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Effect of Heavy Metals on Inhibition of Root Elongation in 23 Cultivars of Flax (*Linum usitatissimum* L.)

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Abstract The effect of toxic metals on seed germination was studied in 23 cultivars of flax (*Linum usitatissimum* L.). Toxicity of cadmium, cobalt, copper, zinc, nickel, lead, chromium, and arsenic at five different concentrations (0.01–1 mM) was tested by standard ecotoxicity test. Root length was measured after 72 h of incubation. Elongation inhibition, EC_{50} value, slope, and NOEC values were calculated. Results were evaluated by principal component analysis, a multidimensional statistical method. The results showed that heavy-metal toxicity decreased in the following order: $As^{3+} \geq As^{5+} > Cu^{2+} > Cd^{2+} > Co^{2+} > Cr^{6+} > Ni^{2+} > Pb^{2+} > Cr^{3+} > Zn^{2+}$.

The number of areas contaminated with heavy metals has increased enormously during the last century, mainly due to urban and other industrial activities, such as mining, smelting, and manufacturing. Currently heavy-metal toxicity and accumulation represents a serious ecologic burden. These elements can be leached into water, taken up by plants, and released as gases into the atmosphere, or bound semipermanently by soil components, such as clay or organic matter, and thus consequently affect human health (Saether et al. 1997; Acero et al. 2003).

Increased levels of heavy metals represent a significant stress factor for the environment and for humans. Their toxicities impose different physiologic effects, e.g., indigestions, different types of dermatitis, changes in blood count, damage to fundamental organs (brain, liver, and kidney), and cancerous processes. The characteristic feature of most cations is strong binding with –SH, –COOH, and $–NH_2$ groups (Kafka and Punčochářová 2002). Therefore, it is important to diminish concentrations of heavy metals to the least harmful levels.

Heavy-metal toxicity is usually tested by root-elongation inhibition (Öncel et al. 2000; Ouariti et al. 1997). Fargašová (1998) tested the impact of heavy-metal treatment (Cu^{2+}, Ni^{2+}, Mn^{2+}, MoO_4^{2-}, and VO_4^{3-}) on the inhibition of root elongation and formation of photosynthetic pigments of *Sinapis alba*. Root elongation was most affected by Cu^{2+} and MoO_4^{2-}. Fargašová and Beinrohr (1998) and Fargašová (1999) investigated metal–metal cross-interactions affecting the accumulation of the previously mentioned metals. Lead uptake and its effect on seed germination and plant growth in was measured in lead hyperaccumulator *Brassica pekinensis* (Xiong 1998). Germination rate as well as root length were substantially decreased at a concentration of 1000 μg Pb/mL. The inhibitory effects of cadmium, copper, zinc, lead, and iron ions on root elongation and contents of photosynthetic pigments as well as metal accumulation were tested in roots and shoots of *S. alba* (Fargašová 2001). The metals were arranged on the basis of elongation inhibition in the following order: Cu > Cd > Fe = Zn > Pb. Peralta et al.

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(2001) reported a stimulatory effect of low-dose (0.005 mg/L) heavy metals (Cd^{2+}, Cr^{6+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}) on root and shoot elongation of alfalfa plants (cultivar Malone), whereas doses of 0.04 mg/L of Cd^{2+}, Cr^{6+}, Cu^{2+}, and Ni^{2+} significantly decreased seed germination and seedling elongation. Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus* were also studied by Munzuroglu and Geckil (2002). Mercury was found to be the most inhibiting metal. It caused complete inhibition of germination in wheat and cucumber seeds, respectively, at concentrations of 1.7 mM and $\geq$ 1.5 mM. Blažek et al. (2003) tested cadmium uptake and accumulation in different plant organs (root, stalk, capsule, and seeds) of fibre flax and oily flax.

The aim of the present work was to evaluate the effect of toxic metals on the germination of flax (*Linum usitatissimum* L.) seeds in order to select resistant cultivars for phytoremediation purposes.

Materials and Methods

Plant Material and Chemicals

Twenty-three cultivars of *L. usitatissimum* L. (Merkur, Flanders, Bonet, Jordán, Super, Hermes, Agáta, Laura, Viking, Ilona, Elektra, Jitka, Tábor, Lola, Viola, Escalina, Atalante, Venica, Raisa, Bilt Star, Marylin, Marina, and Recital) seeds (Flax Germplasm Collection, Agritec Ltd., Šumperk, Czech Republic) were used for the germination test. Heavy-metal ions (As^{3+}, As^{5+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) were obtained from salts $NaAsO_2$, $Na_2HAsO_4 \times 7H_2O$, $Cd(NO_3)_2 \times H_2O$, $Co(NO_3)_2 \times 6H_2O$, $Cr(NO_3)_3 \times 9H_2O$, $K_2Cr_2O_7$, $Cu(NO_3)_2 \times 3H_2O$, $Ni(NO_3)_2 \times 6H_2O$, $Pb(NO_3)_2$, and $Zn(NO_3)_2 \times 6H_2O$. Tested concentrations of each metal were 0.01, 0.05, 0.1, 0.5, and 1 mM. All substances were dissolved in distilled water containing 2 mM $CaCl_2 \times 2H_2O$, 0.5 mM $MgSO_4 \times 7H_2O$, 0.8 mM $NaHCO_3$, and 0.08 mM KCl (according to ČSN EN ISO 7346) (all chemicals came from Penta [http://www.pentachemicals.eu]). The pH was adjusted to 7.6 by addition of 0.1 M solution of NaOH.

Semichronic Toxicity Test

The seeds were placed in plastic dishes (10-cm diameter) with a layer of a filter article on the bottom. Seventeen seeds were equally placed into each dish on the surface of filter article, and 5 mL tested aqueous solution with heavy metal was added. Each treatment had four replicates. The exposure lasted for 72 h in the dark at 25°C. Root lengths were measured, and inhibition values of root elongation were calculated according to the following formula (Eq. 1):

$$I = (D_c - D_t)/D_c, \qquad (1)$$

where I is the inhibition of root elongation in %; D_c is the average length of root under control conditions (i.e., without heavy metal treatment) [mm]; and D_t is the average length of root grown under the tested metal concentration [mm].

EC_{50} Calculation

EC_{50} is the effective concentration at which 50% of tested organisms have a significant response to the tested compound. For the purpose of calculation, nonlinear regression with the lower and upper maximum (0 and 100, respectively) was used. Data were processed by software GraphPad Prism (GraphPad, San Diego, CA), and the output was to MS Excel. The NOEC value is the highest tested concentration of a toxic compound at which there is no significant unfavourable impact on tested organisms compared with controls.

Statistics

Statistical analysis was performed based on STATISTICA (StatSoft, Tulsa, OK) software. Results were evaluated by principal component analysis (PCA), which is a multidimensional statistical method.

Results and Discussion

In the initial experiment, the germination of different flax cultivars was tested to optimize time of germination for heavy-metal toxicity evaluation. Different germination abilities among the flax cultivars are shown in Fig. 1. After 2 days of germination, root length was quite uniform among the cultivars, with exception of cultivars Bilt Star, Raisa, Lola, and Atalante. The differences rapidly increased when root growth began after 3 days. Cultivars Tábor, Laura, Jordán, and Bonet showed the longest root lengths. In contrast were the short root lengths of Bilt Star, Raisa, Lola, and Atalante. For the next set of experiments, 3-day germination was chosen because by day 4, the roots were already so long that they could not be compared with results from other published tests (Munzuroglu and Geckil 2002; Fargašová 2001).

On the basis of root-length measurement, EC_{50} values, their slopes, and NOEC values were calculated. The slope is important because it gives us information about intensity of the toxicity of the tested compound. A lower slope value indicates a slow decrease of toxic effect. When two compounds with the same EC_{50} value are compared, higher potential risk for the environment is caused by the

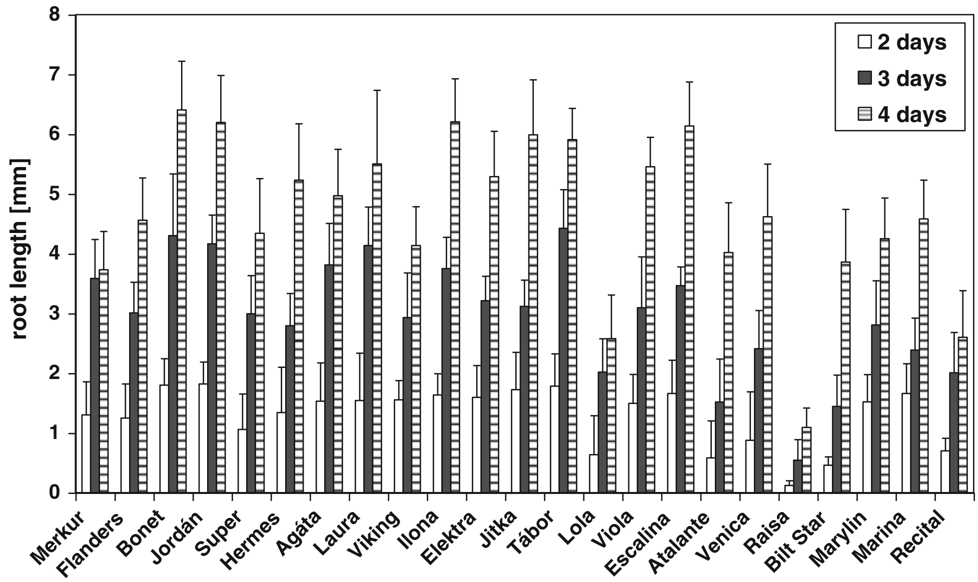

Fig. 1 Time dependence of root elongation for different tested flax cultivars in control solution. Each data point represents the average of four replicates, each with 17 seeds

Table 1 EC_{50} and NOEC values (in mM) and slope for Cd^{2+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.2122	0.1915	0.1395	0.1634	0.1318	0.1754	0.1968	0.1558
Slope	1.641	1.782	1.088	1.405	1.228	1.541	1.213	1.433
NOEC	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.2262	0.1943	0.2190	0.1993	0.2834	0.1655	0.2463	0.1749
Slope	1.90	1.35	1.68	1.77	2.19	2.83	2.65	1.89
NOEC	0.05	<0.01	0.01	0.05	0.01	0.05	0.01	0.01

	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital
EC_{50}	0.1910	0.1892	0.2724	0.1156	0.1949	0.1413	0.1318
Slope	1.77	2.70	1.19	0.88	1.68	1.58	1.35
NOEC	<0.01	0.05	0.05	0.01	0.05	0.01	0.01

compound with a lower decrease of toxic effect (lower slope).

Cadmium

Cadmium ion toxicity did not show any significant differences among the tested cultivars. Table 1 lists EC_{50} values in relatively close range (from 0.13 to 0.24 mM) for most of the cultivars, except for the following four: Bilt Star, which had an EC_{50} value < 0.13 mM (0.1156 mM), and Viola, Tabor, and Raisa, which had EC_{50} values > 0.24 mM (0.2463 mM, 0.2834 mM, and 0.2724 mM, respectively). In general, cadmium toxicity was high. Almost total inhibition of roots and hypocotyls was found at a concentration > 0.1 mM cadmium. Munzuroglu and Geckil (2002) reported similar results for *T. aestivum* and *C. sativus* (threshold = 2.5 mM cadmium). High cadmium toxicity for *Pinus pinea* and *P. pinaster* was also found by Arduini et al. (1994).

Lead

Slight stimulation of root growth was observed at a lead concentration of 0.01 mM. The majority of EC_{50} values for lead ion were found to be in the range of 0.80 to 0.99 mM. Seven cultivars (Atalante, Lola, Raisa, Bilt Star, Marylin, Marina, and Recital) showed a higher sensitivity to lead (two or three times lower EC_{50} compared with the rest of cultivars) (Table 2). Compared with cadmium, lead posed lower toxicity to the tested cultivars. This could have been

Table 2 EC_{50} and NOEC values (in mM) and slope for Pb^{2+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.9672	0.8009	0.978	0.9749	0.9552	0.8585	0.9120	0.9541
Slope	27.48	4.397	21.37	19.58	17.91	6.317	5.24	20.91
NOEC	0.50	0.05	0.50	0.10	0.50	0.10	0.10	0.50
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.9635	0.9645	0.988	0.9366	0.9913	0.6149	0.9679	0.972
Slope	18.76	17.69	25.51	14.29	20.67	3.53	17.79	23.50
NOEC	0.50	0.50	0.50	0.50	0.50	0.10	0.50	0.50
	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital	
EC_{50}	0.7699	0.9678	0.6776	0.3266	0.3	0.3101	0.4167	
Slope	0.97	18.55	1.77	0.31	0.98	0.71	1.16	
NOEC	0.01	0.10	0.10	< 0.01	< 0.01	< 0.01	0.01	

Table 3 EC_{50} and NOEC values (in mM) and slope for Ni^{2+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.9677	0.6555	0.614	0.5973	0.6751	0.5686	0.7579	0.6367
Slope	0.86	2.86	1.75	2.06	1.74	1.33	1.51	1.72
NOEC	0.01	0.10	0.05	0.05	0.05	0.05	0.10	0.10
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.6671	0.6364	0.8031	0.487	0.5551	0.122	0.4958	0.5081
Slope	2.58	1.39	1.17	2.20	1.65	0.89	2.38	1.17
NOEC	0.10	0.05	0.05	0.05	0.05	0.05	0.10	0.05
	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital	
EC_{50}	0.5413	0.6339	0.2121	0.1005	0.1419	0.1783	0.207	
Slope	1.01	1.69	0.94	0.54	1.44	1.41	1.154	
NOEC	0.01	0.10	<0.01	<0.01	<0.01	<0.01	<0.01	

caused by the lower solubility of almost all lead compounds. Xiong (1998) tested lead uptake and effect on roots growth and plant growth in *B. pekinensis*. He found approximately 90% inhibition at a concentration of 0.005 mM Pb^{2+}. Thus, flax seems to be more tolerant. Wierzbicka and Obidzinska (1998) studied the effect of lead on seed imbibition and germination. Their results showed that more important than lead concentration is the amount of lead per seed mass unit. The penetration of lead is also dependent on permeability of the seed coat. This can explain the relatively high resistance of flax to lead.

Nickel

The variability of EC_{50} values for nickel ions were relatively wide compared with cadmium ions (the difference between extreme EC_{50} values was nearly 0.9 mM) (Table 3). The toxicity of nickel ions was comparable with that of lead ions. Cultivars Lola, Raisa, Bilt Star, Marylin, Marina, and Recital exhibited high sensitivity to nickel ions (EC_{50} 0.1 to 0.2 mM). Carlson et al. (1991) treated seeds of six plant species with solutions containing beryllium, nickel, thalium, or vanadium. They determined that low concentrations of nickel stimulated root elongation in most species. In contrast, high concentrations caused decreased root elongation. Peralta et al. (2001) tested the effects of five heavy metals on the root and plant growth of alfalfa (*Medicago sativa*). They measured root length and found approximately 51% inhibition of alfalfa root elongation at an nickel concentration of 0.68 mM, which is similar to our results in flax (30–70% inhibition of flax root elongation at a concentration of 0.5 mM).

Table 4 EC_{50} and NOEC values (in mM) and slope for Cr^{3+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.9703	0.9975	0.9737	0.9193	0.996	0.971	0.9962	0.9996
Slope	18.17	21.64	20.84	5.39	23.62	18.05	21.43	27.40
NOEC	0.50	0.50	0.50	0.10	0.50	0.50	0.50	0.50
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	1.002	0.9804	0.9868	0.9633	0.9958	0.9802	0.9719	0.9844
Slope	22.77	21.61	26.70	17.68	31.78	20.27	18.49	22.38
NOEC	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital	
EC_{50}	0.9468	0.9959	0.9551	0.6945	0.5683	0.7186	0.996	
Slope	20.42	31.54	20.67	2.13	6.05	3.74	32.62	
NOEC	0.50	0.50	0.50	0.10	0.10	0.10	0.50	

Table 5 EC_{50} and NOEC values (in mM) and slope for Cr^{6+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.2061	0.2016	0.4755	0.3571	0.2354	0.1498	0.4341	0.1362
Slope	1.33	1.94	1.96	2.13	1.66	0.88	1.27	0.94
NOEC	0.01	0.05	0.01	0.01	<0.01	<0.01	<0.01	<0.01
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.4857	0.3804	0.4005	0.2935	0.4446	0.1696	0.2892	0.3945
Slope	1.75	1.19	2.07	1.60	2.27	1.22	1.70	1.05
NOEC	0.10	<0.01	0.05	0.01	0.01	<0.01	0.10	<0.01
	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital	
EC_{50}	0.1378	0.4362	0.4779	0.2624	0.2844	0.3729	0.3731	
Slope	0.64	2.48	3.24	1.24	1.58	3.33	4.13	
NOEC	<0.01	0.01	0.10	0.01	0.01	0.10	0.10	

Chromium

Comparison of EC_{50} values for chromium (III) and chromium (VI) ions showed large differences (Tables 4 and 5). Chromium (III) toxicity did not show significant differences among cultivars, and the average value was approximately 0.9 mM (except for cultivars Bilt Star, Marylin, and Marina). In contrast, the EC_{50} values for chromium (VI) exhibited higher differences. From a toxicologic point of view, the best cultivar was Viking, which showed the highest EC_{50} value for both ions ($Cr^{6+} = 0.4857$ and $Cr^{3+} = 1.002$ mM). Peralta et al. (2001) studied chromium (VI) toxicity to alfalfa (*M. sativa*). His root-length measurement was similar to the ones we obtained for flax (approximately 92% inhibition of alfalfa root elongation at a concentration of 0.77 mM compared with 66% to 91% inhibition of flax root elongation at a concentration of 1 mM).

Arsenic

EC_{50} values for arsenic (III) (Table 6) and arsenic (V) (Table 7) ions showed no significant differences among the cultivars. Among the tested cultivars, the highest sensitivity to As^{3+} was observed in Bilt Star (0.0065 mM). Higher sensitivity to As^{5+} ions was also determined in the cultivar Marina (0.0087 mM). However, the differences between EC_{50} values were only minor, as seen for cadmium ions. Due to conversion of the latter ion in plants into the former ion, the higher toxicity of As^{3+} than As^{5+} is well known.

Table 6 EC_{50} and NOEC values (in mM) and slope for As^{3+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.0165	0.0169	0.0315	0.0232	0.0239	0.0162	0.0232	0.0159
Slope	1.24	2.26	1.74	1.99	2.11	1.61	1.42	1.45
NOEC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.0260	0.0221	0.0304	0.0228	0.0310	0.0073	0.0217	0.0156
Slope	1.62	1.18	2.48	1.86	1.97	1.51	1.51	1.22
NOEC	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01

	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital
EC_{50}	0.0106	0.0186	0.0144	0.0065	0.0257	0.0148	0.0121
Slope	1.69	1.72	1.82	1.37	3.3273	2.0674	2.22
NOEC	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01

Table 7 EC_{50} and NOEC values (in mM) and slope for As^{5+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.0175	0.0190	0.0284	0.0256	0.0114	0.0139	0.0221	0.0131
Slope	1.32	1.88	1.44	1.74	1.06	1.51	1.15	1.18
NOEC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.0291	0.0235	0.0290	0.0198	0.0330	0.0090	0.0297	0.0139
Slope	1.57	1.11	2.15	1.36	1.51	1.49	1.73	0.97
NOEC	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital
EC_{50}	0.0158	0.0151	0.0109	0.0135	0.0094	0.0087	0.0174
Slope	1.40	1.20	1.23	1.78	1.33	1.02	2.34
NOEC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

During this conversion, reactive oxygen species (ROS) are produced, and plants synthesize enzymatic antioxidants, e.g., superoxide dismutase, catalase, and glutathion-S-transferase, as well as nonenzymatic antioxidants, e.g., glutathione and ascorbate (Hartley-Whitaker et al. 2001). Marin et al. (1992) discussed arsenic accessability in experiments with two rice cultivars. Availability of arsenic to rice followed the following trend: dimethylarsenic acid (DMAA) < As(V) < monomethylarsonic acid (MMAA) < As(III). Similar results were found by Carbonell et al. (1998) in experiments of wetland plants *Spartina alterniflora* and *Holcus lanatus* with the following trend: DMAA < MMAA < As(V) < As(III). Surprisingly we did not find differences between As(III) and As(V) ions in our experiments in flax.

Copper

In the case of copper, a wide range of EC_{50} values was observed. The tested set of flax cultivars involved sensitive cultivars, such as Jordan or Hermes (with EC_{50} close to 0.001 mM), as well as relatively tolerant cultivars, such as Recital, Lola, Atalante, and Bilt Star ($EC_{50} > 0.2$ mM) (Table 8). Although Cu is an essential micronutrient for plant growth, it can be more toxic than cadmium, chromium, or lead. High concentrations of Cu can affect key enzymes, such as glutamine synthetase and glutamate synthetase, and change nitrogen metabolism in higher plants (Llorens et al. 2000). Fernandes and Henriques (1991) published that excessive Cu appears to be associated with structural damage in plants. Shu et al. (2002)

Table 8 EC_{50} and NOEC values (in mM) and slope for Cu^{2+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.0125	0.0614	0.0138	0.0007	0.0377	0.0067	0.0890	0.0101
Slope	0.54	0.82	0.52	0.33	0.51	0.49	0.73	0.42
NOEC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.0175	0.0369	0.0615	0.0409	0.0505	0.1857	0.0415	0.0117
Slope	0.44	0.71	0.75	0.74	0.78	1.90	0.79	0.43
NOEC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital
EC_{50}	0.1982	0.0619	0.0917	0.1696	0.1282	0.1220	0.3627
Slope	1.70	0.54	0.84	1.44	1.27	1.49	4.34
NOEC	0.05	<0.01	<0.01	<0.01	<0.01	0.01	<0.01

Table 9 EC_{50} and NOEC values (in mM) and slope for Co^{2+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.1372	0.5022	0.0156	0.0327	0.0331	0.1859	0.1647	0.0622
Slope	0.21	0.99	0.27	0.30	0.27	0.43	0.71	0.36
NOEC	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	0.4212	0.1321	0.1597	0.1071	0.2466	0.7100	0.3016	0.0224
Slope	0.58	0.34	0.57	0.68	0.46	2.64	0.71	0.33
NOEC	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital
EC_{50}	1.0510	0.4420	0.2997	0.1193	0.2204	0.4961	0.8000
Slope	2.19	0.64	2.43	1.83	0.83	1.65	1.09
NOEC	0.10	0.01	<0.01	0.50	<0.01	<0.01	0.05

found high sensitivity of grasses (*Paspalum distichum* and *Cynodon dactylon*) to copper. Our results showed high flax sensitivity to the presence of copper, with the differences between cultivars being rather large. Generally, the data were comparable with published results, and the obtained EC_{50} values were lower than for cadmium, chromium, or lead, all of which are considered highly toxic metals.

Cobalt

The highest inhibition effect of cobalt on root elongation was found in the case of cultivar Bonet ($EC_{50} = 0.0156$ mM). The range of obtained EC_{50} values was wide, as in case of copper, but the values were on average 10 times higher (Table 9). Cobalt is known to be an essential element for animals and microorganisms in the form of vitamin B_{12}, but in plants it was found essential only for some of them (Liu et al. 2000). Only leguminous plants, e.g., *Rhizobia* on their roots, and some species of nitrogen-fixing blue–green algae need cobalt (Holm-Hansen et al. 1954; Lowe & Evans 1962). It can have potential beneficial effects, but it can also be toxic. Munzuroglu and Geckil (2002) determined higher germination inhibition for wheat than for cucumber. They found a significant decrease in wheat germination at concentrations between 3.0 and 8.0 mM cobalt. Zeid (2001) tested the toxicity of cobalt and chromium to *Phaseolus vulgaris* seeds, and he found a higher toxicity of cobalt than chromium. These results are in agreement with our study.

Zinc

The inhibition of root elongation of flax cultivars in the presence of varying concentrations of zinc was very low. In

Table 10 EC_{50} and NOEC values (in mM) and slope for Zn^{2+} ion

	Merkur	Flanders	Bonet	Jordan	Super	Hermes	Agata	Laura
EC_{50}	0.54	1.25	2.39	5.04	1.05	3.04	8.62	1.89
Slope	11.94	3.57	1.55	1.39	1.56	0.86	0.68	1.93
NOEC	0.10	0.05	0.10	0.10	0.10	0.10	0.10	0.10

	Viking	Ilona	Elektra	Jitka	Tabor	Lola	Viola	Escalina
EC_{50}	2.28	3.56	5.46	3.69	0.63	2.56	0.68	0.93
Slope	1.85	0.94	2.01	1.16	11.35	0.89	4.88	2.41
NOEC	0.10	0.05	0.10	0.10	0.50	0.10	0.10	0.05

	Atalante	Venica	Raisa	Bilt Star	Marylin	Marina	Recital
EC_{50}	0.46	1.35	1.06	0.76	0.89	0.90	0.68
Slope	0.98	2.15	2.57	2.75	4.88	3.41	0.88
NOEC	0.05	0.50	0.10	0.01	0.05	0.10	0.05

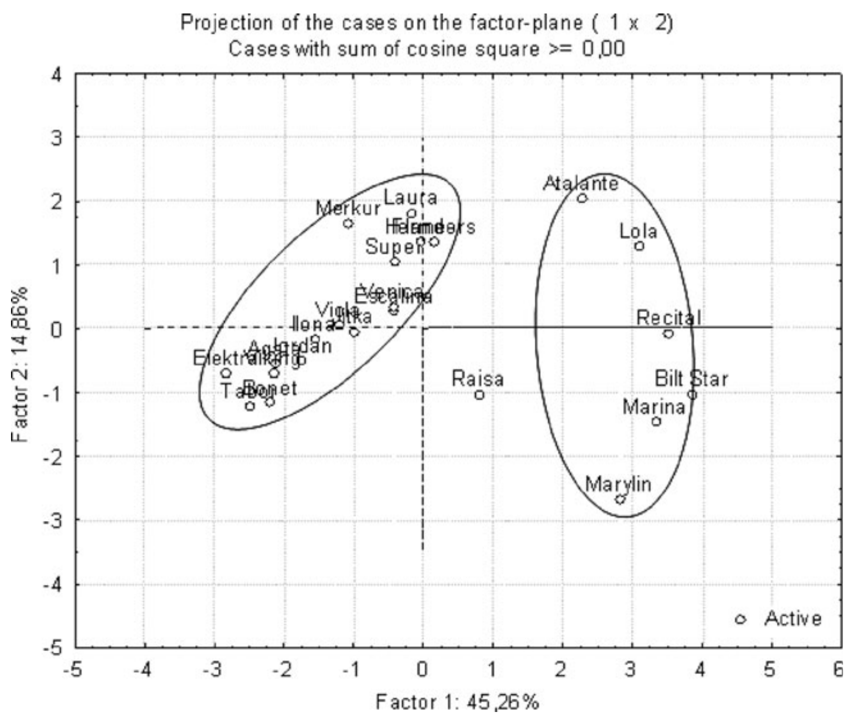

Fig. 2 PCA-centered score plot of cultivars on the basis of EC_{50} values for all tested toxic metals. Projection of the cases, where factors 1 and 2 explain 45.26 and 14.86% of total variability of the data. Cultivars are distributed into the two groups (in *ellipses*) according to their response (EC_{50}) after 3 days of root growth to treatment with metal ions

this case, the calculations of EC_{50} and NOEC values were rather problematic. Most of the obtained values were extrapolated due to the low range of tested zinc concentrations (Table 10). The most sensitive cultivar was Atalante ($EC_{50} < 0.5$ mM). On the basis of inhibition effect, the cultivars can be divided into three different groups. Cultivars in the first group showed stable low inhibition of root elongation for almost all tested concentrations. The second group contains cultivars with slightly increasing inhibition with increasing zinc concentrations. These cultivars were more sensitive to the presence of zinc. The third group includes flax cultivars that showed a slow increase of inhibition of root elongation at low concentrations, an inhibition decrease (elongation stimulation) in the middle of the concentration range, and an inhibition increase again at the end of concentration range (i.e., the highest concentrations). This phenomenon is known as "hormesis," in which small doses of toxicants increase responses, whereas greater doses diminish responses (Calabrese & Baldwin 1999). On the basis of previously mentioned results, the metals can be arranged in the following sequence from the highest toxicity to zero toxicity: $As^{3+} \geq As^{5+} \geq Cu^{2+} > Cd^{2+} > Co^{2+} > Cr^{6+} > Ni^{2+} > Pb^{2+} > Cr^{3+} > Zn^{2+}$.

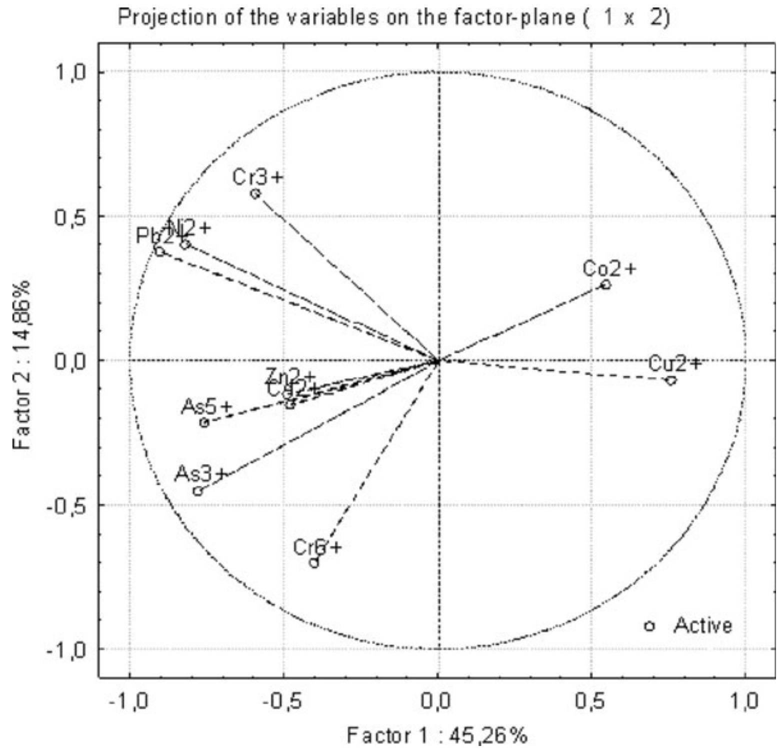

Fig. 3 PCA-loading plot of metal ions on the basis of EC_{50} values for all tested flax cultivars. Ions with positively correlated EC_{50} values are shown close to each other (Pb^{2+}, Ni^{2+}), whereas the negatively correlated metals are oriented oppositely (Co^{3+} and As^{3+})

Statistical Analyses of Results

The obtained results were processed using PCA. This method shows similarity among tested cultivars and among tested toxic metals. Figure 2 illustrates similarities between the cultivars on the basis of EC_{50} values for all tested toxic metals. There are two groups of cultivars: one containing most of the tested cultivars and the second one containing six cultivars (Atalante, Lola, Recital, Bilt Star, Marina, and Marylin). Cultivar Raisa exhibited greater distance from both main groups of tested cultivars.

When relations among tested ions were compared (again on the base of EC_{50} values) for all tested cultivars of flax (Fig. 3a) strong negative correlation was found between ions Cr^{3+} and Cr^{6+}. Strong correlations were found also for ions Cd^{2+} and Zn^{2+} as well as for ions Pb^{2+} and Ni^{2+}. Close correlations were found for ions Co^{2+} and Cu^{2+} as well as for ions As^{3+} and As^{5+}. The strong relations between these ions are in accordance with the published data, e.g., for hyperaccumulators cadmium and zinc, lead and nickel, and copper and cobalt (Baker and Brooks 1989). The differences between Cr^{3+} and Cr^{6+} can be caused by different mechanisms of chromium ions uptake through the cell membrane (Theil and Raymond 1994). In the case of arsenic, it is known from experiments with two rice cultivars that arsenic availability depends on the type of arsenic compound. It has been estimated that As^{5+} is less available than As^{3+} (Marin et al. 1992). In our case, we did not find any differences between arsenic ions.

Conclusion

Our results showed a high diversity in the response of flax cultivars to the presence of toxic metals. We found wide differences in toxicity for cultivars in the case of Cd^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, and Pb^{2+}, including a large difference between Cr^{3+} and Cr^{6+}. The difference between ions As^{3+} and As^{5+} was not confirmed. Some cultivars (Atalante, Lola, Recital, Bilt Star, Marina, Marylin, and Raisa) had different responses to metal toxicities compared with the rest of the cultivars. Our results proved the toxicity test to be a useful tool for the selection of cultivars suitable for phytoremediation purposes. In contrast, if certain varieties of adult plants had not (hyper)accumulated the toxic metals, they could be ideal varieties for flax production in heavy-metal contaminated soils.

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