
THE SENSITIVITY OF *BRASSICA NAPUS* SSP. *OLEIFERA* TO CADMIUM (Cd) AND LEAD (Pb) CONTAMINATION AT DIFFERENT pH OF MINERAL AND ORGANIC SOILS

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Abstract

In a model pot experiment, the influence of elevated levels of cadmium and lead in soils on rapeseed (*Brassica napus* L.) growth, development, biomass (g DM pot⁻¹), cationic ratios (Cd²⁺:Ca²⁺, Cd²⁺:Mg²⁺, Pb²⁺:Ca²⁺, Pb²⁺:Mg²⁺) and tolerance index Ti, has been examined. The tested soils were: two organic soils (acidic and neutral) and a mineral one with pH differentiated into neutral and acidic. The metals were spiked into soils in doses of 10 mg Cd kg⁻¹ DM and 100 mg Pb kg⁻¹ DM of soil. Under the applied doses, the sensitivity of rapeseed to Cd was higher than to Pb. The application of Cd caused visible symptoms of chlorosis on plant leaves and a statistically significant ($p < 0.01$, $p < 0.05$) decrease in aerial biomass. The influence of Pb on *B. napus* biomass yield was not significant. The soil type and its reaction differentiated the biomass of plants in the following decreasing order: mineral neutral > organic neutral > mineral acidic > organic acidic. The Cd added to soil increased the Cd²⁺:Ca²⁺ and Cd²⁺:Mg²⁺ ionic ratios, while Pb caused an increase in Pb²⁺:Ca²⁺ and Pb²⁺:Mg²⁺ ratios compared to the control. The significantly higher values for the above ions ratios were recorded in rapeseed roots rather than in shoots. The results showed a crucial role of the soil type in determining the millimolar ionic relations in *B. napus* plants. The plant was more vulnerable to chemical composition changes in contaminated treatments comparing to control when grown on mineral soils (both acidic and neutral) than on organic ones. The high Ti value proves generally low sensitivity of *B. napus* to soil Cd and Pb contamination.

Key words: *Brassica napus* biomass, sensitivity, Cd, Pb, tolerance index, ionic ratios, mineral soil, organic soil, pH.

INTRODUCTION

Atmospheric emissions caused by mining, smelting, coal combustion and other activities such as the chemical industry, contaminate soils by transporting heavy metals like Cd, Pb, Cu, Ni, Zn and other pollutants in the air and subsequently depositing them on soils and the earth surface overgrown by plants (MEDYŃSKA-JURASZEK, KABALA 2012, ADAMCZYK-SZABELA 2013). Direct application of some fertilizers, sewage sludge, composted solid wastes or industrial residues additionally increases the load of metals in agricultural environment (DOMAŃSKA 2006, MEDYŃSKA-JURASZEK, KABALA 2012, DOMAŃSKA et al. 2013). Cadmium and lead are non-essential elements and can be phytotoxic to plants when present in soils in elevated amounts. Their uptake and toxicity to plants depend on many factors such as plant species or variety, chemical form (speciation of metal in the soil), pH, organic matter content and presence of other ions (DAS et al. 1997, DOMAŃSKA 2002, 2006, NOUAIRI et al. 2006, DOMAŃSKA et al. 2013, WYSZKOWSKA et al. 2013). However, the safe level of Cd or Pb concentration at plants can be harmful to humans and animals.

The advantages and possibilities of using plants from *Brassicaceae species* (*B. juncea*, *B. campestris*, *B. napus*) in phytoremediation and phytoextraction were reported by many authors (MARCHIOL et al. 2004, GHOSH, SINGH 2005, GISBERT et al. 2006, GRISPEN et al. 2006). Phytoextraction of heavy metals by *B. napus* also seems to be possible and promising (MARCHIOL et al. 2004), especially on moderately contaminated soils (GRISPEN et al. 2006). Phytoremediation studies often focus on understanding the mechanisms of plant tolerance to a particular metal and on important plant parameters like degree of accumulation of contaminants, plant growth rate, biomass production, etc. In this work, the toxicity of Cd or Pb to *B. napus* was examined on a basis of a pot experiment designed to determine if this plant is sufficiently tolerant to these metals, when grown on contaminated soils. The research was carried out on two types of organic soils and a mineral one, which differed in pH values. The growth, development and biomass of the tested plant, depending on soil type, pH and cadmium or lead addition, were determined. The experiment design also allowed us to evaluate the influence of soil type on *B. napus* chemical composition, when cultivated on Cd or Pb contaminated soil. This can indicate the usefulness of the plant for phytoremediation on particulate soil types or possibilities of cropping *B. napus* for other purposes on studied types of soils.

MATERIAL AND METHODS

Soils

The research was based on analysis of plant and soil materials gained from a pot experiment performed on two organic soil types and a mineral one with different pH. Soil material was taken from the surface layer (0-20 cm). Organic peat-bog soil developed from short peat with neutral reaction (Fibric Histosols) (*Taxonomy...* 2011) was taken from arable lands near municipal and industrial purification plant in Hajdów near Lublin (Poland). Acidic soil (Dystri-Fibric Histosol - FAO – WRB 1998) developed from high peat was taken from the surroundings of Łukcze Lake in Lublin region. The mineral soil Haplic Podzols (*Taxonomy...* 2011), with the granulometric composition of light loamy sand was taken from agricultural areas of the Lubelskie region. Its neutral reaction was obtained by adding CaCO_3 applied according to 2.0 hydrolytic acidity. The main properties of the soils, determined before the experiment, are listed in Table 1.

Table 1

Selected properties and concentrations of metals in soils used for experiment

Soil		pH_{KCl}	C org. (%)	Available total					Granulometric composition		
				P	K	Mg	Pb	Cd	share (%) of fraction of diameter (mm)		
				(mg kg ⁻¹)					1-0.1	0.1-0.2	<0.02
Mineral	acidic	4.6	1.32	82	89	42	5.49	0.10	74	18	8
	neutral	7.2									
Organic	acidic	3.6	64.0	94	141	385	20.98	0.30	not determined		
	neutral	7.2	18.7	786	183	1662	16.65	0.43	not determined		

Pot experiment

The soil material was placed in pots of 5 dm³ volume. Pots contained 4.8 kg of organic acidic soil, 5.5 kg of organic neutral soil and 6.4 kg of mineral soil. During the experiment, mineral soil humidity was adjusted to 60% of maximum water capacity and 80% in the case of organic soils by adding water to the constant weight. Mineral fertilization in the form of mineral salts: NH_4NO_3 – 0.10 g N kg⁻¹ soil, CaHPO_4 – 0.07 g P kg⁻¹ soil and KCl – 0.15 g K kg⁻¹, was applied in all pots, dividing the NH_4NO_3 dose into two equal parts – before and after sowing. Cd and Pb as $\text{Cd}(\text{NO}_3)_2$ and $\text{Pb}(\text{NO}_3)_2$ were introduced once before sowing at doses: 10 mg Cd kg⁻¹ and 100 mg Pb kg⁻¹ dry mass (DM). The metals were applied into soils according to the following design:

0 – control treatment with no metal addition,

Pb = Pb(II) treatment – lead dose of 100 mg kg⁻¹ DM,

Cd = Cd(II) treatment – cadmium dose of 10 mg kg⁻¹ DM.

Spring rapeseed (*Brassica napus* ssp. *oleifera* L.) (20 seeds) was sown on 30 of April and plants were harvested at the flowering stage. During the growing period, the growth and development of plants were observed. After harvesting, underground biomass (roots) was rinsed with deionized water and aerial biomass (shoots) and roots were separated and weighed. The plant samples were oven-dried, after which DM of roots and shoots of *B. napus* was determined.

Chemical analyses

Collected soil samples were air-dried, crushed in porcelain mortar and sieved through 1 mm mesh. The following were determined in the prepared samples: granulometric composition (mineral soil) by means of the Casagrande's areometric method with modifications by Prószyński, pH – potentiometry in 1 M KCl (Polish Standard 1997), content of organic matter (C_{org}) – the Tiurin's method, content of available P and K using the Egner and Riehm's method, and content of available Mg using the Schachtschabel's method, all being standard methods used by Polish Agricultural Stations. The content of organic matter in organic soils was determined by annealing (BEDNAREK et al. 2004). The total content of Cd and Pb in soils was analyzed after digesting the soil samples in a mixture of concentrated acids HCl and HNO_3 in the ratio of 3:1 (*aqua regia*) (Polish Standard 2001, 2002). The total metal concentrations in the solution were determined by means of flame atomic absorption spectrometry (AAS).

Concentrations of Ca and Mg in plants were determined using AAS after mineralization in concentrated H_2SO_4 . Cadmium and lead determinations in plant samples were achieved by applying the AAS technique after plant material digestion in concentrated H_2SO_4 with H_2O_2 addition (OSTROWSKA et al. 1991).

Data analysis

The experiment including 6 plots in four replicates on mineral soil, and 6 plots in four replicates on organic soils was set up using the randomised block method.

Data obtained from the experiments were statistically processed by means of variance analysis with Tukey confidence intervals. Differences were considered to be significant at $p = 0.05$ and highly significant at $p = 0.01$ level of significance.

Molar ratios of $Cd^{2+}:Ca^{2+}$, $Cd^{2+}:Mg^{2+}$, $Pb^{2+}:Ca^{2+}$, $Pb^{2+}:Mg^{2+}$ were calculated for root and shoot in *B. napus* as well as the tolerance index (Ti) of *B. napus* to Cd and Pb, which specifies the degree of inhibition of plant growth in contaminated soil. $Ti = \text{biomass of plants on treated (Cd or Pb) soil (g DM pot}^{-1}) / \text{biomass of plants on untreated (0 - control) soil (g DM pot}^{-1})$.

RESULTS

Effects of cadmium and lead on growth of plants

The growth and development of plants in particular treatments varied. The poorest emergence and uneven germination were observed on mineral acidic soil. On the mineral soil, de-acidified in a dose equivalent to 2.0 Hh, and on the organic ones, the emergence of rapeseed plants was simultaneous. Initially, there was also no difference in the growth of plants in the variants with metals. As the plant grew, 1.5 months after sowing, plants in the treatments on the mineral neutral soil were the highest, most developed and all started the flowering phase at the same time. In the Cd treatments on mineral acidic soil, *B. napus* leaves had yellowing edges and the generative phase was delayed. In *B. napus* plants from treatments on organic soils, chlorosis on older leaves occurred. On acidic peaty soil, the *B. napus* leaves had slightly purple colour, which indicates P deficiency. Much lower shoots grown on the organic acidic soil, when comparing with the other soils, suggest also the shortage of other elements. Lead did not affect the overall growth of the plant. The symptom of cadmium toxicity was the chlorosis of leaves.

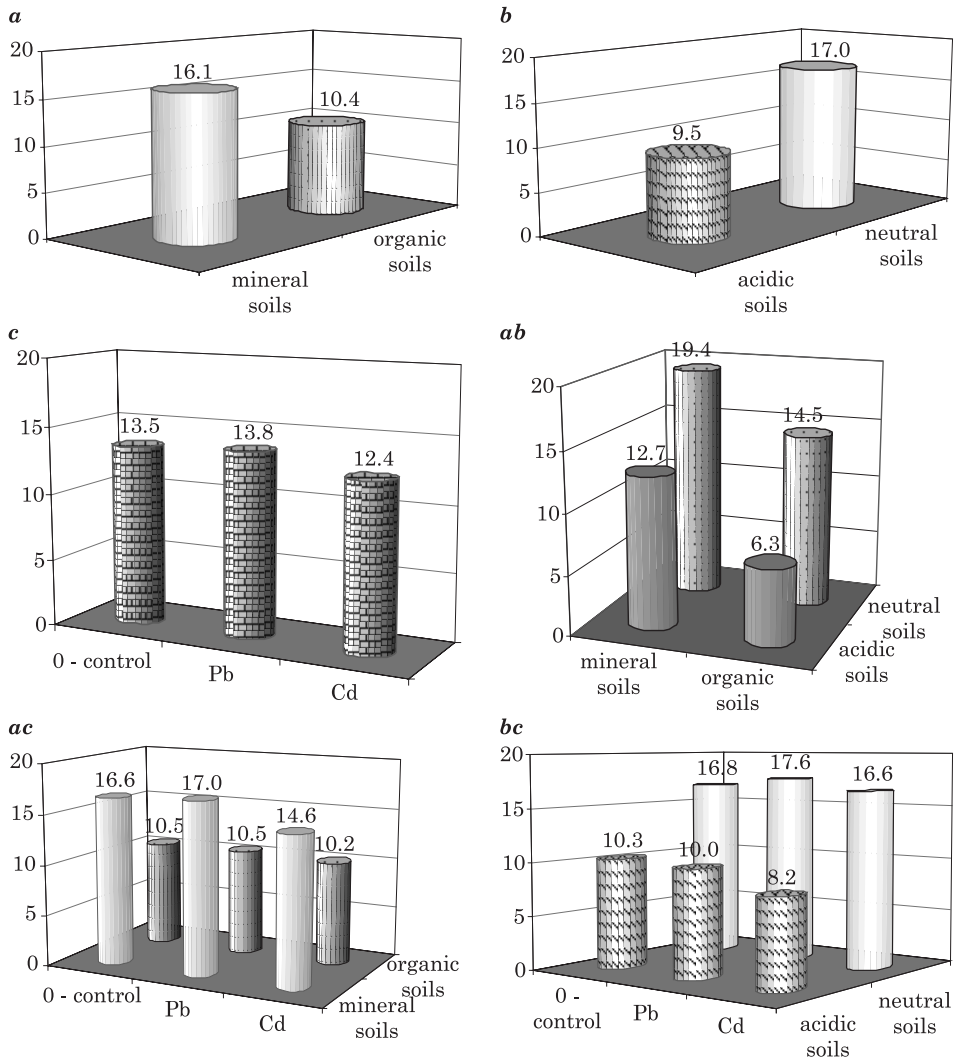
Effects of cadmium and lead on biomass of plants

The experimental factors (type of soil, pH, Cd and Pb addition) significantly influenced the rapeseed aerial (shoots) biomass (g DM pot⁻¹) – Figure 1. Highly significant interactions between these factors were also recorded. Much higher average biomass of plants was obtained on mineral (16.1 g pot⁻¹) than on organic (10.4 g pot⁻¹) soils. There was also statistically significant higher shoot biomass obtained from neutral soils (17.0 g pot⁻¹) than from acidic ones (9.5 g pot⁻¹). The soil type and pH significantly affected the changes of above sequence of obtained plant's biomass as follows: mineral neutral > organic neutral > mineral acidic > organic acidic.

In plants grown on mineral acidic soil, Cd caused a significant decrease (10.2 g pot⁻¹) in rapeseed biomass, compared with the control (14.6 g pot⁻¹), while on mineral neutral soil, there was a considerable increase in the plant biomass (20.7 g pot⁻¹) under the influence of Pb, as compared to the control (18.6 g pot⁻¹). With regard to the control, a significant DM decrease also occurred in Cd and Pb treatments on neutral organic soil.

Considering the mean values, a statistically significant fall of *B. napus* DM (12.4 g pot⁻¹) in comparison with the control (13.5 g pot⁻¹) was observed in the Cd treatment.

The amount of underground (roots) DM was significantly dependent on the type of soil and its pH (Figure 2). More DM of roots was produced on mineral (3.2 g pot⁻¹) than on organic soil (2.4 g pot⁻¹). On mineral neutral soil, the growth of rapeseed roots was better than on mineral acidic one, which



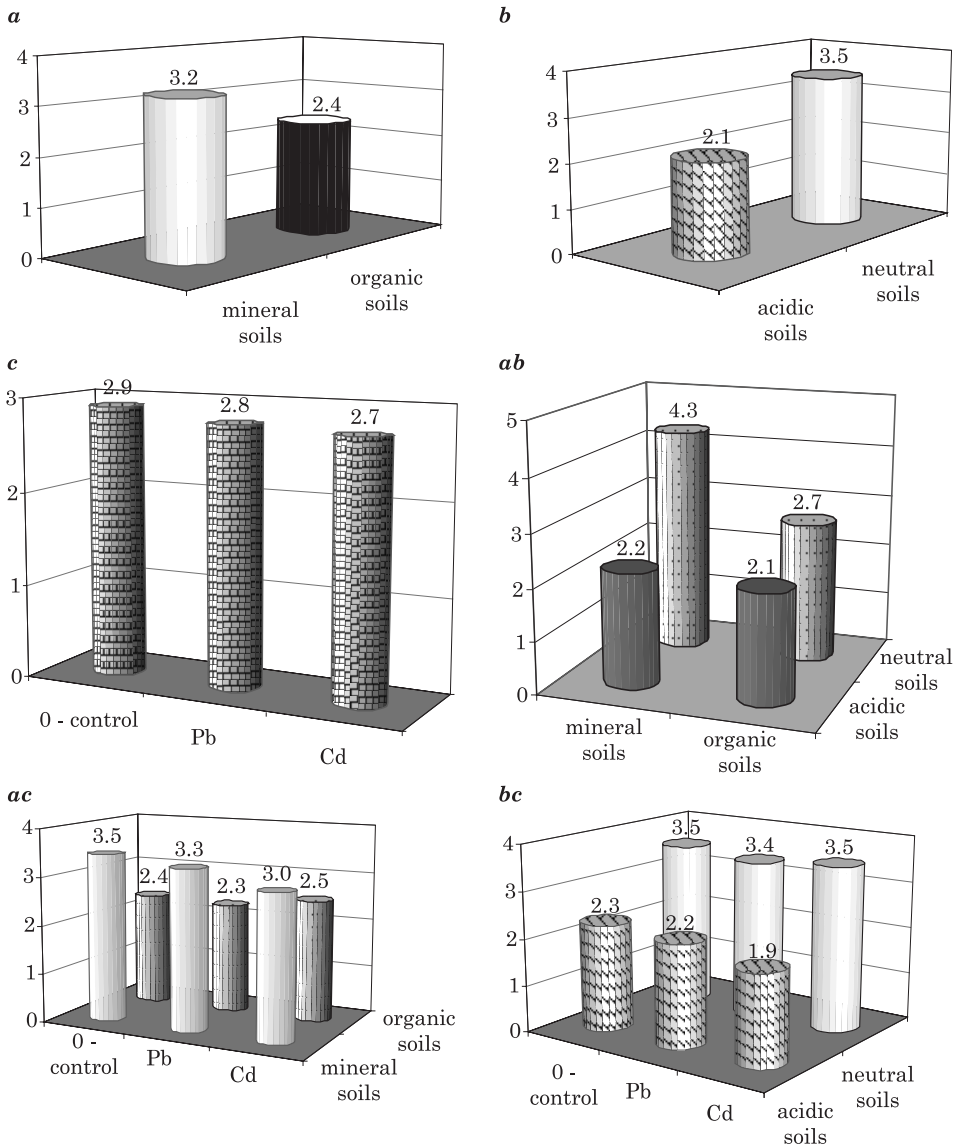
LSD $p = 0.05^*$; $p = 0.01^{**}$ (a – soil, b – soil reaction, c – metal); a 0.45^{**} , b 0.45^{**} , c 0.67^{**} , ab 0.85^{**} , ac 1.16^{**} , bc 1.16^{**}

Fig. 1. Mean aerial biomass of *Brassica napus* (g DM pot⁻¹)

resulted in an almost 2-fold increase in biomass, as compared to the acidic soil. The metals did not influence remarkably the biomass of rape roots.

Tolerance index (Ti)

The tolerance index Ti is fundamental to the evaluation of the plant's sensitivity towards toxic metals (Table 2). The lowest Ti value (0.7) for rapeseed (shoots and roots) was recorded on mineral acidic soil contaminated



LSD $p = 0.05^*$; $p = 0.01^{**}$, a 0.25^{**} , b 0.25^{**} , ab 0.48^{**}

Fig. 2. Mean underground biomass of *Brassica napus* (g DM pot⁻¹)

with Cd. The index value $Ti = 0.9$ implicating a slightly reduced tolerance of plants was also reported for mineral soil in the Pb treatment and in treatments with the addition of Cd (shoots) and Pb (roots) on neutral organic soil. In the remaining treatments, Ti ranged from 1.0 to 1.1. High Ti values for most of the contaminated treatments demonstrate high tolerance of rapeseed to cadmium and lead.

Table 2

Tolerance of *Brassica napus* to Cd and Pb in dependence on soil pH, metal addition and the content of organic matter

Object			Ti*	
Soil	pH	metal	shoots	roots
Mineral	acidic	0	1.0	1.0
		Pb	0.9	0.9
		Cd	0.7	0.7
	neutral	0	1.0	1.0
		Pb	1.1	1.0
		Cd	1.0	1.0
Organic	acidic	0	1.0	1.0
		Pb	1.1	1.0
		Cd	1.1	1.1
	neutral	0	1.0	1.0
		Pb	1.0	0.9
		Cd	0.9	1.1

* Ti = biomass of plants on treated (Cd or Pb) soil (g DM pot⁻¹)/biomass of plants on untreated (0 - control) soil (g DM pot⁻¹).

Cationic ratios

An effect of Cd and Pb soil contamination on the cationic ratios (Cd²⁺:Ca²⁺, Cd²⁺:Mg²⁺, Pb²⁺:Ca²⁺, Pb²⁺:Mg²⁺) in *B. napus* plants was observed (Table 3). The ratio values of Cd²⁺ to Ca²⁺ in analyzed plant shoots and roots varied from 0.01 to 13.41. The highest ratios were recorded in plants from Cd contaminated treatments on mineral soil, which were within the range from 1.34 in plant shoots from neutral soil to 13.41 in plant roots from acidic soil. The Cd²⁺:Ca²⁺ ratios in plants from Cd-uncontaminated soils (both mineral and organic) did not exceed 0.08. The results indicate that in plants growing on organic soils, the Cd²⁺:Ca²⁺ ratio values were the same or much lower than in analogous treatments on mineral soil and even in treatments contaminated by Cd, in which it did not exceed 0.63. The ratio of Cd²⁺ to Ca²⁺ in plant roots was usually higher than in the aerial parts of plants. There was no effect of Pb addition on Cd²⁺:Ca²⁺ ratios in *B. napus* shoots comparing to the control treatments, but slight differentiation occurred in roots.

Mg and Ca have many chemical similarities. Therefore, as in the case of Cd²⁺:Ca²⁺, the highest Cd²⁺:Mg²⁺ ratios were found in plants from Cd-contaminated treatments, especially on mineral acidic soil (16.64 – shoots and 21.43 – roots). The *B. napus* roots absorbed more Cd than shoots and showed the highest values of Cd²⁺:Mg²⁺. There was hardly any effect of Pb addition to soil on the Cd²⁺:Mg²⁺ ratio in *B. napus*, in comparison to the control.

Table 3

Relationship between shoot and root divalent cation ratios of *Brassica napus* in response to cadmium or lead contamination

Soil	Treatment		Shoots				Roots			
	pH	metal	Pb ²⁺ : Ca ²⁺	Pb ²⁺ : Mg ²⁺	Cd ²⁺ : Ca ²⁺	Cd ²⁺ : Mg ²⁺	Pb ²⁺ : Ca ²⁺	Pb ²⁺ : Mg ²⁺	Cd ²⁺ : Ca ²⁺	Cd ²⁺ : Mg ²⁺
Mineral	acidic	0	0.03	0.11	0.03	0.10	0.30	0.53	0.04	0.08
		Pb	0.22	0.78	0.03	0.12	11.47	19.70	0.08	0.15
		Cd	0.02	0.07	4.33	16.64	0.49	0.78	13.41	21.43
	neutral	0	0.01	0.03	0.01	0.04	0.33	0.52	0.06	0.09
		Pb	0.08	0.29	0.01	0.03	4.61	8.94	0.06	0.11
		Cd	0.01	0.06	1.34	5.32	0.11	0.20	9.46	16.82
Organic	acidic	0	0.01	0.02	0.01	0.02	0.26	0.58	0.02	0.05
		Pb	0.03	0.04	0.01	0.02	0.91	1.94	0.01	0.03
		Cd	0.01	0.02	0.16	0.27	0.27	0.58	0.41	0.87
	neutral	0	0.01	0.05	0.01	0.02	0.32	0.17	0.06	0.03
		Pb	0.02	0.07	0.01	0.02	0.98	0.56	0.05	0.03
		Cd	0.01	0.05	0.04	0.15	0.17	0.11	0.63	0.43

The results show that the soil type played a crucial role in determining the molar ratios between ionic ratios of Pb²⁺:Ca²⁺ and Pb²⁺:Mg²⁺ in *B. napus* (Table 3). In plants from Pb treatments on organic soils, the Pb²⁺:Ca²⁺ and Pb²⁺:Mg²⁺ ratios were much lower than on mineral soil, and often close to the control value. In *B. napus* shoots from Cd contaminated organic soils, the Pb²⁺:Ca²⁺ and Pb²⁺:Mg²⁺ ratios were the same as in the control treatment.

The concentration of Pb significantly affected the ionic relations in *B. napus* roots for all Pb-contaminated treatments. A particularly high increase in the Pb²⁺:Ca²⁺ and Pb²⁺:Mg²⁺ ratios occurred in *B. napus* roots from mineral acidic soil: 11.47 and 19.70, respectively (Table 3). The Cd added to neutral soils decreased the Pb²⁺:Ca²⁺ and Pb²⁺:Mg²⁺ ratios in *B. napus* roots, but when added to acidic soils, the heavy metal most often slightly increased these values as compared to the control.

DISCUSSION

In recent years, a number of studies have examined the ability of *Brassica* species to take up heavy metals from contaminated soils, growth media or hydroponic culture (SALT et al. 1995, GRISPEN et al. 2006, MEYERS et al. 2008, SELVAM, WOON-CHUNG 2009, QIU et al. 2011), and indicated the potential of plants in absorbing and translocating metals to the aerial parts (SELVAM,

WOON-CHUNG 2009). GAMBUS (1997) reported lower sensitivity of rapeseed than other plants to Cd, 15 mg Cu, 12 Ni, 90 mg Pb and 60 mg Zn, when applied jointly into the soil.

The soil type and its pH seem to be basic factors in evaluating the usefulness of *B. napus* plant for phytoremediation purposes. In the present study, the soil type and pH significantly differentiated the biomass yield in the following decreasing order: mineral neutral > organic neutral > mineral acidic > organic acidic. This sequence proves high sensitivity of *B. napus* to soil acidity and shows the importance of soil type in controlling the plants growth.

The soil pH is one of the most important factors affecting the metal mobility and availability to plants. It was found that on mineral acidic soil (Cd treatment), the generative phase was delayed and leaves had yellowing edges. Chlorosis resulting from excess Cd can be due to iron deficiency caused by the interaction of the metals with foliar iron (DAS et al. 1997). According to SELVAM, WOON-CHUNG (2009), *B. napus* plants cultivated on fine loamy soil did not show any toxicity symptoms up to 50 mg Cd kg⁻¹ DM.

On acidic peaty soil, the *B. napus* leaves had slightly purple colour, which may indicate lower availability of P (P immobilization processes) for plants in the conditions of high activity of H⁺. P deficiency symptoms can be also a result of P and Cd interactions within the plant (DAS et al. 1997). QIU et al. (2011) reported an important role of P in the Cd uptake and translocation via the processes involved in the bonding Cd to the cell wall fraction and forming the Cd-phosphate complexes.

The highest sensitivity of *B. napus* to soil Cd contamination resulting in biomass depletion was found in the treatment on acidic mineral soil. It was probably the result of nutrient imbalances caused by higher Cd uptake from acidic soil. As recorded by SELVAM and WOON-CHUNG (2009), differences in *B. napus* shoots dry weights were significant only at 50 mg Cd kg⁻¹ soil treatment. The significant *B. napus* roots reduction was observed at 6 mg Cd kg⁻¹ soil treatment in *B. napus* – *B. parachinensis* co-cropping system.

In the Pb treatment on mineral neutral soil, a statistically significant increase of biomass, when compared to the control, was recorded. At an elevated soil pH value, the solubility of Pb in soil decreases (DOMAŃSKA 2006, BADORA 2012) and limits the availability of the metal to plants.

The usefulness of the tolerance coefficient (Ti) in an assessment of plants' vulnerability to soil contamination with heavy metals was underlined by PIKUŁA and STĘPIEŃ (2007). In our studies, Ti was in the range of 0.7-1.1, which proves that *B. napus* was generally tolerant to applied doses of cadmium and lead.

The results show that the soil type played a crucial role in determining the molar ratios between studied ions (Cd²⁺:Ca²⁺, Cd²⁺:Mg²⁺, Pb²⁺:Ca²⁺, Pb²⁺:Mg²⁺) in *B. napus*. The highest values were recorded for plants from the Cd treatments on mineral soil – (Cd²⁺:Mg²⁺) up to 21.42 in roots.

A large increase in the $\text{Pb}^{2+}:\text{Ca}^{2+}$ and $\text{Pb}^{2+}:\text{Mg}^{2+}$ ratios occurred in *B. napus* roots from mineral acidic soil. It was probably the result of higher Pb mobility in acidic soil. BADORA (2012) reported a significant increase of Pb mobility observed at pH below 5. Significantly higher values of $\text{Cd}^{2+}:\text{Ca}^{2+}$, $\text{Cd}^{2+}:\text{Mg}^{2+}$, $\text{Pb}^{2+}:\text{Ca}^{2+}$, $\text{Pb}^{2+}:\text{Mg}^{2+}$ ratios were found in *B. napus* roots rather than in shoots. Most of Pb taken up by a plant accumulates in roots (GAMBUŚ 1997), hence roots are the first barrier to the Pb translocation to shoots. Those properties were also reflected in the $\text{Pb}^{2+}:\text{Ca}^{2+}$, $\text{Pb}^{2+}:\text{Mg}^{2+}$ ionic ratios in roots, which were higher by 1 or even 2 orders of magnitude than in shoots, respectively. Cadmium, as more mobile element than lead, was easily absorbed and translocated to shoots. *B. napus* was less susceptible to mineral composition changes when grown on contaminated organic soil rather than on mineral one. The research on Cd and Pb fractionation in organic soil prove that organic compounds may affect the binding Pb and Cd in organic fraction (DOMAŃSKA et al. 2013) and limit the metal uptake. The studied mmol ratios in *B. napus* plants from organic soils were low (below 0.30 in shoots) and according to obtained data, this obviously excludes this plant from Cd and Pb phytoextraction purposes, when cropping on that types of soils at given levels of Cd and Pb contamination.

CONCLUSIONS

1. The highest sensitivity of *B. napus* to soil Cd contamination (reduction of DM from shoots, poor growth and delayed generative development) was observed in the treatment on acidic mineral soil.

2. On mineral soil under Cd and Pb stress, *B. napus* produces higher biomass than on organic soil, but its mineral composition (ionic ratios) distinctly changes as compared to the control, while the chemical composition of plants from contaminated organic soils changed slightly.

3. The high Ti values (except from mineral acidic soil) prove that *B. napus* was generally tolerant to metals.

4. The soil type played a crucial role in determining the milimolar ratios of the examined ratios of ions: $\text{Pb}^{2+}:\text{Ca}^{2+}$ and $\text{Pb}^{2+}:\text{Mg}^{2+}$ in *B. napus*. Large changes in the mineral composition were observed in plants from mineral soils: typically an increase in ratios, as compared to the control. In plants from organic soils, the changes were small. This indicates the protective role of soil organic substance on *B. napus* chemical composition and excludes the plant species from Cd, and Pb phytoextraction purposes on that type of soil.

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