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EVALUATION OF THE USE OF SPRING RAPESEED IN PHYTOREMEDIATION OF SOILS CONTAMINATED WITH TRACE ELEMENTS AND THEIR EFFECT ON YIELD PARAMETERS

ABSTRACT

The experimental material was made up by the plant organs of *Brassica napus* L. from a pot experiment during one vegetation period. There was investigated the effect of relatively high concentration of zinc, copper, lead and cadmium in soil on the rapeseed yield, the content of protein and oil in seeds. The impact of metals was defined based on the content of selected fatty acids in oil extracted from seeds. The highest contents of zinc and copper were found in leaves, lead – in roots and cadmium – in stems. The biological concentration factor values were respectively calculated for all the rapeseed organs. For Cu and Pb the values of biological concentration factor were low and very low for all the plant organs. The doses of Zn (300 mg × kg^{-1}, 600 mg × kg^{-1}) and Cu (80 mg × kg^{-1}, 160 mg × kg^{-1}) applied in the pot experiment resulted in the translocation of metals from the roots to the leaves. The doses of lead (400 mg × kg^{-1}, 1600 mg × kg^{-1}) did not trigger any translocation of that metal from the roots to the above-ground rapeseed plant parts, however, after the application of the cadmium doses (2 mg × kg^{-1}, 6 mg × kg^{-1}), there was recorded a clear translocation of Cd to the rapeseed stems and the leaves. A relatively high content of zinc, copper, lead and cadmium in soil had a significant effect neither on the yield parameters and nor on the qualitative characters of the rapeseed seed. Neither did they affect the content of protein, fat and fatty acids in seed-extracted oil. The results of the pot experiment suggest that spring rapeseed is suitable for the phytoremediation of moderately heavy-metal-contaminated soils.

Key words: *Brassica napus* L., heavy metals, phytoextraction, pot experiment

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INTRODUCTION

Certain trace elements are essential microelements for plants but elevated concentration of these metals cause toxic effects. Civilisation progress often results in irrevocable environmental changes, seen in an increase in the content of trace elements. They penetrate into soil and surface waters due to industrial gas emissions, fumes, fossil fuel burning, and the accumulation of household and industrial waste, emission of automotive fumes (Kabata-Pendias 2001).

An important source of contamination with trace elements is made up by artificial fertilisers and pesticides applied in agriculture (Susarla *et al.* 2002). The accumulation of trace elements in soils, especially in a form of easily available to plants, can result in their excessive uptake and concentration in plant tissues (Jahangir *et al.* 2008), which results in a decrease in the plant yield and in producing plant material of quality characters not corresponding to the standards applied in food processing and animal feed production (Basta *et al.* 2005, Brunetti *et al.* 2011,. Vamerali *et al.* 2010, Podar *et al.* 2004, Su and Wong 2004).

One of the methods of biological soil clean-up technologies is phytoremediation which uses the capacity of some plant species for uptaking considerable amounts of contaminants from soil and water and their accumulation in tissues at the amounts a few-fold greater than the ones found in the tissues of other plants (Blaylock *et al.* 1997, Ensley 2000). Currently applied technologies for trace elements phytoremediation include:

1) phytostabilization - the use of plants to reduce metal mobility in polluted soil due to the concentration by roots or precipitation within the rhizosphere;
2) phytoextraction - the use of plants to extract trace elements from contaminated soils and to translocate and to accumulate the trace element in aboveground parts of plants;
3) phytovolatilization - the use of plants to turn volatile chemical species of soil metals (Chaney *et al.* 2007, Liang *et al.* 2009, Pulford *et al.* 2002).

Phytoextraction of metal-contaminated soil relies on the use of plants to reduce the concentration of metals in contaminated soils to regulatory levels within a reasonable time frame. Trace element accumulation is described by the metal biological absorption coefficient, i.e. the plant (harvestable)-to-soil metal concentration ratio. Both the value of biological concentration factor (BCF); organs of plant-to-soil metal concentration ratio and the translocation factor (TF); organs of plant (seeds, leaves, stems)-to-roots metal concentration ratio can positively affect phytoextraction.

Plants with high value of biological absorption coefficient are suitable for phytoextraction; whiles with higher than 1 value of BCF and low translocation factor have potential for phytostabilisation (Yoon *et al.* 2006). The plants demonstrate, at the same time, a high increase in the biomass and resistance to unfavourable environmental conditions.

Hyperaccumulator plants are hypertolerant to the metals they accumulate in the plant parts and usually show a higher metal concentration in the shoots than in the roots (the shoot to root metal concentration ratio of >1). So far the most common hyperaccumulators have included indian mustard *(Brassica juncea*), penny-cress (*Thlaspii* L.), especially *Thlaspi caerulescens* and common tumble weed (*Amaranthus retroflexus*) (Salt *et al.* 1995, Kärenlampi *et al.* 2000, Perronnet *et al.* 2003, Bogs *et al.* 2003, Qadir *et al.* 2004). Numerous studies have been performed into the use of other plant species to be used in phytoremediation (Mijovilovich *et al.* 2009, Prasad 2003).

For phytoremediation the ideal plant should possess multiple traits like fast growing, deep roots, have high biomass and should tolerate and accumulate a range of trace elements. *Brassica* plants are well known as trace element accumulators and are being used for phytoremediation of contaminated soils. The aim of the present research was to evaluate spring rape, double-improved cv. Heros, in the phytoremediation of soils relatively contaminated with high doses of copper, cadmium and lead. The evaluation was made based on pot experiments under controlled conditions of the vegetation hall. The experiment also determined the contents of zinc, copper, lead and cadmium in seeds, leaves, stems and roots of *Brassica napus* L.

MATERIAL AND METHODS

The research material was made up by vegetative organs and seeds of spring rapeseed, double-improved cv. Heros. Spring rapeseed accessions of *Brassica napus* L. were grown under standard conditions in the greenhouse laboratory. The pot experiment was set up in completely randomised design in four replications on the soil material sampled from the topsoil of Phaeozem (sandy loam texture). The soil material was placed at the amount of 10 kilograms to each pot and then the trace elements were introduced into the pots. The soil was mixed in each pot after metals addition. The forms and doses of zinc, copper, lead and cadmium are given in Table 1. The fertilisation with NPK and Mg ($Ca(H_2PO_4)_2{\cdot}H_2O$ = 36.60 mg × $kg^{-1}$of soil, K_2SO_4 = 76.4 mg × kg^{-1} of soil, $MgSO_4{\cdot}7\ H_2O$ = 62.8 mg × kg^{-1} of soil) was applied pre-sowing in spring. The nitrogen dose was 34.5 mg N × kg^{-1} of soil. Seeds of rapeseed were sown in the second decade of April a year after the contamination with trace elements. After the plant emergence the selection was made, leaving 8 same developed seedlings in each vegetation pot. The plants were poured with distilled water, keeping the soil moisture in the pot at 60% of field water capacity.

Table 1

Doses of metals [mg × kg^{-1}]

Metal doses	Form of metals	Concentration [mg × kg^{-1}]
Zn-$_{I}$	$ZnSO_4 \cdot 7\ H_2O$	300
	$Zn(NO_3)_2 \cdot 6\ H_2O$	
	$Zn(CH_3COO)_2 \cdot 2\ H_2O$	
Zn-$_{II}$	$ZnSO_4 \cdot 7\ H_2O$	600
	$Zn(NO_3)_2 \cdot 6\ H_2O$	
	$Zn(CH_3COO)_2 \cdot 2\ H_2O$	
Cu-$_{I}$	$CuSO_4 \cdot 5\ H_2O$	80
	$CuCl_2 \cdot 2\ H_2O$	
Cu-$_{II}$	$CuSO_4 \cdot 5\ H_2O$	160
	$CuCl_2 \cdot 2\ H_2O$	
	$Cu(NO_3)_2 \cdot 3\ H_2O$	
Pb-$_{I}$	$Pb(CH_2COO)_2 \cdot 3\ H_2O$	400
Pb-$_{II}$	$Pb(CH_2COO)_2 \cdot 3\ H_2O$	1600
Cd-$_{I}$	$CdCl_2 \cdot 2½\ H_2O$	2
Cd-$_{II}$	$CdCl_2 \cdot 2½\ H_2O$	6

During the plant growth chemical control of rapeseed against pathogens and pests was applied. The plants were harvested in the 3rd decade of July after 14 weeks of rapeseed-growing. The following were determined: the seed yield per pot, 1000 seed weight, number of seeds collected per pot and per plant. For the seeds collected there were defined the percentage content of protein with the Kjeldahl method, percentage content of fat and fatty acids in oil extracted from seeds. The content of fatty acids (palmitic, stearic, oleic, linolic, linolenic, eicosic and erucic acids) in oil from the seeds was determined following the oil extraction compliant with the applicable methods provided for in EN ISO 5508 (European Standard ISO 5508: 1990) and EN ISO 5509 (European Standard ISO 5509: 2000). The analysis of the methyl derivatives of fatty acids was made with the gas chromatography method. The soil study was performed following the applicable procedures, applying the methods used in soil sciences. The soil material dried at room temperature was screened through the sieve with the mesh 2 mm in diameter. The following were assayed in the soil samples (at the beginning of the experiment and after 18 months): texture with the areometric method; soil pH in 1M KCl was determined in 1:2.5 soil/solution suspensions; the cation exchangeable capacity (CEC) in 1 M CH_3COONH_4; the content of total organic carbon (TOC) and nitrogen (N_t) using the TOCN Primacs Skalar Analyser. The plant materials were oven dried for 48 h at 70°C, then plant tissues dry weight were measured. The soil samples were dried at 105°C to reach constant dry weight and then analysed for trace elements content.

The content of metals forms in soil available to plants was determined following the Lindsay and Norvell (1978) method after the extraction with the solution of diethylene triamine pentaacetic acid (DTPA). To determine the total content of trace elements, soil samples were mineralised in the mixture of hydrofluoric acid and perchloric *acid*, while the plant material was mineralised in the microwave oven exposed to the mixture of HNO_3 and H_2O_2 (Kalra 1998).

Applying the atomic absorption spectroscopy method, using Philips PU 9100, there were determined the total contents of Zn, Cu, Pb, and Cd in soil and the content of their forms available to plants. The content of trace elements in the plant material (seeds, leaves, stems and roots) was defined using the emission spectrometry with inductively coupled plasma (ICP), with Jobin Yvon Emission JY 38 S. The biological concentration factor (BCF) values for the metals content in aboveground plant parts depending on the total accumulation of those metals in soil were calculated. The bioconcentration factors were calculated respectively for the roots, stems, leaves and seeds of rapeseed.

There was used the single-factor completely randomised design, while the significance of the differences between treatment means was verified using the Tukey test.

RESULTS

The pot experiment was set up on sandy loam. There were found relatively inconsiderable changes in the basic soil parameters caused by the metals doses applied 18 months after being added to soil. After 18 months the following were observed in control pots: mean pH in 1 M KCl - 7.18; TOC mean content 15.0 $g \times kg^{-1}$ and N_t 1.27 $g \times kg^{-1}$; CEC 158.2 mmol(+) $\times kg^{-1}$. The total contents of Zn, Cu, Pb and Cd and their forms extracted with DTPA solution were typical for soils not contaminated with trace elements (Table 2).

Table 2

Metal content in soil from control pots

Time period	Zn_t	Zn_{DTPA}	$\frac{Zn_{DTPA}}{Zn_t}$	Cu_t	Cu_{DTPA}	$\frac{Cu_{DTPA}}{Cu_t}$	Pb_t	Pb_{DTPA}	$\frac{Pb_{DTPA}}{Pb_t}$	Cd_t	Cd_{DTPA}
	(mg × kg^{-1})		(%)	(mg × kg^{-1})		(%)	(mg × kg^{-1})		(%)	(mg × kg^{-1})	
3 days	47.30	4.40	9.40	7.20	0.85	11.80	12.10	0.80	6.60	<0.20	n.a.
18 months	47.10	5.50	11.70	7.30	0.80	11.30	12.20	0.90	7.40	<0.20	n.a.

$_t$ – total metal content; $_{DTPA}$ – DTPA extractable metal content; n.a. - not analysed

Table 3

Selected soil properties in pots (n = 4). (A) zinc and copper, (B) lead and cadmium

(A) — zinc and copper content								
Properties	Zn_{-I}		Zn_{-II}		Cu_{-I}		Cu_{-II}	
	A	B	A	B	A	B	A	B
pH 1M KCl	7.07	7.31	7.17	7.38	7.13	7.35	7.28	7.23
TOC [g × kg^{-1}]	14.4	14.5	14.5	14.4	14.6	14.7	14.6	14.6
Nt [g × kg^{-1}]	1.2	1.3	1.3	1.3	1.3	1.4	1.2	1.4
CEC [mmol × kg^{-1}]	163.2	163.2	163.0	160.3	164.5	148.0	148.0	151.0
Total content [mg × kg^{-1}]	356.5	296.4	667.2	651.8	83.9	81.1	173.9	142.6
DTPA content [mg·kg^{-1}]	n.a	151.9	n.a	313.3	n.a	48.6	n.a	113.6
% DTPA in total metal content		51.2		48.4		59.8		79.7

(B) — lead and cadmium content								
Properties	Pb_{-I}		Pb_{-II}		Cd_{-I}		Cd_{-II}	
	A	B	A	B	A	B	A	B
pH 1M KCl	7.14	7.28	7.12	7.27	7.14	7.28	7.12	7.27
TOC [g × kg^{-1}]	15.1	14.9	14.6	14.7	15.1	14.9	14.6	14.7
Nt [g × kg^{-1}]	1.2	1.3	1.3	1.3	1.2	1.3	1.3	1.3
CEC [mmol × kg^{-1}]	160.5	164.2	153.7	162.0	160.5	164.2	153.7	162.3
Total content [mg × kg^{-1}]	418.3	400.5	1651.7	1632,0	2.03	2.0	6.01	6.01
DTPA content [mg·kg^{-1}]	n.a.	331.0	n.a.	985.2	n.a.	1.91	n.a.	5.04
% DTPA in total metal content		82.6		60.4		95.4		89.6

A – 3 days; B – 18 months; TOC – total organic carbon; N_t – total content of nitrogen, CEC – cation exchangeable capacity; n.a. - not analysed

Applied fertilisation and the addition of trace elements to soil resulted in a slight decrease in the exchangeable acidity and slightly decreased the content of exchangeable cations (Table 3 a, b). After the extraction with the DTPA solution, it was shown that a considerable part of the trace elements occurred in a form directly available to plants and the mean percentage share of forms of those elements in contaminated soil (after 18 months) was as follows: for $Zn\text{-}_{I}$ = 51.2% and $Zn\text{-}_{II}$ = 48.4%; $Cu\text{-}_{I}$ = 59,8% and $Cu\text{-}_{II}$ = 79.7 %; $Pb\text{-}_{I}$ = 82.6% and $Pb\text{-}_{II}$ = 60.4%; $Cd\text{-}_{I}$ = 95.4% and $Cd\text{-}_{II}$ = 89.6% of their total content (Table 3 a, b). The amount of trace elements added upon the start of the pot experiment as well as their total content in soil found after 18 months much exceeded the admissible amounts for non-contaminated soils. The content of metals in non-contaminated Polish soils should not exceed 300 mg × kg^{-1} of soil for zinc; 150 mg × kg^{-1} of soil for copper; 100 mg × kg^{-1} of soil for lead and 4 mg × kg^{-1} of soil for cadmium (Journal of Laws No 165, item 1359. Regulation of Minister of Environment, dated 9 September 2002).

Table 4

Metal content in the plant organs (d.w.) and selected rapeseed yield parameters

Treatment	Seeds	Leaves	Stems	Roots	Seeds yield per pot	1000 mass seeds per pot	Seeds number per pot	Seeds number per plant
	Metal content				Yield parameters			
	[mg × kg^{-1}]				[g]	[g]		
$Zn_{control}$	22.83	67.35	21.77	36.87	5.19	2.89	1793	224
$Zn\text{-}_{I}$	80.12	320	253.9	98.82	5.13	3.01	1700	212
$Zn\text{-}_{II}$	101.3	583.4	347.1	210.2	5.33	2.89	1851	231
$LSD_{p=0.05}$	8.78	1.71	45.15	13.93	n.s.	n.s.	n.s.	n.s.
$Cu_{control}$	1.37	2.96	0.00	2.51	5.19	2.89	1794	224
$Cu\text{-}_{I}$	2.06	5.22	1.19	4.32	5.09	3.11	1648	206
$Cu\text{-}_{II}$	3.51	9.74	1.24	7.21	5.40	3.00	1804	225
$LSD_{p=0.05}$	0.41	2.49	1.30	1.16	n.s.	n.s.	n.s.	n.s.
$Pb_{control}$	0.00	0.00	0.00	0.00	5.19	2.89	1794	224
$Pb\text{-}_{I}$	3.02	3.34	0.00	27.99	5.11	2.85	1798	225
$Pb\text{-}_{II}$	3.85	6.63	7.11	113.8	4.53	3.00	1516	189
$LSD_{p=0.05}$	2.35	1.70	4.33	35.88	n.s.	n.s.	n.s.	n.s.
$Cd_{control}$	0.00	0.00	0.00	0.00	5.19	2.89	1794	224
$Cd\text{-}_{I}$	0.00	2.22	2.39	1.80	5.12	2.77	1843	230
$Cd\text{-}_{II}$	0.81	4.27	5.94	3.23	4.48	3.01	1488	186
$LSD_{p=0.05}$	0.15	3.64	0.27	2.18	n.s.	n.s.	n.s.	n.s.

n.s. – non-significant

Metals found in the soil materials demonstrated a significant increase in their concentration in the plant material analysed as compared with the control plants (Table 4). The pot experiment involved the contents of Zn, Cu, Pb, Cd and their translocation in rapeseed organs (stems, leaves, roots and seeds) depending on the doses of metals applied. The highest content of Zn (583.39 mg × kg^{-1} d.w.) and Cu (9.74 mg × kg^{-1} d.w.) were recorded in the leaves of rapeseed growing in the pots where zinc at the dose of 600 mg × kg^{-1} of soil and 160 mg × kg^{-1} of soil were added (Table 4). The lowest content of those metals was reported in the seeds, as compared with the other rapeseed organs. The highest Pb content in the rapeseed plants growing in the soil with Pb-$_{I}$ and Pb-$_{II}$ doses was identified in the roots, while the lowest Pb content, irrespective of the load dose applied, was observed in the seeds. Most lead (113.8 mg × kg^{-1}) was found in the dry weight of roots of rapeseed growing in the soil containing 1600 mg × kg^{-1} (Pb-$_{II}$) of soil. After the application of cadmium at the 6.0 mg × kg^{-1} dose, its highest content was noted in stems, as compared with the Cd content in the other organs. The lowest Cd content after the application of this metal was identified in the seeds (Table 4).

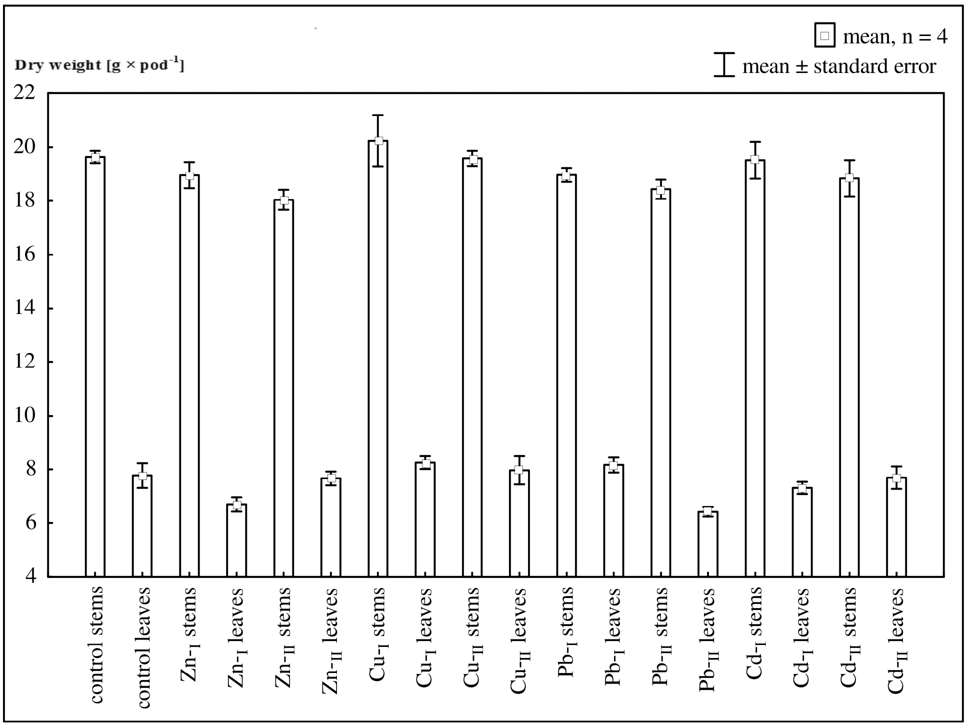

Fig. 1. Leaves and stems biomass production [dry weight per pot]

No significant effect of the zinc, copper, lead and cadmium doses applied on the selected plant seeds parameters was found. The highest seeds yield

(5.40 g) was collected from the plants grown in pots in the soil contaminated with zinc at the Cu-$_{II}$ dose, and the lowest (4.48 g) – for rapeseed growing in the soil contaminated with cadmium at the dose of 6 mg·kg^{-1} of soil (Table 4). It was found that the 1000 seeds weight was highest (3.11 g) in the rapeseed collected from pots contaminated with copper at the dose of 80 mg × kg^{-1} of soil, and the lowest (2.77 g) – for rapeseed collected from the pots to which cadmium at the dose of 2 mg·kg^{-1} of soil was added. The other agrotechnical yield parameters, the seeds number per pot and the number of seeds per plant were highest in the rapeseed collected from the pots contaminated with zinc at the dose Zn-$_{II}$ and the lowest – for the plants of rapeseed collected from the soil contaminated with cadmium at the dose of 6 mg × kg^{-1} of soil.

Table 5

Aboveground plant parts translocation factor (organ/roots ratio) and metals removal by rapeseed, for Zn, Cu, Pb, Cd [mg× pot^{-1}]

Treatment	Translocation factor (TF)			Metals removal per pot (dry weight)			
	Seeds/ roots ratio	Leaves/ roots ratio	Stems/ roots ratio	Seeds	Leaves	Stems	Aboveground plant parts
Zn $_{control}$	0.62	1.83	0.59	0.12	0.52	0.43	1.07
Zn-$_{I}$	0.81	3.24	2.57	0.41	2.14	4.81	7.36
Zn-$_{II}$	0.48	2.77	1.65	0.54	4.47	6.26	11.27
Cu $_{control}$	0.55	1.18	0.0	0.007	0.023	0.0	0.030
Cu-$_{I}$	0.48	1.21	0.28	0.010	0.043	0.024	0.077
Cu-$_{II}$	0.49	1.35	0.17	0.019	0.078	0.024	0.121
Pb $_{control}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pb-$_{I}$	0.11	0.12	0.0	0.015	0.027	0.0	0.042
Pb-$_{II}$	0.03	0.06	0.06	0.017	0.042	0.131	0.190
Cd $_{control}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cd-$_{I}$	0.0	1.23	1.33	0.0	0.016	0.047	0.063
Cd-$_{II}$	0.25	1.32	1.84	0.004	0.033	0.112	0.149

The highest biomass stems and leaves (dry weight) per pot (20.24 and 8.24 g) was collected from the plants grown in pots contaminated with copper at the Cu-$_{II}$ dose (Fig. 1), however the lowest (18.43 and 6.42 g) – for rapeseed growing in the soil contaminated with cadmium at the Pb-$_{II}$ dose.

The spring rapeseed cultivar showed the highest capacity for the bioaccumulation of zinc in its aboveground parts. In the pots to which 600 mg Zn × kg^{-1} was added, the average content of its bioavailable forms for plants was 313.3 mg × kg^{-1} (Table 3a). The plants from those pots contained

11.27 mg of zinc in the aboveground parts (dry weight), which accounts for an average of 1.41 mg per plant. The aboveground parts of rapeseed plants grown in the pots to which 160 mg Cu × kg^{-1}, 1600 mg Pb × kg^{-1} as well as 6 mg Cd × kg^{-1} were added, contained 0.121 mg Cu in the dry weight of plants per pot, 0.190 mg Pb per pot as well as 0.149 mg Cd per pot, respectively (Table 5).

Table 6

Effect of trace elements on selected quality parameters in rapeseed

Treatment	Protein content	Fat content	Mean fatty acids content						
			Palmitic	Stearic	Oleic	Linolic	Linolenic	Eicosic	Erucic
					[%]				
$Zn_{control}$	23.47	43.05	4.52	2.25	70.47	16.22	5.67	0.85	0.00
$Zn\text{-}_{I}$	23.50	43.67	4.55	2.15	69.67	15.95	5.57	2.12	0.00
$Zn\text{-}_{II}$	23.82	43.12	4.40	2.27	71.67	15.45	5.32	0.80	0.00
$LSD_{p=0.05}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
$Cu_{control}$	23.47	43.05	4.52	2.25	70.47	16.22	5.67	0.85	0.00
$Cu\text{-}_{I}$	24.12	42.17	4.45	2.40	70.62	15.97	5.62	0.97	0.00
$Cu\text{-}_{II}$	23.97	43.07	4.37	2.42	70.57	15.97	5.70	0.95	0.00
$LSD_{p=0.05}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
$Pb_{control}$	23.47	43.05	4.52	2.25	70.47	16.22	5.67	0.85	0.00
$Pb\text{-}_{I}$	23.55	43.37	4.75	2.27	70.65	15.87	5.62	0.85	0.00
$Pb\text{-}_{II}$	23.50	44.37	4.40	2.30	70.60	15.85	5.92	0.97	0.00
$LSD_{p=0.05}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
$Cd_{control}$	23.47	43.05	4.52	2.25	70.47	16.22	5.67	0.85	0.00
$Cd\text{-}_{I}$	22.90	43.80	4.45	2.15	70.97	15.92	5.70	0.82	0.00
$Cd\text{-}_{II}$	23.30	43.22	4.47	2.37	70.97	15.85	5.52	0.77	0.00
$LSD_{p=0.05}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

n.s. – non-significant

It was demonstrated that the heavy metal doses applied did not have a significant effect on the percentage content of protein, fat and respective fatty acids in spring rapeseed seeds (Table 6). The content of protein ranged from 22.9 % in the seeds collected from pots contaminated with cadmium - 2 mg × kg^{-1} of soil to 24.1% in the case of seeds collected from rapeseed growing in the soil contaminated with copper at the dose of 80 mg × kg^{-1} of soil. It was found that the seeds of rapeseed with the highest percentage protein content contained least fat. The highest fat content (44.37%) was found in the seeds collected from the plants growing in pots contaminated with lead – $Pb\text{-}_{II}$ (Table 6). The increase in the cadmium dose from 2 to 6 mg·kg^{-1} of soil resulted in a significant increase in the content of stearic acid in seeds-extracted oil. The zinc, copper, lead and cadmium doses applied in the experiment did not have a significant effect on the percentage content of fat and fatty acids in seed-extracted oil, which is favourable if considering their technological use in biofuel production. In the experiment, however, there was identified a significant effect of the heavy metal doses on their content in the rapeseed organs investigated.

The values of the biological concentration factor (BCFs) were highest for cadmium and zinc. Higher doses of those metals (Cd_{II} - 6.0 and Zn_{II} - 600 mg × kg^{-1}), respectively, resulted in a decrease in the BCFs value, pointing to their lower bioaccumulation (Table 7).

Table 7

Biological concentration factor [Dry weight plant part/soil ratio]

Treatment	BCF – biological concentration factor [metal total content mg × kg^{-1}]			
	Seeds /soil ratio	Leaves/ soil ratio	Stems/ soil ratio	Roots/ soil ratio
$Zn_{control}$	0.48	1.43	0.46	0.78
Zn_{-I}	0.27	1.08	0.86	0.33
Zn_{-II}	0.15	0.86	0.52	0.31
$Cu_{control}$	0.19	0.41	0.00	0.34
Cu_{-I}	0.03	0.06	0.01	0.05
Cu_{-II}	0.02	0.07	0.01	0.05
$Pb_{control}$	0.00	0.00	0.00	0.00
Pb_{-I}	0.01	0.01	0.0	0.07
Pb_{-II}	0.003	0.004	0.005	0.07
$Cd_{control}$	0.00	0.00	0.00	0.00
Cd_{-I}	0.00	1.11	1.19	0.90
Cd_{-II}	0.13	0.71	0.99	0.54

DISCUSSION

The experiments, which involved rapeseed exposed to a contamination with trace elements show that the degree of soil contamination differentiates their content in the plant organs considerably. There was found a stimulating effect of zinc in soil on the increase in the content of this element in the rapeseed stems and the roots and leaves. The content of zinc and copper in rapeseed increased with an increase in the concentration of metals in soil, however, the content of those elements in stems and leaves decreased during seed ripening as compared with their content at the rapeseed flowering stage (Rossi *et al* 2004). A considerable part of the trace elements applied occurred in a form directly available to plants. Generally bioavailability of trace elements decreases with increasing residence time.

Brassica species are well known as metal accumulators and especially *Brassica juncea* has been investigated for the accumulation of rage of trace elements in its shoots (Ebbs and Kochian 1997, Bogs *et al.* 2003, Qadir *et al.* 2004). In the present experiment the content of trace elements varied across spring rapeseed organs; the highest contents of Zn and Cu were found in rapeseed leaves, Pb in roots and Cd in stems. Grispen *et al.* (2006) report on rapeseed grown in the soils (pH from 4.2 to 5.7) containing cadmium from 2.5 to 5.5 mg × kg^{-1} contained in the stems, on average, from 5.2 to 7.6 mg Cd in kg of dry weight of plant. Marchiol *et al.* (2004) found that the main organ accumulating trace elements were the roots. Rossi *et al.* (2004) and Szulc *et al.* (2010) showed that the rapeseed roots accumulated Zn and Cu, and their contents in the roots depended on the amount of those metals in the soil.

Although many plant species accumulate much higher amounts of trace elements in its organs than rapeseed but *Brassica napus* L. is one of the plant, which can be used in phytoremediation on a large scale (Ebbs 1997, Marchiol *et al.* 2004). Hyperaccumulator plants are hypertolerant to the metals they accumulate in the plant parts and usually show a higher metal concentration in the shoots than in the roots (the shoot to root metal concentration ratio of >1).

The directive 2003/30/EC of the European Parliament (on the promotion of the use of biofuels or other renewable fuels for transport) guarantees an increase in the acreage of crops for industrial purposes, referring to fuel and power industry, and which is connected with biofuel production (Cardone *et al.* 2003). For that reason rapeseed growing can involve arable fields of a higher content of metals. *Brassica* species have been mainly used for their nutritional qualities, but phytoextraction combined with biofuel production is increasingly becoming profitable enterprise (Grispen *et al.* 2006).

The rapeseed application to phytoremediation of the areas contaminated with trace elements is also justified by the results of this experiment. It was found that the contamination of the soil with trace elements did not result in significant changes in the content of protein and fat as well as fatty acids in the seed-extracted oil. Bidar *et al.* (2008) report on trace elements in contaminated soil resulting in significant changes in the content of fatty acids both in the roots and stems of perennial ryegrass and white clover. The composition of fatty acids in oilseed rape seeds is determined by fertilisation with microelements (Kotecki *et al.* 2001, Spychaj-Fabisiak *et al.* 2011). The percentage share of respective fatty acids in oil extracted from analysed rapeseed was comparable with a typical composition of fatty acids in the oil produced from Canola, used to produce biodiesel (Moser 2009).

Brassica napus L. to be a possible candidate species for phytoremediation of moderately heavy metal contaminated soils (Rossi *et al.* 2004).

CONCLUSIONS

Trace elements applied to the soil material significantly increased their concentration in the analysed plant material. The highest content of zinc and copper was observed in the leaves, however, lead – in the roots, and cadmium – in stems in *Brassica napus* L. Investigated spring rapeseed cultivar demonstrated the greatest capacity for the accumulation of zinc in the aboveground plant parts. The plants collected from pots, to which 600 mg $Zn \times kg^{-1}$ was added, contained 11.27 mg of zinc in the dry weight of aboveground plant parts, which, on average, accounts for 1.41 mg Zn per plant. The Zn and Cu doses applied in the pot experiment resulted in the translocation of metals from the roots to the leaves as well as for the $Zn\text{-}_{I}$ dose – in a clear translocation of zinc to stems. The leaves and the stems of control plants contained neither Pb nor Cd. The $Pb\text{-}_{I}$, $Pb\text{-}_{II}$ doses did not cause any translocation of this metal from the roots to the aboveground parts of rapeseed plants, while, after the application of $Cd\text{-}_{I}$ and $Cd\text{-}_{II}$ doses, there was a clear translocation of cadmium to the stems and leaves of rape. The plants referred to as hyperaccumulators usually demonstrate a higher concentration of metals in the shoots than in the roots (metal translocation factor >1). The spring rapeseed plants met this condition in terms of the Zn bioaccumulation in the leaves and in the stems, Cu in the leaves and Cd in the stems and leaves. The $Zn\text{-}_{II}$ dose, however, resulted in a decrease in the value of the translocation factor, as compared with the $Zn\text{-}_{I}$ dose. A relatively high content of zinc, copper, lead and cadmium in soil affected significantly neither the parameters of the seed yield nor the quality characteristics of the spring rapeseed seeds; namely the content of protein, fat and fatty acids. The $Zn\text{-}_{II}$ and $Cu\text{-}_{II}$ doses applied in the pot experiment resulted in a slight increase in the seed yield, as compared with the rapeseed seed yield from the control pots, while the $Pb\text{-}_{II}$ and $Cd\text{-}_{II}$ doses decreased the seed yield. However, the differences were non-significant. The results of research point to high spring rapeseed application potential for the phytoremediation of soils contaminated with Zn, Cu, Pb and Cd; and so a practical application of spring rapeseed to the phytoextraction seems well justified.

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