VERSITA
DOI: 10.2478/ssa-2013-0004

SOIL SCIENCE ANNUAL
Vol. 64 No. 1/2013: 19-23

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Uptake of heavy metals by darnel multifloral (*Lolium multiflorum* Lam.) at diverse soil reaction and organic matter content

Abstract: Organic matter takes part in all soil processes and influences the physical, chemical and biological properties of soil. The paper presents the analysis of heavy metal contents (Zn, Pb, Cd) in soil and biomass of darnel multifloral (*Lolium multiflorum* Lam.) cultivated at diverse conditions of soil reaction and organic matter content. A brown coal preparation (Rekulter) was applied as the source of organic matter in autumn 1999 to the studied soil, which was contaminated with cadmium, lead and zinc. The limiting influence of Rekulter on the uptake of heavy metals by darnel multifloral (two cuts of the test plant) was the highest in the case when the largest dose was applied to soil with the highest pH (about 6.0). Bioaccumulation indexes (BI) for Cd, Pb, Zn indicate the mobility and bioavailability of Cd, Pb and Zn in soils. The BI for particular heavy metals were generally low, with the lowest in the case when the largest dose of Rekulter was applied to all cuts of the test plant.

Key words: organic matter, heavy metals, darnel multifloral, bioaccumulation index

INTRODUCTION

Heavy metals, including cadmium and lead, belong to metal elements that are most toxic to plants and animals (Symanowicz et al., 2012). The excess of heavy metals in fodder plants negatively influences their growth and development as well as animal health (Broos et al., 2005; Tujaka and Terelak, 2005). There are a number of factors increasing the phytoavailability of these metals, including their very high concentration in soil and the acidic reaction of soil (Kabata-Pendias and Pendias, 1999; Sady et al., 2000; Szatanik-Kloc, 2004). Reduction of soil reaction to slightly acidic and acidic results in the increase of the concentration of bioavailable, active forms of heavy metals in the soil, thus increasing their bioaccumulation indexes. This results from increase of solubility of the chemical bonds between these elements as well as decrease of their adsorption on soil colloids at low soil reaction. The number of phytoavailable forms of heavy metals is influenced also by the soil type, grain size composition, organic matter content, sorption properties and redox potential. Soil organic matter, represented by humus substances, introduced to soil with natural, organic and organic-mineral fertilizers restricts the amount of bioavailable heavy metal forms (Zaniewicz-Bajkowska, 2000; Kwiatkowska-Malina and Maciejewska, 2009). Soils enriched in organic matter contain a lower amount of phytoavailable fractions of heavy metals (Wołoszyk et al., 2005; Kwiatkowska, 2006; Skłodowski et al., 2006; Kwiatkowska-Malina and Maciejewska, 2011; 2012).

The paper was focused on the analysis of the content and uptake of heavy metals (Zn, Pb, Cd) in darnel multifloral (*Lolium multiflorum* Lam.) at variable soil reaction and organic matter content.

MATERIAL AND METHODS

The studies were conducted in soil containers in form of stoneware pots without a bottom, 0.4 m in diameter and 1.2 m long, inserted in the soil. The containers were filled with lessive soil (56 kg) developed from clayey sand on light clay. According to the WRB, the soil was classified as Haplic Luvisol (IUSS Working Group WRB, 2006). The soil reaction (pH in 1 mol·dm^{-3} KCl) was about 6.0, 5.0 and 4.0, respectively. In 1999, a brown coal preparation (Rekulter) was added once and mixed with the soil, at doses of: 180, 360, and 720 g per container, respectively, which corresponded to 75, 150, and 300 g Corg per container, respectively. In dry mass, Rekulter contained 85% brown coal, 10% peat and 4% brown coal ash. At the same time, heavy metals were added to the soil in the following amounts: zinc – 90 mg·kg^{-1} as

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$ZnSO_4 \cdot 7\ H_2O$, lead – 60 mg·kg^{-1} as $Pb(NO_3)_2$ and cadmium – 0.8 mg·kg^{-1} as $Cd(NO_3) \cdot 4\ H_2O$. According to the IUNG classification, the soil was characterized by an increased content of heavy metals and represented the I stage of pollution (Kabata-Pendias et al. 1993).

Darnel multifloral (*Lolium multiflorum* Lam.), Kroto variety, was grown in 2005 during the experiment. The average doses of the applied nutrients were: N – 2.8 g, P – 0.7 g, and K – 2.6 g per container. After harvest (two independent cuts), the fresh yield was weighed, whereas the dry yield was weighed after drying. In fragmented plant samples mineralized in a mixture of concentrated acids (HNO_3+$HClO_4$) at 4:1, the total content of zinc, lead and cadmium was determined using the ICP-AES method.

Soil samples were collected after harvest from the sub-surface horizon (0–20 cm). In soil samples mineralized in a mixture of concentrated acids (HCl + HNO_3 at 3:1 + 30% H_2O_2), the contents of the sum of all forms of zinc, lead and cadmium were determined. The bioavailable forms of zinc, lead and cadmium in soil were established by selective extraction using 0.05 M solution of EDTA (Ure, 1996). The bioaccumulation index (BI) of the heavy metals was determined as the ratio of their content in plant dry mass and the total content in soil. The influence of the addition of organic matter at variable reaction on the uptake of heavy metals by darnel multifloral was tested by variance analysis using Statgraphics 4.1.

RESULTS AND DISCUSSION

Addition of organic matter to soil in form of a brown coal preparation (Rekulter) favoured the yield of darnel multifloral (Table 1). The highest increase of the yield sum (I and II cut) for the fresh mass (272.1 g) in comparison to the control batch was obtained after application of the highest dose of Rekulter (720 g) to slightly acidic soil. Increase of yield with the dose of organic matter (Rekulter) introduced to the soil resulted from the fact that it was a source of macro- (N, P, K, Mg and Ca) and microelements to the plants. This observation conforms with earlier studies (Wong et al. 1999; Maciejewska and Kwiatkowska, 2005; Tujaka and Terelak, 2005). Due to porosity, brown coal has a large sorption capacity in regard to water and nutrients, acting as a buffer for the soil reaction and concentrations of nutrients in the soil solution, thus resulting in better conditions of plant vegetation. The favourable influence of organic matter addition to soil on plant yielding was also confirmed by other authors (Marcote et al., 2001; Sienkiewicz et al., 2005; Wołoszyk et al., 2005).

TABLE 1. Yield of fresh and dry mass (g·pot^{-1}) of darnel multifloral (*Lolium multiflorum* Lam.)

Dose of Rekulter [g·pot^{-1}]	pH KCl	I cut		II cut	
		fresh mass	dry mass	fresh mass	dry mass
Control batch	6.0	120.5	71.5	116.2	58.7
	5.0	115.6	67.5	96.8	57.5
	4.0	103.4	63.5	90.4	47.5
180	6.0	123.5	87.8	123.7	64.0
	5.0	119.5	78.5	118.7	59.1
	4.0	115.3	66.5	111.5	54.5
360	6.0	128.4	94.8	131.5	69.0
	5.0	124.3	86.5	126.8	65.8
	4.0	117.5	78.5	118.4	61.5
720	6.0	130.6	96.8	141.5	71.3
	5.0	127.3	86.5	131.2	66.0
	4.0	120.1	79.5	121.2	62.5
LSD $\alpha = 0.05$		1.55	1.86	2.91	2.38

Notation: LSD – least significant difference.

Introduction of organic matter in form of Rekulter to soil caused reduction of the content of cadmium, lead and zinc in darnel multifloral (Table 2). The bioavailability of heavy metals decreased, which caused lower uptake by plants, resulting in lower pollution of the first level in the food chain. The heavy metal content in plants largely depends on its species or even variety (Tyksiński & Kurdubska, 2004; Tujaka & Terelak, 2005). Diverse sensitivity of plants to cadmium and zinc allows a selection of plants capable of growth at amounts that are toxic to other plants (Baran & Jasiewicz, 2009). Restricted uptake of heavy metals is strongly influenced by increase of organic matter in the soil. This is linked with immobilization of the elements by macromolecular organic colloids

TABLE 2. Contents of heavy metals in darnel multifloral (mg·kg^{-1} dry mass)

Dose of Rekulter [g·pot^{-1}]	pH KCl	I cut			II cut		
		Cd	Pb	Zn	Cd	Pb	Zn
Control batch	6.0	0.79	7.98	91.0	0.74	7.85	93.5
	5.0	0.85	8.54	99.3	0.84	8.2	98.7
	4.0	0.95	10.64	107.7	0.99	10.9	108.2
180	6.0	0.61	7.79	81.4	0.62	5.24	83.0
	5.0	0.72	8.01	87.2	0.73	8.47	88.1
	4.0	0.81	9.04	98.6	0.85	9.01	91.5
360	6.0	0.52	5.72	80.1	0.53	4.89	82.7
	5.0	0.65	7.16	85.2	0.68	5.28	85.4
	4.0	0.75	8.19	89.7	0.79	7.93	92.3
720	6.0	0.30	4.42	77.0	0.37	4.48	77.2
	5.0	0.50	6.88	81.1	0.46	6.13	78.1
	4.0	0.68	7.38	84.4	0.63	7.50	85.8
LSD $\alpha = 0.05$		0.093	0.37	1.72	0.090	0.52	1.98

Notation: see Table 1.

and general improvement of physical and chemical soil properties, which is also reflected in the plant yielding (Table 1).

Cadmium is a metal that is most toxic to plants and animals (Kabata-Pendias, 2000; Kabata-Pendias and Pendias, 2001). The average content of cadmium in the dry mass of darnel multifloral (I and II cut) varied within 0.3–0.99 mg·kg^{-1} dry mass (Table 2) and showed significant variability under the influence of the studied factors (reaction and organic matter) and their interactions. The lowest cadmium content was determined in biomass of the studied plant collected in the I cut in the variant with the largest dose of Rekulter and at pH 6.0. The highest cadmium content was determined in the test plant collected from the control batch, in which the lowest yield of darnel multifloral was obtained (Table 1). Most probably, the element was concentrated in the obtained yield. Regardless the soil reaction, subsequent increase of Rekulter doses caused reduction of the cadmium content in the biomass of darnel multifloral, which may be the result of yield increase after application of Rekulter (Kabata-Pendias and Pendias, 2001; Symanowicz et al., 2012). In the variant with the largest dose of Rekulter on slightly acidic soil, the cadmium content in darnel multifloral (I cut) was reduced by 62% in comparison to the control batch. The obtained results have confirmed earlier studies (Kwiatkowska-Malina and Maciejewska, 2009; 2011), in which the influence of organic matter on Cd uptake by radish, phacelia, sinapis and napa cabbage was studied. In the studies with grass, cadmium content was within 0.27–0.32 mg·kg^{-1} dry mass (Symanowicz, 2005), which confirms the suggestion of lower cadmium uptake by non-papilionaceous plants (Kabata-Pendias, 2000; Baran and Jasiewicz, 2009). The best fodder value in regard to cadmium content was observed in plants (I and II cut) only from the variant with the largest dose of Rekulter on slightly acidic soil (Kabata-Pendias et al., 1993; Kabata-Pendias and Pendias, 2001).

The lead content determined in the test plant was within 4.42–10.90 mg·kg^{-1} dry mass (Table 2) and significantly varied in diverse conditions and interactions of the environmental factors. The highest lead content was determined in the biomass of the test plant collected during the II cut (10.90 mg·kg^{-1} dry mass) in the variant without Rekulter on very acidic soil. In the variant with the largest dose of Rekulter on slightly acidic soil, the lead content in darnel multifloral was by 15% lower in comparison to the control batch. Significantly higher lead contents were determined in darnel multifloral fertilized with sludge and brown coal wastes (Symanowicz, 2005) and in sinapis and napa cabbage (Kwiatkowska-Malina and Maciejewska, 2011). With regard to the critical values for lead accepted at the level below 10.0 mg·kg^{-1} dry mass, it was noted that only plants grown without Rekulter on very acidic soil cannot be applied as fodder (Kabata-Pendias et al., 1993; Kabata-Pendias and Pendias, 2001). It is generally accepted that fertilization plays a significant role in yield planning and plant chemical composition (Nardi et al., 2004; Wołoszyk et al., 2005), which was confirmed by the obtained results.

Zinc content in darnel multifloral was within 77.0–108.2 mg·kg^{-1} dry mass (Table 2), thus at amounts typical of grass plants (12–72 mg Zn·kg^{-1}) or close to this range (Kabata-Pendias and Pendias, 1999). The zinc content was the highest in the variant without Rekulter on very acidic soil and the lowest in the variant with the largest dose of Rekulter on slightly acidic soil. Reduction of Zn bioavailability for plants on organic soils have been also determined by other authors (Domańska and Filipek, 2011). Brown coal, which is the main component of Rekulter, and the products of its humification in soil are capable of forming complex compounds with heavy metals of variable durability. The highest durability is observed in complexes with copper and lead, as well as nickel, cadmium and zinc. The obtained results confirm the studies of other authors (Ciećko et al., 2001; Martyniuk and Więckowska, 2003), in which addition of brown coal caused reduction of the heavy metals content in plants. Moreover, the results indicate the widely recognized positive influence of acidic soil reaction on the bioavailability of zinc. Darnel grown on very acidic soil contained more zinc than on slightly acidic soil.

Introduction of Rekulter to the soil did not significantly influence the total content of all forms of cadmium, lead and zinc in soil; in turn, the content of bioavailable forms (0.05 M EDTA) of these metals was reduced (Table 3). In regard to the content of zinc, lead and cadmium, plants cultivated in soil with increased contents of heavy metals with addition of Rekulter have fodder properties in most variants.

Contents of the bioavailable forms of cadmium, lead and zinc from the control batch were: 0.53–0.59, 37.8–42.1 and 71.7–78.5 mg·kg^{-1} dry mass, respectively, and in relation to the total content of all forms reached: 66–72, 92–96 and 86–89%, respectively. In variants, in which the highest dose of Rekulter was added, the content of bioavailable forms of cadmium, lead and zinc in soil were: 0.38–0.51, 24.6–33.1 and 47.1–53.2 mg·kg^{-1} dry mass, respectively, and in relation to the total content of all forms reached: 51–63; 66–84 and 53–60%, respectively.

TABLE 3. Total (HCl + HNO_3 at 3:1 + 30% H_2O_2) and bioavailable (0.05 M EDTA) contents of heavy metals in the studied soil samples (mg·kg^{-1} dry mass)

Dose of Rekulter [g·pot^{-1}]	pH KCl	Total content			Bioavailable content		
		Cd	Pb	Zn	Cd	Pb	Zn
Control batch	6.0	0.80	41.2	89.9	0.53	37.8	71.7
	5.0	0.81	41.8	90.1	0.57	41.3	72.9
	4.0	0.82	42.9	88.6	0.59	42.1	78.5
180	6.0	0.78	39.8	88.5	0.45	28.2	65.5
	5.0	0.77	40.2	90.1	0.50	30.1	69.2
	4.0	0.82	39.2	90.2	0.51	38.2	76.2
360	6.0	0.79	39.3	87.3	0.45	27.5	62.8
	5.0	0.76	40.2	86.8	0.61	29.3	76.2
	4.0	0.78	40.3	89.3	0.64	37.2	77.3
720	6.0	0.75	37.2	88.0	0.38	24.6	47.1
	5.0	0.78	39.6	87.5	0.44	29.1	49.2
	4.0	0.80	39.3	88.2	0.51	33.1	53.2
LSD α = 0.05		0.014	0.42	0.65	0.21	1.22	1.20

Notation: see Table 1.

TABLE 4. Bio-accumulation indexes (BI)

Dose of Rekulter [g·pot^{-1}]	pH KCl	I cut			II cut		
		Cd	Pb	Zn	Cd	Pb	Zn
Control batch	6.0	0.99	0.19	1.01	0.93	0.19	1.04
	5.0	1.05	0.20	1.10	1.04	0.20	1.10
	4.0	1.16	0.26	1.22	1.21	0.27	1.22
180	6.0	0.78	0.20	0.92	0.79	0.13	0.94
	5.0	0.94	0.20	0.97	0.95	0.21	0.98
	4.0	0.99	0.23	1.09	1.04	0.23	1.01
360	6.0	0.66	0.15	0.92	0.67	0.12	0.95
	5.0	0.86	0.18	0.98	0.89	0.13	0.98
	4.0	0.96	0.20	1.00	1.01	0.20	1.03
720	6.0	0.40	0.12	0.88	0.49	0.12	0.88
	5.0	0.64	0.17	0.93	0.59	0.15	0.89
	4.0	0.85	0.19	0.96	0.79	0.19	0.97
LSD α = 0.05		0.044	0.012	0.025	0.028	0.039	0.011	0.026

Notation: see Table 1.

Values of the bioaccumulation indexes (BI) for particular metals were similar (Table 4). For cadmium, the BI was within 0.40–1.16. The lowest value of this index (0.40) was calculated for the object with the largest dose of Rekulter on slightly acidic soil. The calculated BI indexes for lead were at the level of 0.12–0.27 for particular objects. The lowest BI value for lead (0.12) was noted after application of the largest dose of Rekulter on slightly acidic soil. The BI values for zinc after application of the largest dose of Rekulter were the lowest and varied within 0.88–0.97. Similar values of bioaccumulation indexes for cadmium and zinc were obtained in studies on dicotyledonous plants and grasses (Kwiatkowska-Malina and Maciejewska, 2011; Symanowicz et al., 2012).

CONCLUSIONS

1. Addition of organic matter with Rekulter to soil caused decrease of the bioavailability of cadmium, lead and zinc, and thus their uptake by darnel multifloral.
2. Contents of cadmium, lead and zinc in darnel multifloral were the lowest in the variant with the largest dose of Rekulter.
3. Contents of cadmium, lead and zinc in darnel multifloral were the lowest in soil and plants in the variant with a slightly acidic reaction.
4. The determined values of cadmium, lead and zinc in the biomass of darnel multifloral were within the range below the boundary values for the admissible amounts of these elements in fodder.
5. Bioaccumulation indexes of zinc, cadmium and lead were generally low; the lowest values were observed for lead.

REFERENCES

Baran A., Jasiewicz C., 2009. Toksyczna zawartość cynku i kadmu w glebie dla różnych gatunków roślin. *Ochrona Środowiska i Zasobów Naturalnych* **40**: 157–164.

Broos K., Beyens H., Smolders E., 2005. Survival of rhizobia in soil is sensitive to elevated zinc in the absence of the host plant. *Soil Biology & Biochemistry* **37**: 573–579.

Ciećko Z., Wyszkowski M., Krajewski W., Zabielska J., 2001. Effect of organic matter and liming on the reduction of cadmium uptake from soil by triticale and spring oilseed rape. *The Science of the Total Environment* **281**: 37–45.

Domańska J., Filipek T., 2011. Akumulacja cynku w kupkówce pospolitej w zależności od rodzaju gleby, pH oraz zanieczyszczenia Cd lub Pb. *Ochrona Środowiska i Zasobów Naturalnych* **48**: 67–73.

IUSS Working Group WRB, 2006. World reference base for soil resources. First update 2007, 2nd ed. World Soil Resources Reports, 103. FAO, Rome, p. 128.

Kabata-Pendias A., 2000. Biogeochemia kadmu. Zesz. Nauk. Kom. PAN „Człowiek i Środowisko" **33**: 11–18.

Kabata-Pendias A., Pendias H., 1999. Biogeochemia pierwiastków śladowych. Wyd. II. PWN, Warszawa: 267 pp.

Kabata-Pendias A., Pendias H., 2001. Trace elements in soils and plants. (3 rd Ed.) CRC Press, Boca Raton, Fl, USA: 413.

Kabata-Pendias A., Motowicka-Terelak T., Piotrowska M., Terelak H., Witek T., 1993. Ocena stopnia zanieczyszczenia gleb i roślin metalami ciężkimi i siarką. Ramowe wytyczne dla rolnictwa. IUNG, Puławy.

Kwiatkowska J., 2006. The effect of organic amendments on the phytoavailability of heavy metals in polluted soil. *Ecohydrology & Hydrobiology* **6**, **1–4**: 181–186.

Kwiatkowska-Malina J., Maciejewska A., 2009. Wpływ materii organicznej na pobieranie metali ciężkich przez rzodkiewkę i facelię. *Ochrona Środowiska i Zasobów Naturalnych* **40**: 217–223.

Kwiatkowska-Malina J., Maciejewska A., 2011. Pobieranie metali ciężkich przez rośliny w warunkach zróżnicowanego odczynu gleb i zawartości materii organicznej. *Ochrona Środowiska i Zasobów Naturalnych* **49**: 43–51.

Kwiatkowska-Malina J., Maciejewska A., 2012. Wpływ węgla brunatnego na aktywność mikroorganizmów w glebach zanieczyszczonych metalami ciężkimi. *Rocz. Glebozn.* **LXIII** 1/2012: 39–41, DOI: 10.2478/v10239-012-0010-7

Maciejewska A., Kwiatkowska J., 2005. Wpływ materii organicznej z różnych źródeł na plon oraz zawartość i rozmieszczenie makroelementów w życie (*Seceale cereale L.*). *Fragmenta Agronomica* (XXII) **1**(85): 484-492.

Marcote I., Hernandez T., Garcia C., Polo A., 2001. Influence of one or two successive annual applications of organic fertilizers on the enzyme activity of a soil under barley cultivation. *Bioresource Technology* **79**: 147–154.

Martyniuk H., Więckowska J., 2003. Adsorption of metal ions on humic acids extracted from brown coals. *Fuel Processing Technology* **84**: 23–36.

Nardi S., Morami F., Berti A., Kosoni M., Giardii L., 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilizers. *Europ. J. Agron.* **21**: 357–367.

Sady W., Rożek S., Domagała-Świątkiewicz I., 2000. Biokumulacja kadmu w marchwi w zależności od wybranych właściwości gleb. *Zesz. Nauk. AR Kraków* **364**: 171–173.

Sienkiewicz S., Krzebietke S., Panak H., Czapla J., 2005. Plony jęczmienia jarego i pszenicy jarej w zależności od nawożenia w wieloletnim doświadczeniu polowym. *Fragmenta Agronomica* (XXII) **1**(85): 244–253.

Skłodowski P., Maciejewska A., Kwiatkowska J., 2006. The effect of organic matter from brown coal on bioavailability of heavy metals in contaminated soils. Soil and Water Pollution Monitoring, Protection and Remediation. NATO Science Series. IV Earth and Environmental Sciences 69: 299–307.

Symanowicz B., 2005. Wpływ węgli brunatnych, osadów ściekowych oraz ich mieszanin I obornika na zawartość kadmu i ołowiu w podłożu glebowym i życicy wielokwiatowej (*Lolium multiflorum* Lam.). *Acta Sci. Pol. Agricult.* **4**(2): 83–92.

Symanowicz B., Kalembasa S., Skorupka W., 2012. Wpływ nawożenia fosforem i potasem na pobieranie metali ciężkich przez rutwicę wschodnią (*Galega orientalis* Lam.). *Ochrona Środowiska i Zasobów Naturalnych* **54**: 131–140.

Szatanik-Kloc A., 2004. Wpływ pH i stężenie wybranych metali ciężkich w glebie na ich zawartość w roślinach. *Acta Agrophysica* **4**(1): 177–183.

Tujaka A., Terelak H., 2005. Wpływ rekultera na skład chemiczny, plonowanie i przydatność roślin uprawianych na glebach zanieczyszczonych Cd, Pb i Zn. *Zesz. Probl. Post. Nauk Rol.* **506**: 497–505.

Tyksiński W., Kurdubska J., 2004. Różnice odmianowe w akumulacji kadmu i ołowiu przez rzodkiewkę (*Raphanus Sativus* L.). *Roczniki AR w Poznaniu CCCLVI*: 209–215.

Ure A.M., 1996. Single extraction schems for soil analysis and related applications. *Sc. Total Environ.* **178**: 3–10.

Wołoszyk C., Krzywy E., Iżewska A., 2005. Ocena wartości nawozowej kompostów sporządzonych z komunalnego osadu ściekowego w trzyletnim zmianowaniu roślin. *Fragmenta Agronomica* (XXII) **1**(85): 631–642.

Wong J.W.C., Ma K.K., Fang K.M., Cheung C., 1999. Utilization of manure compost of organic farming in Hong Kong. *Bioresource Technology* **67**: 43-46.

Zaniewicz-Bajkowska A., 2000. Zależność zawartości kadmu i ołowiu w glebie oraz w korzeniach buraka ćwikłowego od nawożenia organicznego i wapnowania. *Ann. UMCS Sectio EEE Hort.* **8**, Supp.: 123–128.

Received: March 6, 2013
Accepted: April 12, 2013