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RESPONSE OF *ARMERIA MARITIMA* (MILL.) WILLD. TO CD, ZN AND PB

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The response of *Armeria maritima* to heavy metals was examined in plants from two populations, one from metalliferous and the other from non-metalliferous soil. Concentrations of metals (Cd, Zn, Pb) were determined in organs of plants growing on 100-year-old calamine spoil (S Poland) and in a "clean" area (central Poland), and in the first generations of those plants grown in hydroponic culture with addition of metals. Large differences between root and green leaf concentrations of metals were found, indicating restriction of transport of metals from roots to aboveground parts in *A. maritima*, and an exclusion strategy. Even under full availability of metals (hydroponic culture), adult plants effectively limited the flow of metals to their aboveground parts. In *A. maritima* from calamine populations, part of the metals transported to aboveground plant organs were directed to the oldest leaves. Plants from the "clean" population growing in a non-metalliferous area did not accumulate heavy metals in ageing leaves. It seems that the ability to accumulate metals in withering leaves characterizes plants growing under strong environmental pressure from metal contamination, in which one may expect intensification of metal detoxification processes.

Key words: *Armeria maritima*, heavy metals, calamine spoils, hydroponic culture.

INTRODUCTION

Adaptation of plants to an environmental stress factor may be the result of different strategies based on preventing or tolerating the operation of a given factor. The tolerance strategies of plants to heavy metals have been widely described (e.g., Antosiewicz, 1992; Streit and Stumm, 1993; Ernst, 1998; Leopold et al., 1999; Clemens et al., 2002). On the level of the organism one may distinguish two main strategies of plants in response to an excess of heavy metals in the environment: exclusion and accumulation. Exclusion consists in immobilizing metals in roots and considerably limiting their transport to shoots. This strategy is most often used by pseudometallophytes, plants inhabiting areas with high concentrations of heavy metals in soil as well as areas without these elements (Punz, 1995). Accumulation consists in accumulating metals in aboveground shoots and is observed more often in metallophytes, plants growing exclusively in areas with high concentrations of heavy metals in soils (Punz, 1995).

Armeria maritima (Mill.) Willd. (Plumbaginaceae) is a perennial occurring commonly in Europe. In Poland it is common in the western part of the lowland, becoming rarer to the east and south. It grows mostly in sandy places. In the environs of Olkusz it occurs numerously on calamine spoils (zinc-lead) where soils contain large amounts of Cd, Zn, and Pb (Grodzińska and Szarek-Łukaszewska, 2002).

This paper investigates the response of *Armeria maritima* from two populations, one from calamine

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spoils and the other from a non-metalliferous area, to heavy metals. The concentrations of metals were determined in plants growing in calamine spoils and in the 'clean' control area, as well as in first-generation plants from the two populations, grown in hydroponic culture with the addition of metals.

MATERIALS AND METHODS

Samples of *Armeria maritima* were collected from a calamine spoil more than 100 years old in Bolesław near Olkusz (S Poland) and a 'clean' site in Olsztyn near Częstochowa (central Poland). Total heavy metals content in the soil from the calamine spoils was high (~200 mg/kg Cd, 3100 mg/kg Pb, 52000 mg/kg Zn), associated with the high concentrations of metals in the material forming the mine spoils (Szarek-Łukaszewska and Niklińska, 2002). The amount of heavy metals in soils from the 'clean' area was low and did not exceed the geochemical background for Polish soils (0.03–1 mg/kg Cd, 0.5–21.0 mg/kg Pb, 5–59 mg/kg Zn; Czarnowska, 1996).

Collected individuals were divided into green leaves, brown leaves (dead and withering), lower multi-annual part of shoots (underground) and roots. Plants were rinsed with deionized water and cleaned by ultrasound to remove soil and dust particles. Air-dried samples were ground and dried to constant weight (105°C), and then ~0.1 g of each sample was mineralized in $HClO_4$+HNO_3 in a 1:4 ratio. Heavy metal concentrations were determined using flame (FAAS) and graphite furnace (GFAAS) atomic absorption spectrometry (Varian 220 SS). As the control, elements were determined in reference materials SRM 1575 and SRM 1573a.

Seeds of *A. maritima* were collected from the calamine spoil in Bolesław (calamine spoil population, identified also as *A. maritima* subsp. *halleri*; Szafer and Zarzycki, 1972) and from a 'clean' area in Osowiec near the Biebrza National Park (population determined as *A. maritima* subsp. *elongata*; Szafer and Zarzycki, 1972). Plants were grown from these seeds in garden mold under greenhouse conditions. After old leaves and generative shoots were removed, one-year-old plants were transplanted to hydroponic culture, initially for 7 days in pure medium (culture in 1/2 Knop medium) and then with metal chlorides added to the culture. Individuals in groups of five (average biomass ~10 g per plant at the beginning of cultivation) were grown in 6-liter containers in culture solution (control) or in solutions with the following concentrations of metals: 50 mg/l Zn^{+2}, 5 mg/l Pb^{+2}, 5 mg/l Cd^{+2}. Mineral culture did not contain phosphates, so that precipitation of insoluble metal salts could be avoided. Stable concentrations of metals were maintained in the culture. Based on daily AAS measurements of removal of heavy metals from cultures during cultivation days, the solutions in the experimental groups were changed in the following order: with Pb^{+2} after 2 days, with Zn^{+2} after 4 days, and with Cd^{+2} after 7 days of cultivation. The control solution was also changed every 7 days. The solutions were continuously aerated. At the first symptoms of weakening of plants treated with metals, that is, after 30 days, cultivation was stopped. Then the plants were divided into young green leaves (developed during growth in culture), mature green leaves, brown (withering) leaves, and roots. During the experiment the plants in every experimental group flowered, so generative shoots and inflorescences also were separated. The concentration of metals (Zn, Cd, Pb) were determined in the particular parts of plants by the methods described above.

The localization of metals in plants grown in hydroponic mineral culture with Zn, Pb and Cd was determined using the histochemical method of detecting metals with dithizone (diphenylthiocarbazone) (Lipiec and Szmal, 1980; Seregin et al., 2002), as modified and described by Pielichowska and Wierzbicka (2004).

Metal concentrations were compared in plant organs with Friedman's test and in populations with the Kolmogorov-Smirnov test (Birkit, 1987). The Statgraphics package was used.

RESULTS

METAL CONCENTRATIONS IN SPECIMENS GROWING IN THE FIELD

Concentrations of metals in plant organs collected from the calamine spoil and the non-polluted area are shown in Figure 1. Plants from the calamine spoil contained much higher levels of metals than plants from the 'clean' area. Cadmium and lead were accumulated in plants from both populations, following a similar pattern. The largest amounts of both elements were found in roots (R) and brown leaves (Bl). In the mine spoil population they were 6 times higher than in green leaves (Gl) and underground shoots (Us) in the case of Cd, and 9 times higher in the case of Pb. In the 'clean' population the differences in concentrations of cadmium between

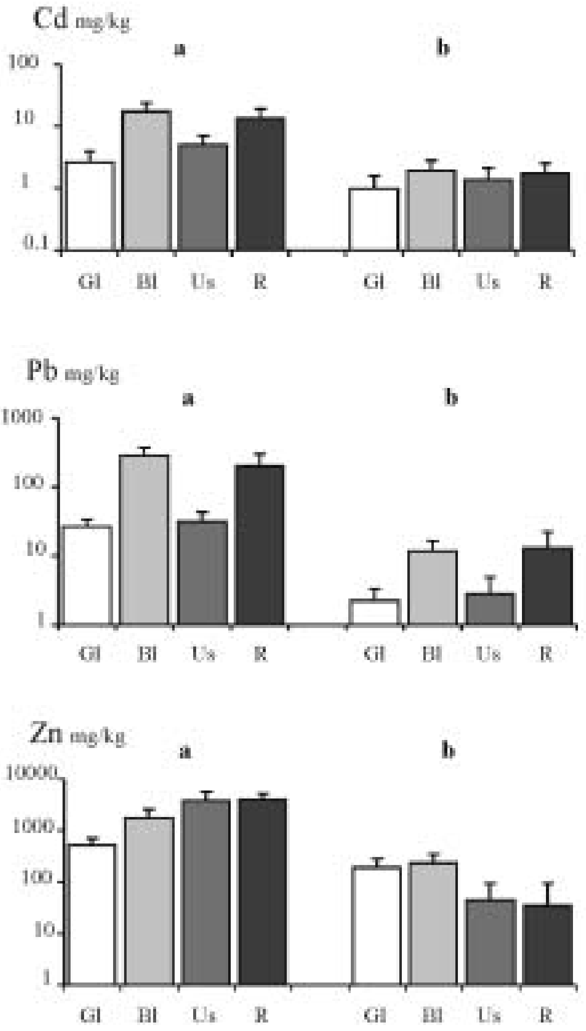

Fig. 1. Concentrations of heavy metals (Cd, Pb, Zn) in *Armeria maritima* from calamine spoils (**a**) and a non-metalliferous area (**b**); Gl – green leaves; Bl – brown leaves; Us – upper part of shoots; R – roots.

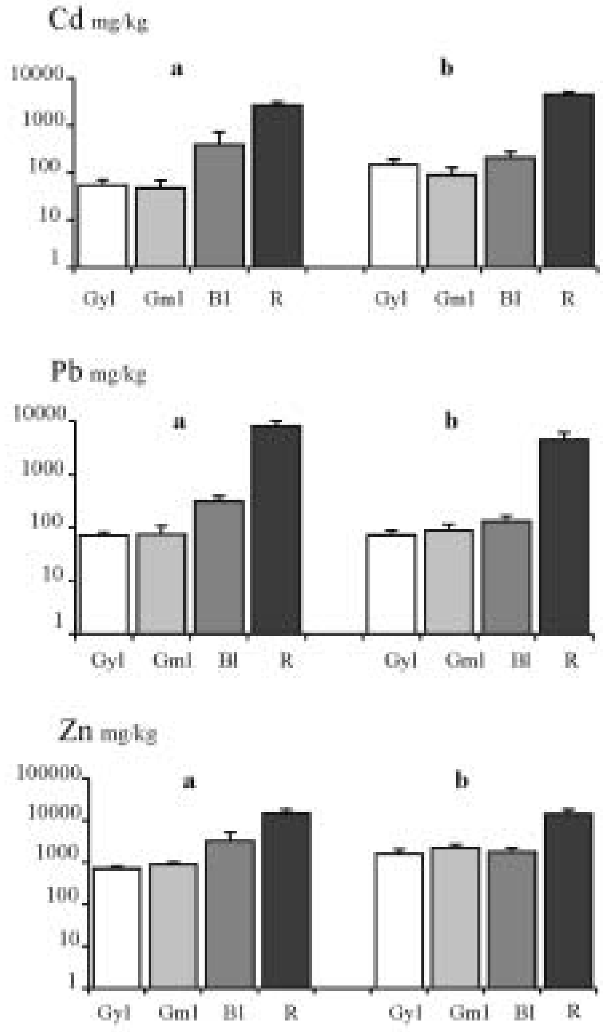

Fig. 2. Concentrations of heavy metals (Cd, Pb, Zn) in *Armeria maritima* plants from hydroponic cultures with metals. (**a**) Plants of calamine population, (**b**) Plants of population from non-metalliferous area. Gyl – green young leaves; Gml – green mature leaves; Bl – brown leaves; R – roots. Bars indicate arithmetic mean ± standard deviation.

organs were small; only green leaves contained significantly less Cd ($p < 0.05$). Concentrations of lead were 5 times higher in roots and brown leaves than in green leaves and underground shoots. In plants from the mine spoil population, zinc was accumulated 3 times less in brown old leaves and 8 times less in green leaves than in roots and underground shoots. In the 'clean' population, Zn was accumulated mainly in leaves.

To determine any differences in the response to heavy metals of plants from the *A. maritima* populations from calamine spoils and from the 'clean' area, we performed laboratory experiments on the first plant generations from both populations.

METAL CONCENTRATIONS IN SPECIMENS TREATED WITH METALS IN HYDROPONIC CULTURE

Plants grown in hydroponic culture with the addition of metals, like plants growing in the field, accumulated metals in roots in amounts much higher than in green leaves (Fig. 2). In withering leaves of the mine spoil population there were about 6 times more Cd, twice more Pb and 4 times more Zn than in green leaves after 30 days in culture. On the other hand, in the 'clean' population the concentrations of Cd and Zn were similar in withering and green

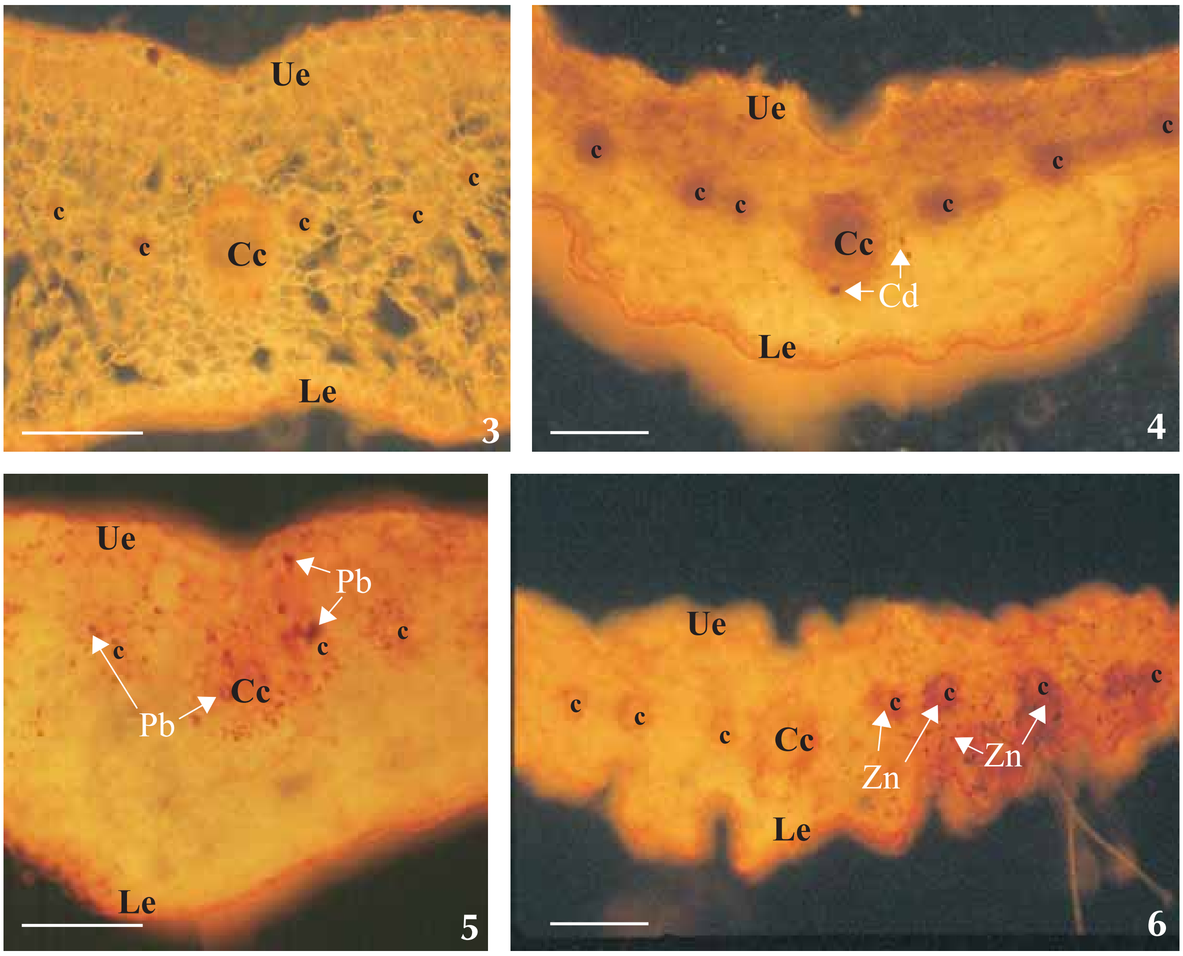

Fig. 3. Old leaves of *Armeria maritima* plants from calamine population in hydroponic culture – control plants (without metals); c – conductive tissue; Cc – central conductive tissue; Ue – upper epidermis; Le – lower epidermis. Bar = 0.1 mm. **Fig. 4.** Localization of Cd in old leaves of *Armeria maritima* using dithizone method – plants from calamine population in hydroponic culture – treatment with 5 mg/l Cd^{2+}. c – conductive tissue; Cc – central conductive tissue; Ue – upper epidermis; Le – lower epidermis; Cd – metal-dithizone complex with Cd^{2+}. Bar = 0.1 mm. **Fig. 5.** Localization of Pb in old leaves of *Armeria maritima* using dithizone method – plants from calamine population in hydroponic culture – treatment with 5 mg/l Pb^{2+}. c – conductive tissue; Cc – central conductive tissue; Ue – upper epidermis; Le – lower epidermis; Pb – metal-dithizone complex with Pb^{2+}. Bar = 0.1 mm. **Fig. 6.** Localization of Zn in old leaves of *Armeria maritima* using dithizone method – plants from calamine population in hydroponic culture – treatment with 50 mg/l Zn^{2+}. c – conductive tissue; Cc – central conductive tissue; Ue – upper epidermis; Le – lower epidermis; Zn – metal-dithizone complex with Zn^{2+}. Bar = 0.1 mm.

leaves, and Pb concentrations were slightly higher in withering leaves. In generative shoots and inflorescences the metal concentrations were low (< 0.1 mg/kg Cd, < 0.5 mg/kg Pb, < 0.5 mg/kg Zn).

LOCALIZATION OF METALS IN LEAVES

Localization of metals was analyzed in the oldest withering leaves of *A. maritima* treated with Cd, Zn and Pb, because these leaves were the aboveground organs accumulating the largest amounts of metals. In withering leaves the largest concentrations of metals were found in conductive tissues and in parenchymal cells surrounding conductive tissues (Figs. 3–6). Metals were also detected in cell walls in the parenchyma and epidermis.

DISCUSSION

In *Armeria maritima* growing on the calamine spoil in Bolesław the amounts of metals (Cd, Zn, Pb) found in leaves were within or exceeding the ranges of metal concentrations considered toxic for plants (8–12 mg/kg Cd, to 30 mg/kg Pb, 200–400 mg/kg Zn) (Balsberg-Påhlsson, 1989; Kabata-Pendias and Pendias, 1993). Other plant species (e.g., *Biscutella laevigata*, *Plantago lanceolata*, *Dianthus carthusianorum*, *Silene vulgaris*) growing on calamine spoils have shown high concentrations of metals – Pb, Cd, Zn and Tl (Szarek-Łukaszewska and Niklińska, 2002; Wierzbicka et al., 2004).

Armeria maritima growing on calamine spoils takes up large amounts of heavy metals, and applies an exclusion strategy. These plants as well as plants from the calamine population grown in laboratory conditions accumulated the metals in roots; lead, less mobile, seemed to be accumulated the most. In this way, adult plants effectively limited the flow of metals to their aboveground parts, even under full availability of metals (hydroponic culture). Part of the metals transported to aboveground plant organs were directed to the oldest leaves. It seems that in these leaves the metals are transported mainly by conductive tissues, penetrating parenchymal and epidermal cells by both apoplastic and symplastic transport. In the *A. maritima* rosette of leaves there are both green, withering and dead leaves, which remain on the shoot for a long time. Metals may be accumulated gradually in the older leaves. In the plants from the population growing on the metalliferous soil of a calamine spoil, older leaves played an important role in the accumulation of heavy metals. It is a genetically fixed adaptation. The exclusion strategy and the mechanism of detoxification by old leaves was described in *A. maritima* growing near a metal smelter (Dahmani-Müller et al., 2000). Many metal-exposed plants employ a strategy consisting in retaining metals mainly in roots. This has been also found in other species occurring on calamine spoils (Szarek-Łukaszewska and Niklińska, 2002).

Do the *Armeria maritima* populations from the metal-polluted and 'clean' areas respond to exposure to heavy metals in the same way? Our laboratory experiments showed that adult plants from both populations blocked metals in roots. Plants from the calamine population also accumulated large amounts of metals in ageing leaves, unlike the plants from the 'clean' population growing in a non-metalliferous area. Plants from the latter population did not use their old leaves as a dustbin for metals. It seems that the ability to accumulate metals in withering leaves is characteristic of plants growing under the strong environmental pressure of metal contamination, in which one may expect intensification of metal detoxification processes. In plants, one of these processes is directing metals to the oldest withering leaves (Ernst, 1998). A similar phenomenon was observed in *Biscutella laevigata*, another plant species occurring on calamine spoils in Bolesław (Pielichowska and Wierzbicka, 2004). Other adaptations of plants from calamine populations to growth on soil with heavy metals were described by Wierzbicka and Panufnik (1998), Załęcka and Wierzbicka (2002), Wierzbicka and Pielichowska (2004), and Baranowska-Morek and Wierzbicka (2004).

During the laboratory experiment (hydroponic culture with addition of metals), adult individuals of *A. maritima* from both the calamine and 'clean' populations developed new leaves and flowered. Köhl (1997) carried out experiments on the long-term growth response of *A. maritima* from metalliferous and non-metalliferous populations to elevated Zn concentrations in artificial soil, and found that the metal tolerance of all populations was enough for their survival on Zn-rich soils. The high Zn tolerance of all *A. maritima* populations would facilitate colonization of Zn-contaminated sites from adjacent populations, so that it could take place without strong selection of tolerance-related genes.

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