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Metal Hyperaccumulation in *Cardaria draba* (L.) Desv. (*Brassicaceae*) and Heavy Metal Effects on the Nematodes and a Weevil Associated with the Plant Roots in Sites near a Non-Ferrous Metal Smelter in Bulgaria

Abstract

The heavy metal hyperaccumulation in *Cardaria draba* (L.) Desv. was studied in sites with different level of contamination with Pb, Cd, Cu and Zn near a non-ferrous metal smelter in Bulgaria. The hyperaccumulation levels of the metal concentrations in shoots were reached in the extremely contaminated site for Pb and Cd. The Enrichment factor was more than one for Cd in sites with low and medium level of contamination and for Zn in moderately and extremely contaminated sites. The shoot concentrations of all metals in the extremely contaminated site were between 13 and 48 times higher than in the least contaminated site.

The low number of the plant feeding nematodes and the root weevil galls in the highly and extremely contaminated sites were regarded as a result of the successful elemental (heavy metals) defense of the plant against the nematode and insect herbivores. The higher number of the fungal feeding nematodes in the extremely contaminated site in the absence of the weevil root galls, might indicate a better development of the fungi associated with the roots in this site in comparison with the rest of the sites.

Key words: *Cardaria draba*, metal hyperaccumulation, heavy metal, soil pollution, nematode

Introduction

Large areas near metal production and processing facilities have been chronically exposed to high pollution from several heavy metals or metalloids. The fate and behavior of the metals, as well as the biological and biochemical processes that control their mobility and bioavailability in soil were subject of numerous studies (McGrath et al., 1995; Violante et al., 2010).

Soil heavy metals threaten the ecosystems and human health in many ways: direct ingestion, food chains (through plants and animals), contaminated water, reduction in food quality, phytotoxicity and reduction in the agricultural use of land for food and forage production (Wuana and Okieimen, 2011).

Plants growing on the metal contaminated soil have been classified as: excluders, which maintain low level of the shoot heavy metal concentrations over a wide range of soil levels; accumulators, that concentrate metals in their above-ground parts in levels much higher than in the soil and indicators, in which the accumulated shoot concentrations reflect the level of the soil metal contamination (Baker, 1981). Metal accumulation and hyperaccumulation potential of the plants have been extensively discussed in relation to their possible use in phytoremediation of soil metal contamination (Prasad and Freitas, 2003; Alkorta et al., 2004; Suchkova et al., 2014).

Awere et al. (2014) reported 500 plant species – hyperaccumulators of heavy metals, belonging to 101 families and the majority of the plant species accumulated two, few accumulated three and only *Thlaspi caerulescens* (the best known hyperaccumulator) accumulated four heavy metals (Pb, Cd, Ni, Zn). According to Reeves and Baker (2000), Ni is the most commonly accumulated metal, followed by Cu, Pb, Zn and plants – Cd hyperaccumulators are very rare and only five species have been reported till now (Rascio and Navari-Izzo, 2011). About 25% of all hyperaccumulators belong to the family *Brassicaceae* and the potential of the family in phytoremediation and biofumigation have been discussed by Szczgłowska et al. (2011) and Pantola and Alam (2014).

The metal hyperaccumulation in plants might serve as a self-defense mechanism against the herbivores and pathogens, as suggested by Martens and Boyd (1994) and accepted by other authors (Peters, 2004). This hypothesis was supported by several experimental data (Behmer et al., 2005; Coleman et al., 2005; Jiang et al., 2005). Most of the plants in *Brassicaceae* family are known to have also a strong chemical defense, which includes glucosinolates against herbivores and pathogens (Van Dam et al., 2009; Chaplin-Kramer et al., 2011; Bohinc et al., 2012). Rascio and Navari-Izzo (2011) suggested that many hyperaccumulators from the mustard family may have a combined elemental (inorganic) from the heavy metal defense and chemical (organic) from the secondary metabolites defense against herbivores and pathogens.

Cardaria draba (L.) Desv., known as hoary cress, or white top, is a perennial weed that often forms monoculture stands in many disturbed areas, along the roadsides, ditch banks and landmark sites. The hoary cress often invaded pastures and meadows and reduced the biodiversity and forage production (Jacobs, 2007; Graves-Medley and Mangold, 2011) and had been reported to have some forage value in the early growth stages before flowering (McInnis et al., 1993).

The potential of *C. draba* as an accumulator of high heavy metal concentrations in the aboveground parts was studied by Ghaderian et al. (2007), Cheragi et al. (2011) and Mohsenzadeh and Rad (2012). The glucosinolate production was also reported for *C. draba* (Kiemnec and McInnis, 2002; Miri et al., 2013). The herbivore fauna, associated with *C. draba* was studied in attempts to find a biological control agent for this noxious weed species (Fumanal et al., 2004; Cripps et al., 2006a; 2006b, Talmaciu et al., 2010).

The aim of our study was to compare the metal accumulation potential of *C. draba* in sites with low, medium, high and extreme levels of soil contamination in the area of a non-ferrous smelter in Bulgaria and to assess the metal effects on the nematodes, associated with the plant roots, as well as on the present weevil root herbivores.

Material and Methods

Experimental sites

Five experimental sites were selected with different level of soil heavy metal contamination: low (L), medium (M_1, M_2), high (H) and extreme (E) in the area of the non-ferrous metal plant – KCM, Plovdiv, situated in the Central South Bulgaria. Four of the sites were in south-eastern direction at 300 m (E), 800 m (H), 2000 m (M_2) and 5000 m (L) from the smelter and one site at 2000 m (M_1) in north-eastern direction. The soil in all sites was classified as Alluvial (Deluvial) meadow soil (Eutric Fluvisols) with humus content between 1.73% and 2.42%.

Plant and soil sampling and analyses

From each site between 40 and 60 plants of *C. draba* were gathered in May, 2012 during the flowering of the plants and in June, 2013 at the end of the vegetation period during the seed production. The shoots were thoroughly washed by the tap water and were dried and ground into a powder and Zn, Cu, Pb and Cd concentrations were measured according to the relevant ISO methods (ISO 11466:1995).

Bulk soil samples were taken from each experimental sites in May, 2012 and in June, 2013. Soil was sieved through 2.5 mm sieve and the soil samples were analyzed for the total metal concentrations of Pb, Zn, Cd and Cu according to the relevant ISO methods (ISO 11466:1995).

Nematode analysis

The roots of *C. draba* gathered in May, 2012 were thoroughly washed with tap water and were separated in two groups: with or without insect galls of a weevil *Ceutorhynchus* sp. (fam. *Curculionidae*, *Coleoptera*). From each site two mixed sample were made from damaged + undamaged roots (1: 1, by weight), 15 gram each, except in the extremely contaminated site, where no insect-galled roots were found. The weevil larvae were removed from the galls and the root material was cut in small pieces, homogenized and the nematodes were extracted by Baermann funnel technique and 48 h exposure. Nematodes were killed by heat (3 minutes at 60 °C), fixed in 4% formaldehyde and mounted in semi-permanent slides. Nematodes were identified till genus level. They were separated in trophic groups according to Yeates et al. (1993) and fungal to bacterial feeders ratio (Fu/Fu + Ba) was also used. ANOVA and Pearson's correlation test were applied in the data analyses.

Results and Discussion

Heavy metal concentrations in plant shoots and in soil

The lowest concentrations of Cu, Pb and Cd in the plant material were found in the least contaminated site (L) and only for Zn the lowest concentrations were in M_1 site. The highest concentrations for all metals in shoots were measured in the extremely contaminated site (E) – Table 1. For all metals, except for Zn, there were positive correlations of the metal concentrations in shoots and the soil metal concentrations (for Cu: $r = 0.82$, $P < 0.01$, $n = 10$; for Pb: $r = 0.88$, $P < 0.001$, $n = 10$ and for Cd: $r = 0.99$, $P < 0.001$, $n = 10$). In most of the sites the metal concentrations in shoots in 2013 were higher than in 2012.

Pb and Cd in plant material were found in concentrations above the hyperaccumulation level in the extremely contaminated site for Pb (1256 µg/g dw and 4450 µg/g dw) at both samplings and for Cd (137 µg/g dw) at the second sampling. At the same site Zn (3422 µg/g dw) and Cu (475 µg/g dw) in plant material were at the accumulation levels (Table 1). The aboveground plant material in the highly contaminated site contained Pb (499 µg/g dw; 618 µg/g dw) and Cd (36 µg/g dw; 31 µg/g dw) above the accumulation levels for these metals. For Pb this level was also reached in the sites with the medium contamination (M_1, M_2) at the second sampling (Table 1).

The Enrichment factor exceeded one (EF > 1) for Cd (1.5 and 1.6) in the least contaminated site and in M_2 site (2.6) in 2012 and for Zn in M_1 (12 and 17) and in E site (2.2) in 2013 (Table 1).

The heavy metal concentrations in the plant material from the extremely contaminated site were more than ten times (12 – 48) higher than these concentrations in the shoots from the least contaminated site for all metals and for Pb and Cd in highly contaminated site, as well (Table 2).

Nematodes and insects associated with the roots of the plants

Insect root herbivores

The observed galls on the roots of *C. draba* were very similar to the typical galls of the *Ceutorhynchus* sp. (fam. *Curculionidae*, *Coleoptera*). The highest number of the roots with root weevil galls were observed in sites with the medium level of contamination (M_1 – 15% and M_2 – 41%) and less in the least (6%) and highly contaminated sites (1%). No root galls were observed in plants from the extremely contaminated site at both samplings.

Nematodes associated with roots

Total number of the nematodes isolated from the plant roots varied between 25 and 35 ind/g fw in the different sites. These nematodes belonged to 27 genera and more genera (17 – 20) were identified in the less contaminated sites (L, M_1) in comparison with the rest of the sites (12 – 14 genera) – Table 3.

The most abundant nematodes (32 – 69%) in the sites with the medium and high level of heavy metal contamination (M_1, M_2, H) belonged to the genus *Panagrolaimus*. The same nematodes were less represented in L and E sites (10 – 11%), where *Eucephalobus* (24%) dominated in L and *Ditylenchus* (27%) dominated in E site.

The bacterial feeders were the most abundant nematodes in the root material from all sites with the lowest relative abundance (49%) in the extremely contaminated site and higher in the rest of the sites (70 – 84%) – Table 4. The fungal feeders in the E site contained 43% of all nematodes and in the rest of the sites they were less represented (12 – 26%). The fungal to bacterial feeders ratio (0.47) was highest in the extremely contaminated site and had lower values in sites with the medium or high level of

Table 1. Heavy metal concentration (µg/g dw) in shoots of C. draba and in soil and the Enrichment factor (EF) in sites with low (L), medium (M_1, M_2), high (H) and extreme (E) level of heavy metal contamination
Таблица 1. Концентрации на тежките метали (µg/g dw) в надземната част на C. draba и в почви, и фактор на обогатяването (EF) в площадки с ниско (L), средно (M_1, M_2), високо (H) и много високо (E) ниво на замърсяване

		L	M_1	M_2	H	E
Zn	shoots	69	125	175	562	1025
	soil	130	10.5	350	2700	5900
2012	EF	0.53a	11.90b*	0.50a	0.21a	0.17a
Zn	shoots	137	203	281	775	3422
	soil	220	11.75	950	1725	1525
2013	EF	0.62a	17.28b*	0.30a	0.45a	2.24a*
Cu	shoots	6	9	11	24	79
	soil	37a	203.5a	54a	191a	487.5b
2012	EF	0.16	0.04	0.20	0.13	0.16
Cu	shoots	10	15	24	36	475
	soil	46.5a	204a	97.5a	136.5a	617.5b
2013	EF	0.22	0.07	0.25	0.26	0.77
Pb	shoots	33	40	70	499	1256**
	soil	101a	490a	213a	2225a	6000b
2012	EF	0.33A	0.08A	0.33A	0.22A	0.21A
Pb	shoots	97	162	310	618	4450**
	soil	113a	529a	533a	1075a	7175b
2013	EF	0.86B	0.31B	0.58B	0.57B	0.62B
Cd	shoots	3a	4a	14a	36a	93b
	soil	2a	8a	5.5a	45.5a	117.5b
2012	EF	1.50*	0.50	2.55*	0.79	0.79
Cd	shoots	4a	6a	12a	31a	137b**
	soil	2.5a	11.5a	15a	33.5a	147.5b
2013	EF	1.60*	0.52	0.80	0.93	0.93

Small letters indicate significant differences between the sites within a sampling and the capital letters indicate significant differences between samplings within a site, P < 0.05, *EF > 1; **Zn > 10 000 µg/g; Pb, Cu > 1000 µg/g and Cd > 100 µg/g.
Различните малки букви показват достоверните различия между площадките от едно вземане, а главните букви – между вземанията на една и съща площадка, P < 0,05, *EF > 1; **Zn > 10 000 µg/g; Pb, Cu > 1000 µg/g и Cd >100 µg/g.

contamination (Table 4). The abundance of the fungal feeding nematodes correlated positively with the plant concentrations of Cu ($r = 0.94$, $P < 0.05$, $n = 10$), Pb ($r = 0.89$, $P < 0.05$, $n = 10$) and Cd ($r = 0.91$, $P < 0.05$, $n = 10$), as well as with the soil concentrations of Pb ($r = 0.89$, $P < 0.05$, $n = 10$) and Cd ($r = 0.88$, $P < 0.05$, $n = 10$).

The omnivorous nematodes were isolated from the roots in higher abundance (6%) only in the L site and they were less represented in the rest of the sites and were not found in the highly contaminated site. The carnivorous nematodes from the guild of the general opportunists (Ca_2, *Seinura*) were present only in the extremely contaminated site and were not found in the rest of the sites.

The plant feeding nematodes were isolated from the root material in very low number, high-

Table 2. The ratio of the heavy metal concentrations in shoots of C. draba from sites with medium (M), high (H)and extreme (E) contamination to the concentrations in shoots of the plants from the least contaminated (L) site

Таблица 2. Съотношение на концентрациите на тежките метали в надземната част на C. draba от площадките със средно (M_1, M_2), високо (H) и много високо (E) ниво на замърсяване и концентрациите в най-слабо замърсената площадка (L)

Heavy metal	Year	M_1/L	M_2/L	H/L	E/L
Zn	2012	1.81	2.54	8.14	14.86*
	2013	1.48	2.05	5.66	24.98*
Cu	2012	1.50	1.83	4.00	13.17*
	2013	1.50	2.40	3.60	47.50*
Pb	2012	1.21a	2.12a	15.12a*	38.06b*
	2013	1.67a	3.20a	6.37a	45.88b*
Cd	2012	1.33a	4.67a	12.00a*	31.00b*
	2013	1.50a	3.00a	7.75a	34.25b*

Different letters indicate significant differences, P < 0.05, *ratio >10.
Различните букви показват достоверните различия между площадките P < 0,05, *съотношение > 10.

est for the L site (3.3%) and less than 1% in the rest of the sites (Table 4). The root endoparasitic nematodes (*Pratylenchus*) were isolated in very low numbers (0.9%) only from the roots from the least contaminated site (Table 3).

Metal hyperaccumulation in *C. draba*

Reeves and Baker (2000) divided plants into three categories: normal, accumulators and hyperaccumulators, on the base of their heavy metal concentrations in the above ground material. Normal plants, according to them, contain low concentrations of Cd (0.1 – 3 µg/g dw), Pb (0.1 – 5 µg/g dw), Cu (5 – 25 µg/g dw) and Zn (20 – 400 µg/g dw). The accumulator plants contain higher metal concentrations: Cd > 20 µg/g dw; Cu, Pb > 100 µg/g dw and Zn > 2000 µg/g dw. The hyperaccumulators contain remarkably high shoot concentrations of Cd (> 100 µg/g dw), Cu, Pb (> 1000 µg/g dw) and Zn (> 10 000 µg/g dw). The heavy metal concentrations in the shoots have been considered as the first criterion to define a plant as a hyperaccumulator (Baker and Brookes, 1989).

In addition of that two more criteria have been suggested: Transfer factor to be more than one (TF > 1). This means that the concentration of the heavy metals in shoots are higher than the concentrations in roots (Baker et al., 1994) and the Enrichment factor (EF > 1), the heavy metal concentrations in shoots to be higher than in soil (McGrath and Zhao, 2003; Yanqun et al., 2005, Kazemeini et al., 2013). Van der Ent et al. (2013) suggested that the active accumulation of the heavy metals in the shoots was more diagnostic for the hyperaccumulation than the metal accumulation in roots, which was relatively common.

Shen and Liu (1998) identified a plant as a hyperaccumulator when the heavy metal concentrations in shoots from the contaminated soil were between 10 – 500 times higher than the heavy metal concentrations in plants from the non-contaminated soil.

The investigated plant (*C. draba*) contained Pb and Cd in shoots in concentrations above their hyperaccumulation levels in the extremely contaminated site, at the accumulation level in the highly contaminated site and for Pb in M_1 and M_2 sites at the second sampling, as well. The accumulation levels were also found for Zn and Cu at the extremely contaminated sites at the second sampling. Chehregani et al. (2007) and Mohsenzadeh and Rad (2012) reported shoot concentrations of *C. draba* for Pb (776 µg/g dw) at the accumulation level and for Zn (1600 µg/g dw), Cu (26.4 µg/g dw) and Cd (2.4 µg/g dw) below the accumulation levels for these metals. Cheragi et al. (2011) found hyperaccumulation level of Pb (4252 µg/g dw) and low Cu (32.4 µg/g dw) in *C. draba*. Low values of Zn (600 – 675 µg/g dw) and Pb (50 – 90 µg/g dw) in *C. draba* were reported by Ghaderian et al. (2007).

Table 3. Relative abundance (%) of the nematode genera, their trophic and functional groups (G) isolated from the plant root material and total number of genera and nematodes (ind/g root material, TNN) from sites with low (L), medium (M_1, M_2), high (H) and extreme (E) level of heavy metal contamination
Таблица 3. Относителна численост (%) на родовете нематоди, трофичните и функционални групи (G) нематоди, изолирани от корените на растението и общ брой родове и обща численост (TNN) в площадки с ниско (L), средно (M_1, M_2), високо (H) и много високо (E) ниво на замърсяване

Genera	G	L	M1	M2	H	E
Mesorhabditis	Ba_1	-	-	1.08	0.62	0.20
Panagrolaimus	Ba_1	10.37	31.79	41.62	69.06	13.50
Pristionchus	Ba_1	0.13	26.49	-	1.23	-
Rhabditis	Ba_1	-	-	-	0.31	1.96
Acrobeles	Ba_2	5.44	0.88	-	1.44	-
Acrobeloides	Ba_2	8.22a	6.84a	25.14b	7.30a	10.57a
Cervidellus	Ba_2	0.76	-	0.27	1.13	-
Chiloplacus	Ba_2	13.02	2.43	1.62	1.54	-
Eucephalobus	Ba_2	24.02	10.82	1.62	-	21.92
Plectus	Ba_2	7.59	3.97	1.89	1.44	1.17
Tylocephalus	Ba_2	0.38	-	-	-	-
Aphelenchus	Fu_2	5.18	6.84	8.11	11.20	2.35
Aphelenchoides	Fu_2	9.61	3.09	9.19	4.01	14.09
Deladenus	Fu_2	-	0.44	-	-	-
Ditylenchus	Fu_2	6.19a	1.77a	8.38a	0.31a	26.81b
Ecumenicus	Om_4	0.51	-	-	-	-
Eudorylaimus	Om_4	0.76	0.22	-	-	-
Mesodorylaimus	Om_4	3.29	1.77	-	-	-
Thonus	Om_4	1.14	-	0.54	-	1.37
Aporcelaimellus	Om_5	0.13	0.22	-	-	-
Seinura	Ca_2	-	-	-	-	5.09
Clarkus	Ca_4	-	0.22	-	-	-
Filenchus	Pl_2	0.63	1.77	-	-	0.39
Merlinius	Pl_3	1.77	0.44	-	0.41	0.20
Bitylenchus	Pl_3	-	-	0.54	-	-
Pratylenchus	Pl_3	0.88	-	-	-	-
Pungentus	Pl_4	-	-	-	-	0.39
Total genera		20	17	12	13	14
TNN		29	35	25	32	34

Different letters indicate significant differences, $P < 0.05$.
Различните букви показват достоверните различия между площадките, P < 0,05.

Table 4. Relative abundance (%) of the nematode trophic groups and the fungal to bacterial feeders ratio (Fu/Fu + Ba) of the nematodes isolated from the root material of the plants from sites with low (L), medium (M_1, M_2), high (H) and extreme (E) level of heavy metal contamination
Таблица 4. Относителна численост (%) на трофичните групи нематоди и съотношението на фунгифагите и бактериално хранещите се нематоди (Fu/Fu + Ba), изолирани от корените на растенията от площадки с ниско (L), средно (M_1, M_2), високо (H) и много високо (E) ниво на замърсяване

Trophic groups	Site L	Site M_1	Site M_2	Site H	Site E
Bacterial feeders	69.9a	83.2a	73.2a	84.1a	49.4b
Fungal feeders	20.9a	12.1a	25.7a	15.5a	43.3b
Omnivores	5.8	2.2	0.5	-	1.4
Carnivores	-	0.2	-	-	5.1
Plant feeders	3.3	2.2	0.5	0.4	0.9
Fu/Fu + Ba	0.23a	0.13a	0.26a	0.16a	0.47b

Different letters indicate significant differences, $P < 0.05$.
Различните букви показват достоверните различия между площадките, $P < 0,05$.

The Enrichment factor for Cd was more than one in the least contaminated site, for Zn in M_1 site at both samplings and for Zn in the extremely contaminated site in 2013. Cheragi et al. (2011) reported high Enrichment factor (1.7) and high Transfer factor (1.64) of Cu for *C. draba*.

The shoot concentrations of all metals (Zn, Cu, Pb and Cd) in the extremely contaminated site and of Pb and Cd in the highly contaminated site were more than 10 times higher than the shoot concentrations in the least contaminated site. The heavy metal concentrations in the shoots correlated positively with their soil concentrations for all metals except for Zn.

The higher hyperaccumulation potential of the hoary cress for Cd and Pb in comparison with Cu and Zn was in accordance with the results of Anguelova et al. (2003) for the mobile forms of these metals in sites close to the same smelter. The authors reported the contents of the mobile forms, determined by an extract of ammonium acetate to vary between 70 – 92% for Cd, 30 – 55% for Pb, 5 – 26% for Cu and 4 – 21% for Zn in relation to their total soil concentrations. Similar values were also given by Dinev (2012) for the same sites near the smelter.

Plant feeding nematodes and a weevil, associated with the roots

The highest number of plants with the insect damaged roots were observed in sites with the medium level of heavy metal contamination, lower in the least contaminated site, very few in highly contaminated site and no one in the extremely polluted site. The herbivore fauna, associated with *C. draba* was studied and some species of *Ceutorhynchus* were suggested for biological control of *C. draba* (Fumanal et al., 2004; Cripps et al., 2006a, 2006b; Talmaciu et al., 2010).

The reduction of the root-galled incidence in the highly contaminated site and complete absence in the extremely contaminated site might be in connection with the high level of accumulated heavy metals in the feeding plant. Martens and Boyd, 1994 suggested that the metal hyper accumulation in plants might serve as a self-defense mechanism against the herbivores and pathogens and this was supported by several experimental data (Behmer et al., 2005; Coleman et al., 2005; Jiang et al., 2005).

Plant feeding nematodes, isolated from the roots of *C. draba* were in very low number in all sites and especially in sites with the higher metal contamination. This very low number of the nematode root parasites might be a result of both chemical (organic compounds) and elemental (heavy metals) defense of the plant, as suggested by Rascio and Navari-Izzo (2011). Most of the mustard plants are known to have chemical defense, which includes glucosinolate production against herbivores and pathogens (Van Dam et al., 2009; Chaplin-Kramer et al., 2011; Bohinc et al., 2012). The glucosinolate production was reported for *C. draba* and several allelopathic activities were documented (Kiemnec and McInnis, 2002; Powell et al., 2005; Radonić et al., 2011; Miri et al., 2013).

Microbial feeding nematodes associated with the plant roots

The high abundance of the microbial feeding nematodes isolated from the roots in most of the sites were probably associated with the root galls as food microsites for these nematodes. The high abundance of the enrichment opportunists (*Panagrolaimus*) in sites with medium and high level of contamination indicated high bacterial activity either inside the gall, or outside on the other damaged places on the root surface. Boyd and Martens (1998) suggested that the decomposition of the hyperaccumulator residues might be affected by the high metal concentrations as the decomposers might also experience toxic effects. No indication of heavy metal toxicity to the bacterial feeding nematodes were observed in our study. The nematodes from *Panagrolaimus* spp. were also among the main insect associates and commensals of various Orthoptera, Coleoptera, Lepidoptera and Diptera and their phoretic associations with insects were reported by several authors (Steinhaus, 2012).

The increased abundance of the fungal feeders (mainly *Ditylenchus*) with the increasing of the heavy metal concentrations both in soil and in shoots was difficult to explain, unless tolerant fungi were associated with the plant roots in the extremely contaminated site. Associations of soil borne fungi and insect-galled roots of *C. draba* have been described (Caesar et al., 2010) although no gall formation was observed in the extremely contaminated site. The plants from Brassicaceae do not form a functional mycorrhizal associations probably because of the potentially toxic glucosinolates, although Zeng et al. (2003) reported growth stimulation of ectomycorrhizal fungi by root exudates of *Brassicaceae* plants and Rengvar et al. (2003) found a colonization of *Brassicaeans* (*Thlaspi* spp.) by arbuscular mycorrhizal fungi. The high value of the fungal to bacterial feeders ratio in the extremely contaminated site might reflect a higher importance of the fungi associated with roots of *C. draba* in this site in comparison with the rest of the sites.

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Хиперакумулация на тежки метали в *Cardaria draba* (L.) Desv. (*Brassicaceae*) и влияние на металите върху нематоди и бръмбари-хоботници, асоциирани с корените на растението в площадки от околностите на комбинат за цветни метали в България

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Резюме

Изследвано е натрупването на тежки метали в *Cardaria draba* (L.) Desv. в площадки с различна степен на замърсяване с Pb, Cd, Cu и Zn в околностите на комбинат за цветни метали в България. Отчетени са нива на хиперакумулация на Pb и Cd в надземната част на растенията в най-силно замърсената площадка. По-висока концентрация в надземните растителни части отколкото в почвата е отчетена за Cd в площадките с ниско и средно замърсяване и за Zn – в средно и най-силно замърсените площадки. Концентрациите на всички тежки метали в надземните растителни части в най-силно замърсената площадка са между 13 и 48 пъти по-големи отколкото в най-слабо замърсената.

Ниската численост на растителнохранещите се нематоди и малкият брой на коренови гали на бръмбари-хоботници в силно замърсените площадки са индикация за успешна елементна (с тежки метали) защита на растенията срещу коренови растителноядни нематоди и насекоми. По-голямата численост на нематодите – фунгифаги в най-силно замърсената площадка при отсъствие на коренови гали на хоботници е индикатор за по-добро развитие на гъбите, асоциирани с корените в тази площадка в сравнение с останалите.