THALLIUM HYPERACCUMULATION IN POLISH POPULATIONS OF *Biscutella laevigata* (Brassicaceae)

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*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 kg⁻¹ d.m., only (1.1–2.1) mg 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 kg⁻¹ d.m. The largest content was found in leaves – 164.9 mg kg⁻¹ d.m. (max. 588.2 mg 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 kg⁻¹ d.m., max. in leaves – 138.4 mg kg⁻¹ d.m. (max. 1100 mg 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 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

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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·kg⁻¹d.m. of Tl, whereas from the flotation tailings – 30–40 mg·kg⁻¹d.m. (Dmowski and Badurek, 2002). For comparison, Tl content in uncontaminated soil amounts to 0.2-1 mg·kg⁻¹d.m. (Crößmann, 1984) or 0.02–2.8 mg·kg⁻¹d.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 (Escarré 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·kg⁻¹d.m. (from 11 to 57 mg·kg⁻¹d.m.), and in the plants 504 mg·kg⁻¹d.m. (from 65 to 3920 mg·kg⁻¹d.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-Lukaszewska, 2002; Szarek-Lukaszewska 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 scree; 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 open cast 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-Lukaszewska 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; Hanč 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 29ºC/18ºC (day/night). Altogether, 112 plants were cultivated.

PLANT CULTIVATION IN CALAMINE SOIL WITH ADDED EDTA

To enhance the bioavailability of metals, an EDTA solution (Na₂H₂EDTA·H₂O) was added to calamine soil. The salt solubility is equal to 11.1 g/100 ml H₂O at temp. 21ºC. 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 29ºC/18ºC (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
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 HNO₃ : H₂O₂ (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 HNO₃ 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 (Wachalewski, 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 CaCl₂; 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·kg⁻¹·d.m. and 15.2 mg·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·kg⁻¹·d.m. and 3.1 mg·kg⁻¹·d.m., respectively), similarly in CaCl₂ (2.1 mg·kg⁻¹·d.m. and 1.1 mg·kg⁻¹·d.m., respectively). The calamine soil was reasonably rich in potassium (3004 mg·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).

TABLE 2. Thallium (Tl) and potassium (K) content [mg·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).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Tl Total content [mg·kg⁻¹·d.m.]</th>
<th>Tl Extracted by EDTA [mg·kg⁻¹·d.m.]</th>
<th>Tl Extracted by CaCl₂ [mg·kg⁻¹·d.m.]</th>
<th>K [mg·kg⁻¹·d.m.]</th>
<th>pH N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calamine</td>
<td>66.7±0.4 a</td>
<td>16.4±1.6 a</td>
<td>2.1±0.6 a</td>
<td>3004±104 a</td>
<td>7.81±0.06 a</td>
</tr>
<tr>
<td>Vegetated calamine</td>
<td>15.2±1.5 a</td>
<td>3.1±0.6 a</td>
<td>1.1±0.3 a</td>
<td>not studied</td>
<td>7.4±1.4 a</td>
</tr>
<tr>
<td>Garden</td>
<td>0.0 a</td>
<td>0.0 a</td>
<td>0.0 a</td>
<td>417±61 a</td>
<td>4.97±0.08 a</td>
</tr>
<tr>
<td>Geochemical background</td>
<td>0.02–2.8 a</td>
<td>no data</td>
<td>no data</td>
<td>130-20600 a</td>
<td>5–7 a</td>
</tr>
</tbody>
</table>

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.
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

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**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.

<table>
<thead>
<tr>
<th>Plant age [weeks]</th>
<th>C</th>
<th>M</th>
<th>Overall effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Shoots</td>
<td>Roots</td>
</tr>
<tr>
<td>2</td>
<td>93.6±63 a</td>
<td>126.5±173 a</td>
<td>68.2±176 a</td>
</tr>
<tr>
<td>4</td>
<td>19.0±7 a</td>
<td>155.4±54 b</td>
<td>17.4±6 a</td>
</tr>
<tr>
<td>8</td>
<td>17.0±4.5 a</td>
<td>122.5±21 b</td>
<td>13.0±7 a</td>
</tr>
<tr>
<td>40</td>
<td>11.1±4 a</td>
<td>59.8±24 a</td>
<td>17.0±4.5 a</td>
</tr>
</tbody>
</table>

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 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.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Roots</th>
<th>Fresh leaves</th>
<th>Dry leaves</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calamine</td>
<td>51.5±23 a</td>
<td>135.7±45 a</td>
<td>21.2±2.5 a</td>
<td>69.5 a</td>
</tr>
<tr>
<td>Calamine with EDTA</td>
<td>78.7±33.5 a</td>
<td>138.4±70.2 a</td>
<td>37.1±13.3 b</td>
<td>84.8 a</td>
</tr>
</tbody>
</table>

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 [1] 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.
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⁻¹d.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 plasmodesmata (data not presented).

The investigation of the thallium presence in trichomes on the *B. leavigata* 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).

**TABLE 5.** Potassium content [mg·kg⁻¹d.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.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Roots***</th>
<th>Fresh leaves***</th>
<th>Dry leaves***</th>
<th>Overall effect***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calamine</td>
<td>2005±306 a</td>
<td>8063±4866 a</td>
<td>4149±2657 a</td>
<td>4739 a</td>
</tr>
<tr>
<td>Calamine with EDTA</td>
<td>1706±511 a</td>
<td>3608±434 a</td>
<td>3037±548 b</td>
<td>2783 a</td>
</tr>
<tr>
<td>Garden</td>
<td>1081±4008 b</td>
<td>6616±28471 b</td>
<td>4343±7841 c</td>
<td>40136 b</td>
</tr>
<tr>
<td>Garden with EDTA</td>
<td>7956±1670 b</td>
<td>78854±18325 b</td>
<td>49922±12600 c</td>
<td>45577 b</td>
</tr>
</tbody>
</table>

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.
In Poland in the metalliferous post-industrial areas of Olkusz, the amount of thallium in soils amounts to 29 to 44 mg\textcdot kg$^{-1}$d.m., whereas in green plants, from 7.9 to 25.5 mg\textcdot kg$^{-1}$d.m. (Kiciński, 2009). It is noteworthy that in green plants the Tl content should be 0.05 mg\textcdot 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\textcdot kg$^{-1}$d.m., Silene vulgaris – 7 mg\textcdot kg$^{-1}$d.m., Dianthus carthusianorum – 7 mg\textcdot 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; Poščić 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.

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\textcdot 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\textcdot 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\textcdot kg$^{-1}$ EDTA.

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.

**BISCUETILLA 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, the youngest (2-week-old) plants contained on average 108.9 mg·kg⁻¹·d.m., while the oldest drying leaves (which 138.4 mg·kg⁻¹·d.m. and 43 mg·kg⁻¹·d.m., respectively).

In this 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 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 TI amount that was increased but still smaller than the accepted boundary of 100 μg g⁻¹ (100 mg 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 TI 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 TI 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 TI 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 TI 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 TI 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 TI 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 et 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, TI 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 Boleslaw 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 TI 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 TI 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;
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