; Leblanc et al., 1999), however, it exceeds the hyperaccumulation level indicated by van der Ent et al. (2013), i.e., 100 μg·g^{-1} (100 mg·kg^{-1}) d.m. This proves that *B. laevigata* is a thallium hyperaccumulator, especially since the study was conducted in laboratory conditions. The Tl amount in the *B. laevigata* plants much smaller than reported by Anderson et al. (1999) and Leblanc et al. (1999) may be an effect of too low Tl bioavailability in the calamine soil. After the extraction with EDTA, the estimated Tl bioavailability for plants was 3.1 mg·kg^{-1}d.m., and after the extraction with $CaCl_2$, 1.1 mg·kg^{-1}d.m. (Tab. 2). After water extraction, Kicińska (2009) estimated the Tl bioavailability as even lower: 0.1-0.8 mg·kg^{-1} d.m.

The Tl content in the *B. laevigata* plants evidences their ability to hyperaccumulate this element. Of the total thallium content 16% was accumulated in the roots and 84% was transported to the leaves. The Tl accumulation pattern was as follows: fresh leaves > roots > the oldest drying leaves. The translocation factor was high; it reached 6.1 on average. The bioconcentration factor relating the Tl amount in shoots to its content in the soil also turned out high (10.9). It should be pointed out that both high (>1) root-to-shoot translocation and shoot-to-soil bioconcentration factors for metals are characteristic of hyperaccumulators (van der Ent et al., 2013 and references therein). The results of this study indicate that *B. laevigata* is a facultative thallium hyperaccumulator. In our opinion the ability to hyperaccumulate this element is a species-wide trait, common to plants of both subspecies, independent of their natural occurrence in Tl contaminated and uncontaminated habitats. Pošćić et al. (2013), who had studied in laboratory conditions *B. laevigata* plants from different populations – on zinc-lead waste heaps in southern France (Les Avinières), northeastern Italy (Cave del Predil) and southern Poland (Bolesław near Olkusz) as well as an area uncontaminated with metals (Polish Tatra Mts.), came to other conclusions. The authors indicated a notably varied tolerance of the plants to the high Tl content in the soil – from hyperaccumulation (translocation factor >1; population from Les Avinières), through enhanced tolerance (populations from Italy and Bolesław) to inability to survive (Tatra population). According to the authors, the ability to hyperaccumulate thallium is a trait typical of a given population, and it is not common to the whole species. Similarly, after analyzing only elementary composition of the soil and plant specimens collected from metalliferous and uncontaminated areas in northeastern Italy, Pošćić et al. (2015) found a high interpopulation variability in the Tl content in plants. From among 15 populations studied, one was distinguishable by the Tl hyperaccumulation in shoots (Cave del Predil population; translocation and bioconcentration factors >1), and two (copper mine area, Avanza and former zinc and lead mine, Salafossa) had a Tl amount that was increased but still smaller than the accepted boundary of 100 $\mu g \cdot g^{-1}$ (100 $mg \cdot kg^{-1}$) d.m. (van der Ent et al., 2013). Only these three of all populations occurred in metalliferous areas. It should be emphasized that those results were obtained in natural, and not in laboratory conditions. The discrepancy between reports on whether metal hyperaccumulation is a species-wide or population-specific trait was pointed out by Pollard et al. (2014). The authors' conclusion was that this issue certainly needed further study and explanation. Our results strongly support the idea that hyperaccumulation of metals is common for plants at the species rather than population level.

The toxic activity of thallium is due to its similarity to potassium and therefore its competition ability (Scheckel et al., 2007). Our study showed that the K uptake by the *B. laevigata* plants was ca. 10 times lower when plants grew in the calamine soil containing Tl in comparison to the garden soil (Tab. 5). In both soil types the K content was sufficient (Tab. 2).

The fact that the K uptake was much lower in plants having an increased Tl amount indicates competition between the two elements. A similar relationship was discovered during the study on *Sinapis alba*, when thallium was added to the soil as thallium sulphate. There occurred competition between the K and Tl uptake (Vaněk et al., 2010). According to Wenzel and Jockwer (1999), there is some evidence that potassium may play a role in heavy metal ion compartmentation in hyperaccumulating species; however, a precise mechanism has not been described yet.

THALLIUM DISTRIBUTION IN LEAF LAMINAS AND TRICHOMES

As thallium was located in the greatest amount in fully developed leaves of the *B. laevigata* plants, its distribution there was checked by LA-ICP-MS. It turned out that the smallest Tl amount was at the top of a leaf, whereas the largest one was at its base. In the leaves of another species, *Iberis intermedia*, a similar pattern of Tl distribution was detected using synchrotron X-ray differential absorption edge computed microtomography (CMT) (Scheckel et al., 2007).

In this study it was shown by means of the sensitive LA-ICP-MS method that a considerable Tl amount was accumulated in the leaf trichomes of *B. laevigata*. An important role of trichomes in hyperaccumulation of other metals has also been proved in other plant species, e.g., zinc and cadmium in *Arabidopsis halleri* (Küpper at al., 2000) or nickel in the *Alyssum* genus (Broadhurst et al., 2004a, 2004b).

Accumulation of metals in trichomes is a way to protect the metabolism of active cells against metal toxicity (Küpper et al., 2000). The specialized trichome cells trap and accumulate a very high content of metals (15–20% of dry mass in *Alyssum*), which might be a general rule in hyperaccumulating plant species (Broadhurst et al., 2004b).

In this study it was shown for the first time that thallium is accumulated in leaf trichomes of plants from both *B. laevigata* populations. Therefore, the trichomes play an important role in the protection of other leaf cells against the toxicity of thallium, and an increased number of trichomes on leaves favors plant growth in the habitat enriched in this element.

In our previous study (Wierzbicka and Pielichowska, 2004) it was shown that the trichome number on leaves is one of the main traits distinguishing the calamine *B. laevigata* population from the montane one.

The leaves were 50–100% covered with trichomes in the calamine population and 5-50% in the montane one. Thus, the leaf trichomes were more numerous in the calamine than in the montane population. This trait is genetically preserved because it occurred in plants cultivated in the same laboratory conditions. To sum up, Tl accumulation is the most possible in leaf trichomes of *B. laevigata* plants growing on the zinc-lead waste heap.

CONCLUSION

It has been proved in controlled laboratory conditions that Polish *B. laevigata* plants, regardless of their origin (i.e., the Bolesław calamine waste heap vs West Tatra montane populations) are able to hyperaccumulate thallium: to take it up by the roots from the calamine soil and to transport it mainly to fresh leaves. Therefore, we conclude that the ability to hyperaccumulate Tl is a species-wide rather than population-specific trait. This ability turned out to be related to the age of plants and it was the highest in the young ones.

It has been shown for the first time by the LA-ICP-MS method that *B. laevigata* plants detoxify Tl by removing it into leaf trichomes. Though present in both populations, the trichomes were more numerous in the calamine than in the montane plants, which indicates stronger adaptation of the former to growing on the zinc-lead waste heap.

AUTHORS' CONTRIBUTIONS

MW conceptual work, study supervision, manuscript elaboration; MP conducting experiments and processing data; AA chemical analyses of thallium;

BW collecting material and elaborating analytical results; IW chemical analyses by LA-ICP-MS; EB supervising study done by methods of analytical chemistry; DPM final manuscript preparation.

ACKNOWLEDGEMENTS

This research was financially supported by the Polish Ministry of Science and Higher Education as a promoter research project no. 2 PO4C 035 28 and a grant BW no. 501/68/1561/02/50.

REFERENCES

Anderson CWN, Brooks RR, Chiarucci A, Lacoste CJ, Leblanc M, Robinson BH, Simcock R, and Stewart RB. 1999. Phytominig for nickel, thallium and gold. *Journal of Geochemical Exploration* 67: 407–415.

Broadhurst CL, Chaney RL, Scott Angle J, Erbe EF, and Maugel TK. 2004a. Nickel localization and response to increasing Ni soil levels in leaves of the Ni hyperaccumulator *Alyssum murale*. *Plant and Soil* 265: 225–242.

Broadhurst CL, Chaney RL, Scott Angle J, Maugel TK, Erbe EF, and Murphy CA. 2004b. Simultaneous hyperaccumulation of nickel, manganese, and calcium in *Alyssum* leaf trichomes. *Environmental Science and Technology* 38: 5797–5802.

Crößmann G. 1984. Thallium – a new environmental problem? *Angewdte Botanik* 58(1): 3–10.

Dmowski K. 2000. Chapter 17 Environmental monitoring of heavy metals with magpie (*Pica pica*) feathers - an example of Polish polluted and control areas. In: Markert B, Friese K. [eds], *Trace Elements: Their Distribution and Effects in the Environment, Trace Metals and the Environment 4*, 455–477. Elsevier BV, Amsterdam.

Dmowski K, and Badurek M. 2001. Thallium contamination of selected biotic elements of ecosystems neighboring the zinc smelter in Bukowno. In: Gworek B, Mocek A. [eds], *Obieg pierwiastków w przyrodzie*, Monografia. Vol. I. 19–23. Instytut Ochrony Środowiska, Warszawa.

Dmowski K, and Badurek M. 2002. Thallium contamination of selected plants and fungi in the vicinity of the Bolesław zinc smelter in Bukowno (S. Poland). Preliminary study. *Acta Biologica Cracoviensia Series Botanica* 44: 57–61.

Dmowski K, Kozakiewicz A, and Kozakiewicz M. 1998. Small mammal population and community under conditions of extremely high thallium contamination in the environment. *Ecotoxicology and Environmental Safety* 41: 2–7.

Dobrzańska J. 1955. Badania florystyczno-ekologiczne nad roślinnością galmanową okolic Bolesławia i Olkusza [Flora and ecological studies on calamine flora in the district of Bolesław and Olkusz]. *Acta Societatis Botanicorum Poloniae* 24: 357–408.

Epstein AL, Gussman CD, Blaylock MJ, Yermiyahu U, Huang JW, Kapulnik Y, and Orser CS. 1999. EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. *Plant and Soil* 208: 87–94.

Escarré J, Lefèbvre C, Raboyeau S, Dossantos A, Gruber W, Cleyet Marel JC, Frérot H, Noret N, Mahieu S, Collin C, and Van Oort F. 2011. Heavy metal concentration survey in soils and plants of the Les Malines Mining District (Southern France): implications for soil restoration. *Water Air and Soil Pollution* 216: 485–504.

Frattini P. 2005. Thallium properties and behaviour – a literature study. Geological Survey of Finland. http://arkisto.gtk.fi /s41/S41_0000_2005_2.pdf.

Godzik B. 1984. *Tolerancja wybranych gatunków roślin na metale ciężkie* [*Tolerance of selected plant species to heavy metals*]. PhD dissertation, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Godzik B. 1991. Accumulation of heavy metals in *Biscutella laevigata* (Cruciferae) as a function of their concentration in the substrate. *Polish Botanical Studies* 2: 241–246.

Godzik B. 2015. *Natural and historical values of the Olkusz Ore-bearing Region*. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Grodzińska K, Korzeniak U, Szarek-Łukaszewska G, and Godzik B. 2000. Colonization of zinc mine spoils in southern Poland – preliminary studies on vegetation, seed rain and seed bank. *Fragmenta Floristica and Geobotanica* 45: 123–145.

Grodzińska K, and Szarek-Łukaszewska G. 2002. Hałdy cynkowo-ołowiowe w okolicach Olkusza – przeszłość, teraźniejszość i przyszłość [Zinc-lead waste heaps in the environs of Olkusz – the past, the present and the future]. *Kosmos – Problemy Nauk Biologicznych* 51: 127–138.

Hanć A, Barałkiewicz D, Piechalak A, Tomaszewska B, Wagner B, and Bulska E. 2009. An analysis of long-distance root to leaf transport of lead in *Pisum sativum* plants by laser ablation-ICP-MS. *International Journal of Environmental Analytical Chemistry* 89(8–12): 651–659.

Heil DM, Samani Z, Hanson AT, and Rudd B. 1999. Remediation of lead contaminated soil by EDTA. I. Batch and column studies. *Water Air and Soil Pollution* 113: 77–95.

Kabata-Pendias A. 2011. *Trace elements in soils and plants*. CRC Press, Boca Raton, US.

Kicińska A. 2009. Arsen i tal w glebach i roślinach rejonu Bukowna [Arsenic and thallium in the soil and plants in the area of Bukowno]. *Ochrona Środowiska i Zasobów Naturalnych* 40: 199–208.

Küpper H, Lombi E, Zhao FJ, and Mcgrath SP. 2000. Cellular compartmentation of cadmium and zinc in relation to other metals in the hyperaccumulator *Arabidopsis halleri*. *Planta* 212: 75–84.

Lacoste C, Robinson B, and Brooks R. 2001. Uptake of thallium by vegetables: its significance for human health, phytoremediation, and phytomining. *Journal of Plant Nutrition* 24(8): 1205–1215.

Lacoste C, Robinson B, Brooks R, Anderson C, Chiarucci A, and Leblanc M. 1999. The phytoremediation potential of thallium-contaminated soils using *Iberis* and *Biscutella* species. *International Journal of Phytoremediation* 1(4): 327–338.

Leblanc M, Petit D, Deram A, Robinson BH, and Brooks R. 1999. The phytomining and environmental significance of hyperaccumulation of thallium by *Iberis intermedia* from Southern France. *Economic Geology* 94: 109–114.

LIPHADZI MS, KIRKHAM MB, MANKIN KR, and PAULSEN GM. 2003. EDTA-assisted heavy-metal uptake by poplar and sunflower grown at a long-term sewage-sludge farm. *Plant and Soil* 257: 171–182.

LIS J, PASIECZNA A, KARBOWSKA B, ZEMBRZUSKI W, and ŁUKASZEWSKI Z. 2003. Thallium in soils and stream sediments of a Zn-Pb mining and smelting area. *Environmental Science and Technology* 37: 4569–4572.

MINCZEWSKI, J, MARCZENKO Z. 2004. *Chemia analityczna. Chemiczne metody analizy ilościowej* [*Analytical chemistry. Chemical methods of quantitative analysis*]. Vol. II, PWN, Warszawa.

NRIAGU J.O. 1998. Thallium in the environment. *Advances in Environmental Science and Technology 29*, Wiley and Sons, New York.

OLKO A, ABRATOWSKA A, ŻYŁKOWSKA J, WIERZBICKA M, and TUKIENDORF A. 2008. *Armeria maritima* from a calamine heap – Initial studies on physiologic-metabolic adaptations to metal-enriched soil. *Ecotoxicology and Environmental Safety* 69: 209–218.

POLLARD AJ, REEVES RD, and BAKER AJM. 2014. Facultative hyperaccumulation of heavy metals and metalloids. *Plant Science* 217–218: 8–17.

POŠĆIĆ F, MARCHIOL L, and SCHAT H. 2013. Hyperaccumulation of thallium is population-specific and uncorrelated with caesium accumulation in the thallium hyperaccumulator, *Biscutella laevigata*. *Plant and Soil* 365: 81–91.

POŠĆIĆ F, FELLET G, VISCHI M, CASOLO V, SCHAT H, and MARCHIOL L. 2015. Variation in heavy metal accumulation and genetic diversity at a regional scale among metallicolous and non-metallicolous populations of the facultative metallophyte *Biscutella laevigata* subsp. *laevigata*. *International Journal of Phytoremediation* 17(5): 464–475.

SAGER M. 1994. Thallium. *Toxicological and Environmental Chemistry* 45(1–2): 11–32.

SCHECKEL KG, LOMBI E, ROCK SA, and MCLAUGHLIN MJ. 2004. In vivo synchrotron study of thallium speciation and compartmentation in *Iberis intermedia*. *Environmental Science and Technology* 38: 5095–5100.

SCHECKEL KG, HAMON R, JASSOGNE L, RIVERS M, and LOMBI E. 2007. Synchroton X-ray absorption-edge computed microtomography imaging of thallium compartmentalization in *Iberis intermedia*. *Plant and Soil* 290: 51–60.

SEREGIN IV, and IVANOV VB. 1997. Histochemical investigation of cadmium and lead distribution in plants. *Russian Journal of Plant Physiology* 44: 791–796.

SHEORAN V, SHEORAN AS, and POONIA P. 2009. Phytomining: A review. *Minerals Engineering* 22: 1007–019.

SZAFER W. 1927. *Flora Polska: Rośliny naczyniowe Polski i ziem ościennych* [*The Polish flora: Vascular plants of Poland and neighbouring areas*]. Vol. III, Polska Akademia Umiejętności (PAU), Kraków.

SZAREK-ŁUKASZEWSKA G, and NIKLIŃSKA M. 2002. Concentration of alkaline and heavy metals in *Biscutella laevigata* L. and *Plantago lanceolata* L. growing on calamine spoils (S. Poland). *Acta Biologica Cracoviensia Series Botanica* 44: 29–38.

SZMAL ZS, and LIPIEC T. 1996. *Chemia analityczna z elementami analizy instrumentalnej* [*Analytical chemistry with the elements of instrumental analysis*]. Wydawnictwo Lekarskie PZWL, Warszawa.

TURGUT C, PEPE MK, and CUTRIGHT TJ. 2004. The effect of EDTA and citric acid on phytoremediation of Cd, Cr and Ni from soil using *Helianthus annuus*. *Environmental Pollution* 131: 147–154.

XIAO T, GUHA J, BOYLE D, LIU C-Q, and CHEN J. 2004. Environmental concerns related to high thallium levels in soils and thallium uptake by plants in southwest Guizhou, China. *Science of the Total Environment* 318: 223–244.

WĄCHALEWSKI T. 1999. Kwasowość czynna i potencjalna gleby [Active and potential acidity of soil]. In: Szczepaniec–Cięciak E, Kościelniak P. [eds], *Chemia środowiska: ćwiczenia i seminaria*, Vol. II. 21–24. Uniwersytet Jagielloński, Kraków.

WENZEL WW, and JOCKWER F. 1999. Accumulation of heavy metals in plants grown on mineralized soils of the Austrian Alps. *Environmental Pollution* 104: 145–155.

WIERZBICKA M, and PIELICHOWSKA M. 2004. Adaptation of *Biscutella laevigata* L., a metal hyperaccumulator, to growth on a zinc-lead waste heap in southern Poland. I: Differences between waste-heap and mountain populations. *Chemosphere* 54: 1663–1674.

WIERZBICKA M, SZAREK-ŁUKASZEWSKA G, and GRODZIŃSKA K. 2004. Highly toxic thallium in plants from the vicinity of Olkusz (Poland). *Ecotoxicology and Environmental Safety* 59(1): 84–88.

WIERZBICKA M, DUTKIEWICZ J, WYSOCKA I, BULSKA E, and JANSSENS K. 2007. Biotransformacja selenu w roślinach [Biotransformation of selenium in plants]. In: Wierzbicka M, Bulska E, Pyrzyńska K, Wysocka I, Zachara BA [eds], *Selen pierwiastek ważny dla zdrowia, fascynujący dla badacza*, 88-102. Wyd. Malamut. Warszawa.

WÓYCICKI Z. 1913. *Obrazy roślinności Królestwa Polskiego* [*Vegetation in the Kingdom of Poland*]. IV *Roślinność terenów galmanowych Bolesławia i Olkusza* [*The calamine flora of Boleslaw and Olkusz*], Kasa Mianowskiego, Warszawa.

WYSOCKA I. 2004. Badanie specjacji i metabolizmu selenu w roślinach metodą chromatografii cieczowej połączonej ze spektrometrią mas ze wzbudzeniem w plazmie indukcyjnie sprzężonej [The study on speciation and metabolism of selenium in plants with use of the liquid chromatography method coupled with ICP-MS]. PhD dissertation, Wydział Chemii, Uniwersytet Warszawski.

VAN DER ENT A, BAKER AJM, REEVES RD, POLLARD AJ, and SCHAT H. 2013. Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. *Plant and Soil* 362: 319–334.

VANĚK A, KOMÁREK M, CHRASTNÝ V, BEČKA D, MIHALJEVIČ M, ŠEBEK O, PANŭSKOVÁ G, and SCHUSTEROVÁ Z. 2010. Thallium uptake by white mustard (*Sinapis alba* L.) grown on moderately contaminated soils—Agro-environmental implications. *Journal of Hazardous Materials* 182: 303–308.