





SCREENING FLAX FOR IDENTIFYING CADMIUM TOLERANT CULTIVARS FOR GROWTH IN POLLUTED ENVIRONMENT

Simona Ilavská¹, Mária Pavlovičová*^{1,2}, Richard Hančinský¹, Peter Nemeček¹, Miroslav Horník¹, Pavol Hauptvogel², Ildikó Matušíková¹

Address(es): RNDr. Mária Pavlovičová

- ¹University of Ss. Cyril and Methodius in Trnava, Faculty of Natural Sciences, Institute of Chemistry and Environmental Sciences, Nám. J. Herdu 2, SK-917 01 Trnava, Slovak Republic.
- ² National Agricultural and Food Centre, Research Institute of Plant Production, Bratislavská cesta 122, 921 68 Pieštany, Slovak Republic.

*Corresponding author: pavlovicova7@ucm.sk

https://doi.org/10.55251/jmbfs.11634

ARTICLE INFO

Received 1. 8. 2024 Revised 11. 9. 2024 Accepted 18. 9. 2024 Published 1. 10. 2024

Regular article



ABSTRACT

Toxic metal pollutants such as cadmium disrupt all stages of plant development. Flax (*Linum usitatissimum* L.) has demonstrated the ability to tolerate and accumulate high amounts of cadmium, making it suitable for phytoremediation. Within-species variability of cadmium tolerance and uptake needs to be examined in controlled conditions to assess multiple beneficial, or even required traits for use in phytoremediation programs. In this study, 31 flax cultivars were screened for their ability to germinate and grow in the presence of a sublethal concentration of cadmium (20 mg.kg⁻¹). Several parameters, including growth indicators and photosynthesis performance, were analyzed using principal component analysis. A subset of flax cultivars was then selected for measuring cadmium uptake using gamma spectrometry. The results showed that the cultivars Belinka, Diana, Laura, Marina, Flanders, and Jitka have comparable metal uptake and transfer to shoots. Among these, the cultivar Jitka proved to be the best candidate for phytoremediation, consistent with previous reports.

Keywords: bioaccumulation factor, linseed, metal toxicity, metal transfer factor, soil health

INTRODUCTION

Activities such as mining, industrial production, agricultural practices, and military operations contribute significantly to the concentrated presence of toxic metals in the environment (Nedelkoska and Doran, 2000; Castro et al., 2011). Metals such as cadmium (Cd) are significant environmental pollutants and have no known role in organisms. On the contrary, they have negative effects on crop health and productivity because they are non-biodegradable. Cadmium exposure interferes with all stages of plant development, inhibiting seed germination, vegetative growth and reproductive growth. It can disrupt the normal structure and function of cellular components and interferes with a variety of metabolic and developmental processes (Benavides et al., 2021; Hou et al., 2023).

Phytoremediation is a method of cleaning up polluted areas using plants that remove heavy metals from contaminated soil and accumulate them in roots, stems and leaves (Hamzah et al., 2016). Repeated cycles of planting and harvesting plants with accumulated heavy metals gradually reduce the concentration of these toxic substances in the soil to levels acceptable for further use (Thomas et al., 2022). This method offers several advantages, primarily environmental friendliness and relatively low cost. However, it relies on plant species that have low biomass and is therefore time consuming before the toxic elements in the soil are reduced below threshold levels, requires seasonal treatments as well as procedures and costs for disposal of contaminated biomass (Koptsik, 2014; Laghlimi et al., 2015; Farraji et al., 2016; Awa and Hadibarata, 2020). Therefore, modern phytomanagement focuses on growing industrial crops with high biomass and value on contaminated soils, which not only remediates the soil but also generates useful feedstock for bioproducts and bioenergy (Mench et al., 2018; Zine et al., 2020).

The efficiency of phytoremediation varies among plant species because each species has different mechanisms of ion uptake that depend on its morphological, physiological and anatomical characteristics (Rahman and Hasegawa, 2011). The use of fibrous crops (e.g. flax, hemp) has been considered in connection with their ability to tolerate and take up also heavy metals from the soil (Baraniecki and Mankowski, 1995; Griga et al., 2000). Several authors have described the high tolerance of some flax varieties to cadmium ions as well as their ability to accumulate cadmium from the soil (Angelova et al., 2004; Shi and Cai, 2009; Bjelková et al., 2011; Douchiche et al., 2012; Praczyk et al., 2015; Hosman et al., 2017). Although flax does not accumulate cadmium in as high quantities as some species that hyperaccumulate it (such as Thlaspi spp.) (Ueno et al., 2011), it

still tolerates significantly higher cadmium concentrations than most plants and is therefore evaluated as a potentially effective tool for phytoremediation (**Bjelková** *et al.*, 2011).

Metal uptake by flax depends mainly on the concentration of heavy metals in the soil. Also, the level of heavy metal toxicity to crops depends on several factors: crop species, growth conditions, developmental stage, toxicity characteristics of individual elements, soil physical and chemical properties, presence and bioavailability of heavy metal ions in the soil solution, and soil rhizosphere chemistry (Ali et al., 2019; Wan et al., 2024). Due to the complexity of the processes of metal uptake and tolerance by plants, results obtained in the laboratory often fail to reproduce the same outcomes under field conditions. However, tolerance screenings in simple experiments remain the essential starting point for evaluating the tolerance and metal uptake of genotypes (Sánchez-Castro et al., 2023). The complexity of these factors underlines the importance of considering multiple parameters when assessing the tolerance of flax varieties to cadmium stress. Therefore, instead of evaluating a single parameter, here we examined several indicators of cadmium tolerance and using a biplot (Yasar, 2023) we reflect to multiple interactions between genotype and external factors to screen a set of flax cultivars for potential use in remediation programs.

MATERIAL AND METHODS

Plant material

A set of 31 fibre flax (*Linum usitatissimum* L.) varieties, Belinka, Escalina, Laura, Jitka, Ilona, Flanders, Marina, Szeged 30, Azur, Modran, Texa, Rekord, Wiera, Verum, Liral Sussex, Krasnoder, Jugoslavik viner, Ilgermila II, Hohenheim, Gisa, Lilas, Dearo, Rastatter Weiss, Mume, Solido, Diana, Pastel, Stamkanovits, Shakhimskaja, Rembrandt and Purple, were provided by the Gene Bank SK (Research Institute of Plant Production in Piešt'any, NPPC Lužianky). To preliminarily assess the sensitivity of flaxseed and to determine the working Cd concentration for further experiments, seeds of flax purchased at the grocery store (dmBio; product number 1447530, EAN: 4058172389566) were used. The seeds were surface sterilized before germination using a 5% (v/v) sodium hypochlorite solution for 10 min and rinsed three times with sterile deionised water.

1

Germination assays

Flax seeds were sown on Petri dishes with $CdCl_2$ solution at concentrations of 0, 10, 20, 30, 40 and 50 mg.L⁻¹. We evaluated germination of 50 seeds after 6 days of cultivation in the dark at room temperature. As the lowest Cd concentration that markedly inhibited to some extent germination all varieties was 30 mg.L⁻¹, for further tolerance tests of selected flax varieties (including germination assays with 25 seeds) the concentration of 20 mg.L⁻¹ $CdCl_2$ was used. Germination rate was expressed in %.

Testing for flax tolerance to Cd2+

Screening of flax varieties for tolerance was performed in Petri dishes with Hoagland nutrient solution (400 mg.L⁻¹ KNO₃, 350 mg.L⁻¹ MgSO₄. 7H₂O, 300 mg.L⁻¹, NaH₂PO₄.2H₂O, 400 mg.L⁻¹ CaCl₂, 350 mg.L⁻¹ NaNO₃) with/without the addition of a 20 mg.L⁻¹ CdCl₂. Flax (6 seeds for each variety) was cultivated in a cultivation room at a photoperiod of 16 hours light/8 hours dark with maximum intensity 11 450 lx; temperature max. 28°C (light)/min. 18°C (dark) for 8 days. Fresh weight and shoot lengths were measured for experimental plants and tolerance indexes were expressed as ratio of values for metal-exposed plants to corresponding controls (in %).

Determination of photosynthetic pigments

Determination of photosynthetic pigment concentrations was carried out according to **Lichtenthaler** (1987) in 50 mg (fresh weight) of plant tissue. Tolerance indexes (ratio of values obtained for Cd-treated plant/ values for control) were calculated for parameters expressing the Cd impact on photosynthetic apparatus (**Douchiche et al.**, 2012), including sum of chlorophylls A and B (Chl(A+B)), ratio of chlorophylls (ChlA/B), content of total carotenoids (Car) and the weight ratio of chlorophylls to total carotenoids ((A+B)/Car).

Radioanalytical analyses

For radioanalytical studies, the metal solution for plant cultivation was spiked with exact amount of the radioisotope ¹⁰⁹Cd (Czech Metrological Institute, Czech Republic) in 5 mL vial tubes. Metal exposure was for 10 days. Root tissues were separated with a scalpel and immersed in 20 mM Na₂-EDTA for 15 min and rinsed

with deionised water to remove Cd^{2+} from the surface. The shoots and roots of seedlings were frozen with liquid nitrogen. The samples were analysed using a scintillation gammaspectrometer type 76BP76/3 (Scionix, The Netherland) with well type NaI(Tl) crystal. For energy and efficiency calibration, a library of radionuclides was created by selecting the characteristic γ -ray peaks for ^{109}Cd (E γ = 88.04 keV), ^{137}Cs (E γ = 661.66 keV), and ^{60}Co (E γ = 1,173.24 keV). The calibration was performed using standard solutions of $^{109}\text{CdCl}_2$ with known radioactivity and half-life of the radionuclide ($T_{1/2}$ = 462.6 d). The peak areas of the samples and references were determined at the same geometry. The amount of ^{109}Cd accumulated in tissue (Bq.g $^{-1}$ DW) was used to estimate the total Cd allocated in tissues.

Metal transfer factor (TF) was calculated by dividing the concentration of a heavy metal in a plant's aerial parts (shoots) by its concentration in the root (**Douchiche** *et al.*, **2012**).

Bioconcentration factor (BCF) was calculated by dividing the concentration of a heavy metal in organ biomass by concentration in the cultivation medium at the end of the experiment (**Douchiche** *et al.*, **2012**).

Data processing

Data processing, visualisation and correlation analysis were done using Microsoft Office Excel 2016 program. Principal component analysis was done using Statistica program.

RESULTS AND DISCUSSION

Data on growth parameters showed natural variability among the 31 flax varieties. Fiber-type plants are usually taller with fewer branches compared to linseed types grown for seeds (**Diederichsen and Ulrich, 2009**). The significant genetic diversity of flax has been attributed to genetic background, environmental conditions, and breeding practices. Previously, considerable differences have been reported in traits such as seed yield, straw weight, and growth rates among different flax varieties (**You** et al., 2017). Fresh weight values observed for some of the tested varieties exhibited double the fresh weight of others (Figure 1). To account for the significant variability and make the data comparable across different varieties, we expressed tolerance indexes for individual parameters by calculating the ratio of values measured for metal-exposed plants to those of control plants for each variety.

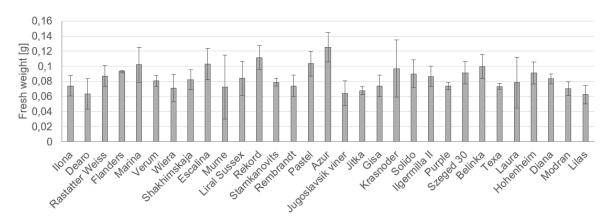


Figure 1 Variability in fresh weight of experimental flax varieties in control conditions. The data represent average values \pm SD (n=6).

A prerequisite for the successful use of flax for soil remediation is its ability to germinate in contaminated soil. We tested the ability of flax varieties to germinate in presence of cadmium (20 mg.L-1) in the growth medium. The results indicated that nine varieties had 100% germination, while germination in four varieties decreased by half due to the presence of the toxic metal (Figure 2A). Genotypic variations of seed coat permeability have been suggested as key determinant for metal impact on seed germination (Cheng et al., 2008). Additional factors like variable sensitivity of seed's key hydrolytic enzymes or variable efficiency of antioxidant defences/metal transport and sequestration likely play role (Carvalho et al., 2023). Previously, Cd strongly inhibited germination of Trigonella foenumgraecum seeds at 10 mg.L⁻¹ (Alaraidh et al., 2018), while 1 mM of CdCl₂ completely inhibited germination of the rice seed cultivar Hwayeong (Ahsan et al., 2007). In contrast positive effects of low Cd concentrations (up to 0.5 mM CdCl₂) on germination has also been reported for some rice cultivars (Cheng et al., 2008). In the screening of six fibre flax and two linseed varieties, Brutch et al., (2022) found that cadmium (Cd) did not influence germination. Cd tolerance of germination varies between- and also within plant species (Ahmad et al., 2012).

Tolerance indexes based on different growth parameters are good indicators of sensitivity/tolerance to stresses, including metal toxicity. Rich literature reports on mostly negative effects of cadmium on plant biomass production, expressed by weight or height of organs (plants), yield, seed production, etc. (Singh et al., 2016; Konotop et al., 2017; Yotsova et al., 2020; Halim et al., 2021; Mészáros et al., 2021). The mechanisms of metal effects are rather well described; however, they are hardly uniformly applicable as the complexity of plant defence strategies, including those at cellular, tissue, and plant levels, result in different tolerance rate values when individual parameters are compared (Maglovski et al., 2019; Maglovski et al., 2021). Such variable pattern we also observed when TI was determined based on shoot weight (Figure 2B) and shoot length (Figure 2C), while the orders of examined flax varieties differed also from that generated by germination efficiency in presence of Cd (Figure 2A). Belinka, Flanders, Verum and Marina repeatedly occur among the most tolerant flax varieties in these tests (Figure 2). In contrast, the Ilona variety germinates poorly in presence of Cd, though the plants later can rather efficiently cope to metal toxicity (Figure 2).

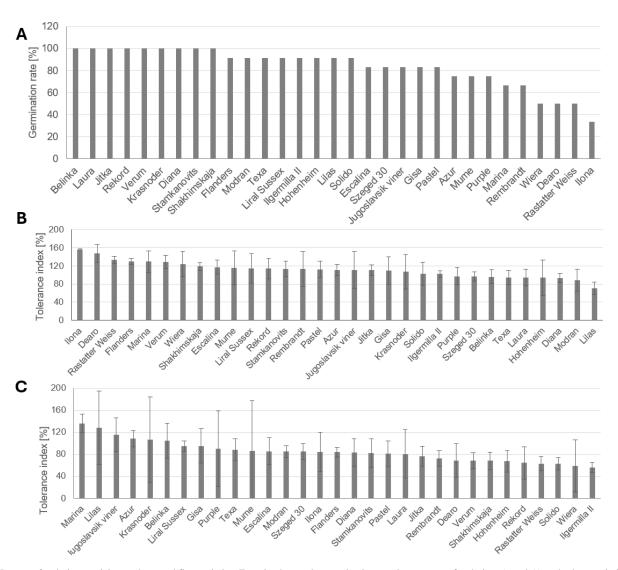


Figure 2 Impact of cadmium toxicity on the tested flax varieties. Examined were the germination rate in presence of cadmium (panel A) and tolerance indexes based on shoot weight (B) and shoot length (C). The data represent average values \pm SD (n=2-6 plants).

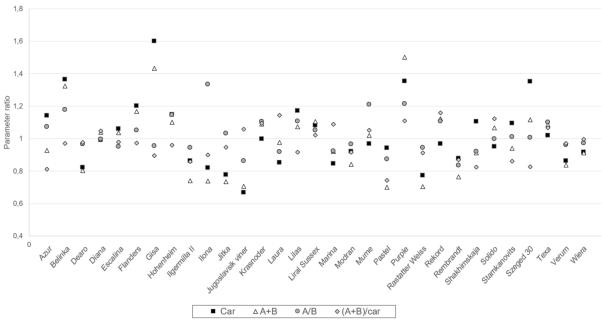


Figure 3 Effect of cadmium on photosynthetic parameters in flax varieties. Indicated are parameter ratios of Cd-stressed to control plants for carotenoid (Car) and total chlorophyll (ChlA+ChlB) content, content ratio of ChlA to ChlB (A/B) and the weight ratio of ChlA and ChlB to total carotenoids ((A+B)/Car). For clarity, only average values are indicated.

Photosynthetic pigments in flax varieties exposed to Cd

Photosynthetic pigment contents were affected in flax as impact of cadmium toxicity (Figure 3). There are many cases in the literature that describe a decrease in chlorophyll content under metal toxicity but also its increase. Photosynthesis is essential for plants to generate biomass and energy for adaptive responses, at the same time it is a process significantly sensitive to metal toxicity impairing chloroplast structure in leaves and enzymatic activity (Yotsova et al., 2020). We assessed the functionality of the photosynthetic apparatus in the plant as a sensitive stress indicator (Kalaji et al., 2016; Kalisz et al., 2023). Total chlorophyll content (ChlA+ChlB) varied in flax plants between 2.5-6.2 mg.g-1 FW; ratio between Cdexposed and control plants ranged between 0.7 to 1.5 indicating diverse tolerance (Figure 3) as has been observed previously in flax (Brutch et al., 2022) as well as other species (Yotsova et al., 2020). The ChlA/B ratio as an indicator of the functional pigment equipment and light adaptation/acclimation of the photosynthetic apparatus decreases in shaded (stressed) leaves (Lichtenthaler et al., 2013). In the control flax plants, the values ranged between 1.6 and 3.3 compared to Cd-stressed plants (1.88 - 3.17), while the corresponding tolerance indexes varied between 0.83 and 1.33 (Figure 3). Previously overall lower ChlA/B values were measured for flax varieties Jitka, Escalina, Belinka, Krasnoder and Marina (both for control and Cd-stressed condition) (Pavlovičová et al., 2020), suggesting different growth conditions.

Considering their unique structure with a system of conjugated double bonds responsible for antioxidative properties, carotenoid (Car) content has been suggested to correlate with defence against stress (Chen et al., 2016). While Car content varies among but also within species (Ninčević Grassino et al., 2023), in flax it ranged 0.5-1.0 mg.g⁻¹ FW in controls and 0.4 to 1.0 mg.g⁻¹ FW in metal-exposed variants. The tolerance indexes derived from these values were 0.7-1.6 (Figure 3) suggesting variable adaptability to metal toxicity. Greenness of plants as expressed by chlorophylls to total carotenoids was also calculated for flax varieties. Normally the values lay between 4.2 and 5 in sun-exposed leaves (Lichtenthaler, 1987). In flax varieties values 3.9-6.2 were measured in both control and Cd-exposed plants. Previously, higher values (<8.3) were detected in Escalina and Marina varieties (Pavlovičová et al., 2020). As lower values (as low as 2.5 to 3.0) indicate senescence and/or damage to the plant structures (Lichtenthaler, 1987), derived tolerance indexes in flax between 0.7-1.2 (Figure 3) indicate to relatively most sensitive parameter reflecting to presence and toxicity of Cd.

Several varieties had TI values for assayed individual parameters above 100%. which is a result of the so-called hormesis effect, i.e. the positive influence of the element at low concentrations as a manifestation of biological plasticity or temporary adaptive response to stress (Calabrese and Mattson, 2011). Varieties Purple, Liral Sussex and Texa appear to benefit most from the applied concentration of Cd (ratios of all pigment parameters increased in stressed plants compared to controls), in contrast to varieties Ilgemilla II, Ilona, Modran, Pastell, Rastatter Weiss, Rembrandt, Verum and Wiera that suffer most (Figure 3) (TIs based on all pigment parameters were below 100%). General increase in photosynthetic system efficiency, for example, determines the final hormetic stimulating effect (e.g., biomass increase), and is considered as one of the main action mechanisms of plant hormetic responses triggered by metal ions (Salinitro et al., 2021). The selected concentration of cadmium used in analyses apparently reaches the threshold of toxicity. Beneficial concentrations widely vary among tested metals and interspecific diversities result in different reactions in plants subjected to the same metal treatment (Salinitro et al., 2021).

Selecting flax varieties with good potential for phytoremediation

The flax defence strategies include involvement of mechanisms at cellular, tissue, and plant levels, which can vary in effectiveness across different varieties (Yaṣar et al., 2023). In addition, different growth parameters, such as shoot weight and length, can respond differently to cadmium stress. Therefore, not surprising, that various flax parameters exhibit different sensitivities to the presence of Cd ions, resulting variations in the sensitivity rankings for the tested varieties (Figures 1-3), as has been observed also previously (Shaari et al., 2022). These rankings may be hardly directly comparable, especially if in addition to genetic background and tolerance mechanisms also environmental factors enter the interactions (e.g., affecting metal bioavailability in soil). TI values and germination data were analysed together to identify the weight of the main components. This analysis sorted the varieties according to component PC 1, which explains 54.8% of the observed variability, and component PC 2, which accounts for 19.2% of the total variability (a combined 73%).

Tolerance to Cd ions, as expressed by the germination rate and fresh weight of seedlings, increases in the direction of PC 2, explaining a relatively high proportion (20%) of the information and variability in the data. Component PC 1 reflects the "condition" of the photosynthetic apparatus in the presence of Cd, or the ability to maintain function; it accounts for almost 55% of the total variability. This analysis enabled the selection of varieties with a positive combination of all tested parameter values, indicating good tolerance during germination and growth in the presence of sublethal concentrations of cadmium ions (quarter with varieties in the

red cluster in the Figure 4). These varieties include Belinka, Diana, Laura, Marina, Flanders, and Jitka.

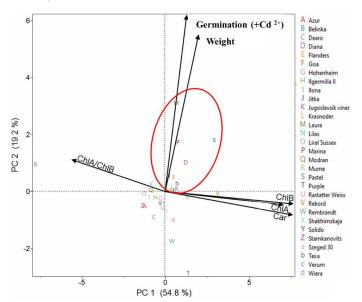


Figure 4 Principal Component Analysis (PCA). The analysis classifies the tested flax varieties based on changes in TI values calculated from different parameters. Arrows refer to graphical representations of the influencing factors for components 1 and 2. The highlighted set (red circle) includes varieties with good properties for soil phytoremediation contaminated with cadmium.

Testing the selected flax varieties for metal uptake

The selected varieties Belinka, Diana, Laura, Marina, Flanders, and Jitka were assayed for metal uptake and accumulation capacity using gammaspectrometry. All these varieties accumulated comparable amounts of >2200 mg.kg⁻¹Cd in roots, the most the variety Flanders (Tab 1). Shoots accumulated ≤ 203 mg.kg⁻¹ (cv. Jitka) but not less than 136 mg.kg-1 Cd (cv. Flanders) (Tab 1). These values are comparable in order of magnitude to the results of an earlier study with the Hermes variety (Douchiche et al., 2012) (Tab 1). In contrast, they are rather high compared to previously studied cvs. Kaliakra (Angelova et al., 2004) and Longya (Shi and Cai, 2009) (Tab 1). Accumulation rates were here much higher also than previously reported data on the same varieties in similar growth stage cultivated in hydropony (Pavlovičová et al., 2020) but also in mature plants grown in soil conditions (Bjelková et al., 2011). Variable accumulation rates were reported within (flax) species depending on several factors including type (fibre flax/linseed), cultivation conditions, growth stage, culture type (laboratory vs. field), plant part, stressing factor etc. (Bjelková et al., 2011 and within; House et al., 2020; Pavlovičová et al., 2020; Yaşar et al., 2023). The metal transfer rate from roots to shoots, however, were very low compared to other studies (Tab 1). At the same time, bioconcentration factor in roots was comparable with the cv. Hermes in both roots (Douchiche et al., 2010, 2012) and shoots (Douchiche et al., 2010). The methodology of metal exposure, duration, and concentration used in different studies can lead to different patterns of metal accumulation and translocation within the plant. For example, composition of the growth media/soil and corresponding metal mobility/bioavailability is likely rate determining factor. Bjelková et al. (2011) noted that artificial increase of soil Cd resulted in dramatic decrease of translocation from root to shoot. House et al. (2020) revealed that two of four canadian flax cultivars (including the cv. Flanders used in this study) exhibited stable Cd concentrations through the first three stages of development, but accumulaton declined significantly at maturity.

For all flax varieties assayed for cadmium uptake and transfer to aboveground tissue, we obtained comparable values. In this aspect, our selection from the set of 31 genotypes was successful. However, some varieties tested in soil experiments could clearly be distinguished in this respect (Bjelková et al., 2011). Bjelková et al. (2011) found that while the cultivars Merkur and Escalina accumulated most Cd in their roots, the cultivar Jitka was the highest Cd accumulator in aboveground tissue among the studied cultivars. In agreement with this, the in vitro screening system identified the cultivar Jitka as the most Cd-tolerant, accumulating high amounts of metal in explants, in contrast to the cultivar Merkur (Smykalova et al., 2010). Incorporating different, seemingly independent parameters that reflect plant adaptability to both internal and external factors is likely useful in identifying novel flax genotypes with good tolerance and high metal accumulation and transfer to shoots (Socha et al., 2015). Bjelková et al. (2011) considered it unlikely to identify high Cd-accumulators within recently grown commercial flax/linseed cultivars.

Table 1 Cadmium uptake by different flax cultivars (according to Douchiche et al. 2012).

Flax cultivar	Cd in medium/soil (mg.kg ⁻¹)	Treatment duration	Organ	Cd content (mg.kg ⁻¹)	BCF	TF*	Reference
Hermes	11	4 months	Roots	753	67	0.48	1
Hermes	56	18 days	Roots Shoot	3147 670	56 12	0.21	2
Kaliakra	12	Seed maturity	Roots	8.7	0.7	0.84	3
Flax, linseed	100	28 days	Roots	203	4	0.17*	4
Longya7	50		Shoots	109	2	0.54	5
Belinka	50	10 days	Roots	31	0.62*	_	6
	30	10 days	Roots Shoots	2386.6±161.6 173.1±49.0	79.52 5.77	0.07	this study
Diana	30	10 days	Roots Shoots	2415.5±273.0 148.2±35.5	80.52 4.94	0.06	this study
Flanders	30	10 days	Roots Shoots	2717.8±276.1 135.9±23.4	90.59 4.53	0.05	this study
	100	Seed maturity Seed maturity	Roots Shoots	9.1 2.7	0.09* 0.03*	0.30*	4
Jitka	50	10 days	Roots	62	1.24*	_	6
	30	10 days	Roots Shoots	2344.6±102.6 202.6±45.9	78.15 6.75	0.09	this study
	100	Seed maturity Seed maturity	Roots Shoots	17.5 4.9	0.17* 0.05*	0.29*	4
	40	3 weeks	Explants	789.9	19.75	_	7
Laura	30	10 days	Roots Shoots	2286.6±96.3 161.9±24.7	76.22 5.40	0.07	this study
	100	Seed maturity Seed maturity	Roots Shoots	18.0 4.2	0.18* 0.04*	0.23*	4
Marína	50	10 days	Roots	42	0.84*	-	6
	30	10 days	Roots Shoots	2212.3±185.1 184.2±97.9	73.74 6.14	0.08	this study

Legends: BCF - bioconcentration factor = [Cd]plant tissue/[Cd]medium. TF - translocation factor = [Cd]shoot tissue/[Cd]roots. TF = Cd accumulation in shoot tissue/Cd accumulation in roots. References: 1 – Douchiche et al., (2012); 2 – Douchiche et al., (2010); 3 – Angelova et al., (2004); 4 – Bjelková et al., (2011); 5 – Shi and Cai, 2009; 6 – Pavlovičová et al., (2020); 7 – Smykalova et al., (2010). Data in this study present average ± SE (n=3). *Values calculated from data reported in the given work.

CONCLUSION

Fast, cheap but reliable screening systems should comprise several parameters as individually they are likely insufficient to estimate the tolerance/metal uptake and transfer rate. Flax plants of early developmental stages were screened in a laboratory for cadmium tolerance. Photosynthesis-related parameters, germination ability in presence of cadmium and growth parameters allowed to identify 6 of 31 flax cultivars, which revealed comparable metal uptake/transfer properties. The results supported previous findings that the cv. Jitka is most tolerant, at the same time accumulates most cadmium in shoots. Such screenings are necessary to identify novel genotypes for remediation purposes.

Acknowledgments: This work was supported by research grants APVV-15-0051 and APVV-21-0504. RH was supported by project from the Research Support Fund at the University of Ss. Cyril and Methodius in Trnava number FPPV -59 – 2024 (in frames of Early Stage Grant No. 09-i03-03-v05-00004, Recovery Plan scheme).

REFERENCES

Ahmad, I., Akhtar, M. J., Zahir, Z. A., & Jamil, A. (2012). Effects of cadmium on seed germination and seedling growth of four wheat (*Triticum aestivum L.*) cultivars. *Pakistan Journal of Botany*, 44, 1569–1574.

Ahsan, N., Lee, S. H., Lee, D. G., Lee, H., Lee, S. W., Bahk, J. D., & Lee, B. H. (2007). Physiological and protein profiles alteration of germinating rice seedlings exposed to acute cadmium toxicity. *Comptes Rendus Biologies*, 330, 735–746.http://dx.doi.org/10.1016/j.crvi.2007.08.001

Alaraidh, I. A., Alsahli, A. A., & Razik, E. S. A. (2018). Alteration of antioxidant gene expression in response to heavy metal stress in *Trigonella foenum-graecum* L. *South African Journal of Botany*, 115, 90–93. http://dx.doi.org/10.1016/j.sajb.2018.01.012

Ali, H., Khan, E., & Sajad, M. A. (2019). Phytoremediation of heavy metals—Concepts and applications. *Chemosphere*, 91(7), 869-881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Angelova, V., Ivanova, R., Delibaltova, V., & Ivanov, K. (2004). Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton, hemp). Industrial Crops and Products, 19, 197–200.http://dx.doi.org/10.1016/j.indcrop.2003.10.001

Awa, S. H. & Hadibarata, T. (2020). Removal of heavy metals in contaminated soil by phytoremediation mechanism: a review. *Water, Air, & Soil Pollution*, 231, 47. https://dx.doi.org/10.1007/s11270-020-4426-0

Baraniecki, P., & Mankowski, J. (1995). Hemp fibre as a raw material for paper production in the aspect of natural environment protection. *Zemedelska Technika*, 3, 85-88

Benavides, M. P., Gallego, S. M., & Tomaro, M. L. (2021). Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology*, 13(3), 51-69.https://doi.org/10.1590/S1677-04202005000100003

Bjelková, M., Genčurová, V., & Griga, M. (2011). Accumulation of cadmium by flax and linseed cultivars in field-simulated conditions: A potential for phytoremediation of Cd-contaminated soils. *Industrial Crops and Products*, 33, 761–774. https://doi.org/10.1016/j.indcrop.2011.01.020

Brutch, E., Zabegaeva, O., Nozkova, J., & Brutch, N. (2022). Cadmium tolerance and its absorption ability in fibre flax and linseed varieties. *Turkish Journal of Agriculture and Forestry*, 46(1), Article 8.http://dx.doi.org/10.3906/tar-2011-118 Calabrese, E. J., & Mattson, M. P. (2011). Hormesis provides a generalized quantitative estimate of biological plasticity. *Journal of Cell Communication and Signaling*, 5, 25-38.https://doi.org/10.1007/s12079-011-0119-1

Carvalho, M. E. A., Agathokleous, E., Nogueira, M. L., Brunetto, G., Brown, P. H., & Azevedo, R. A. (2023). Neutral-to-positive cadmium effects on germination and seedling vigor, with and without seed priming. *Journal of Hazardous Materials*, 448, 130813. http://dx.doi.org/10.1016/j.jhazmat.2023.130813

Castro, R. S. D., Caetano, L., Ferreira, G., Padilha, P. M., Saeki, M. J., Zara, L. F., Martines, M. A. U., & Castro, G. R. (2011). Banana peel applied to the solid phase extraction of copper and lead from river water: Preconcentration of metal ions with a fruit waste. *Industrial & Engineering Chemistry Research*, 50(6), 3446-3451.http://dx.doi.org/10.1021/ie101499e

Diederichsen, A., & Ulrich, A. (2009). Variability in stem fibre content and its association with other characteristics in 1177 flax (*Linum usitatissimum L.*) genebank accessions. *Industrial Crops and Products*, 30(1), 33-39.https://doi.org/10.1016/j.indcrop.2009.01.002

Douchiche, O., Chaïbi, W., & Morvan, C. (2012). Cadmium tolerance and accumulation characteristics of mature flax, cv. Hermes: Contribution of the basal stem compared to the root. *Journal of Hazardous Materials*, 235-236, 101-107.https://doi.org/10.1016/j.jhazmat.2012.07.027

Douchiche, O., Soret-Morvan, O., Chaïbi, W., Morvan, C., & Paynel, F. (2010). Characteristics of cadmium tolerance in 'Hermes' flax seedlings: Contribution of cell walls. *Chemosphere*, 81, 1430–1436.https://doi.org/10.1016/j.chemosphere.2010.09.011

Farraji, H., Zaman, N. Q., Tajuddin, R. M., & Faraji, H. (2016). Advantages and disadvantages of phytoremediation: a concise review. *International Journal of Environmental Technology and Science*, 2, 69-75.http://dx.doi.org/10.5281/zenodo.4668122

Griga, M., Bjelkova, M., & Tejklova, E. (2000). Flax and Hemp – Industrial Crops for Phytoremediation of Contaminated Soils. *In 1st Scientific Workshop COST*

- Action 837: Phytoremediation 2000 State of the Art in Europe (An Intercontinental Comparison), Hersonissos, Greece, 45-46.
- Halim, M. A., Rahman, M. M., Mondal, D., Megharaj, M., & Naidu, R. (2021). Bioaccumulation and Tolerance Indices of Cadmium in Wheat Plants Grown in Cadmium-Spiked Soil: Health Risk. *Frontiers in Environmental Science*, 9, 779588.https://doi.org/10.3389/fenvs.2021.779588
- Hamzah, A., Hapsari, R. I. & Wisnubroto, E. I. (2016). Phytoremediation of cadmium-contaminated agricultural land using indigenous plants. *International Journal of Environmental and Agricultural Research (IJOEAR)*, 2, 2454-1850.
- Hosman, M. E., Elanwar, M., & Mohammed, H. (2017). Mechanism of Phytoremediation Potential of Flax (*Linum usitatissimum* L.) to Pb, Cd and Zn. *Asian Journal of Plant Science and Research*, 7(4), 30-40.
- Hou, L., Ji, S., Zhang, Y., Wu, X., & Zhang, L. (2023). The mechanism of silicon on alleviating cadmium toxicity in plants: A review. *Frontiers in Plant Science*, 14.http://dx.doi.org/10.3389/fpls.2023.1141138
- House, M. A., Young, L. W., Liu, X., Liber, K., Diederichsen, A., & Booker, H. M. (2020). Comparative analysis of cadmium uptake and distribution in contrasting Canadian flax cultivars. *BMC Research Notes*, 13(1), 424.http://dx.doi.org/10.1186/s13104-020-05265-1
- Chen, D., Wang, S., Cao, B., Cao, D., Leng, G., Li, H., Yin, L., Shan, L., & Deng, X. (2016). Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Frontiers in Plant Science*, 6, 1241.https://doi.org/10.3389/fpls.2015.01241
- Cheng, W. D., Zhang, G. P., Yao, H. G., & Zhang, H. M. (2008). Genotypic difference of germination and early seedling growth in response to Cd stress and its relation to Cd accumulation. *Journal of Plant Nutrition*, 31, 702–715.https://doi.org/10.1080/01904160801926764
- Kalaji, H. M., Govindjee, Bosa, K., Kościelniak, J., & Żuk-Gołaszewska, K. (2016). Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum*, 38(4), 102.http://dx.doi.org/10.1007/s11738-016-2113-y
- Kalisz, A., Bartkowiak, A., Banaszak, Z., Czerwińska, E., Praczyk, M., & Nowak, K. (2023). Leaf chlorophyll fluorescence and reflectance of oakleaf lettuce exposed to metal and metal(oid) oxide nanoparticles. *BMC Plant Biology*, 23(1), 329.https://doi.org/10.1186/s12870-023-04305-9
- Konotop, Y., Kovalenko, M., Matušíková, I., Batsmanova, L., & Taran, N. (2017). Proline application triggers temporal redox imbalance, but alleviates cadmium stress in wheat seedlings. *Pakistan Journal of Botany*, 49(6), 2145-2151.
- Koptsik, G. N. (2014). Problems and prospects concerning the phytoremediation of heavy metal polluted soils: a review. *Eurasian Soil Science*, 47, 923-939.https://dx.doi.org/10.1134/S1064229314090075
- Laghlimi, M., Khalil, M., Rihani, M., & Ez-Zariy, L. (2015). Phytoremediation mechanisms of heavy metal contaminated soils: a review. *Open Journal of Ecology*, 5(7), 375-388.http://dx.doi.org/10.4236/oje.2015.58031
- Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148, 350–382.https://doi.org/10.1016/0076-6879(87)48036-1
- Lichtenthaler, H. K., Babani, F., Navrátil, M., & Buschmann, C. (2013). Chlorophyll fluorescence kinetics, photosynthetic activity, and pigment composition of blue-shade and half-shade leaves as compared to sun and shade leaves of different trees. *Photosynthesis Research*, 117(1-3), 355-66.http://dx.doi.org/10.1007/s11120-013-9834-1
- Maglovski, M., Gerši, Z., Rybanský, Ľ., Bardáčová, M., Moravčíková, J., Bujdoš, M., Dobrikova, A., Apostolova, E., Kraic, J., Blehová, A., & Matušíková, I. (2019). Effects of nutrition on wheat photosynthetic pigment responses to arsenic stress. *Polish Journal of Environmental Studies*, 28(3), 1821-1829.http://dx.doi.org/10.15244/pjoes/89584
- Maglovski, M., Rybanský, Ľ., Bujdoš, M., Adamec, L., Bardáčová, M., Blehová, A., & Matušíková, I. (2021). Nitrogenous nutrition affects uptake of arsenic and defense enzyme responses in wheat. *Polish Journal of Environmental Studies*, 30(3), 2213-2231.http://dx.doi.org/10.15244/pjoes/127912
- Mench, M. J., Bes, C. M., Lepp, N., Schröder, P., Smolders, E., Vangronsveld, J., & Bleeker, P. M. (2018). Phytomanagement and remediation of Cu-contaminated soils by high yielding crops at a former wood preservation site: Sunflower biomass and ionome. *Frontiers in Ecology and Evolution*, 6, 123.http://dx.doi.org/10.3389/fevo.2018.00123
- Mészáros, P., Rybanský, L., Bardáčová, M., Roszival, M., & Matušíková, I. (2021). Soybean roots defence against cadmium and its dependence on dose in a non-linear manner. *Israel Journal of Plant Sciences*, 10(1), 13-24.http://dx.doi.org/10.1163/22238980-bja10044
- Nedelkoska, T. V. & Doran, P. M. (2000). Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. *Minerals Engineering*, 13, 549–561.https://doi.org/10.1016/S0892-6875(00)00035-2
- Ninčević Grassino, A., Rimac Brnčić, S., Badanjak Sabolović, M., Šic Žlabur, J., Marović, R., & Brnčić, M. (2023). Carotenoid content and profiles of pumpkin products and by-products. *Molecules*, 28(2), 858.http://dx.doi.org/10.3390/molecules28020858
- Pavlovičová, M., Gerši, Z., Bardáčová, M., Ranušová, P., Horník, M., & Matušíková, I. (2020). Variable accumulation of cadmium in flax (*Linum*

- usitatissimum L.). Nova Biotechnologica et Chimica. 19(1), 70-79. https://doi.org/10.36547/nbc.v19i1.579
- Praczyk, M., Świątek, Ł., Warzecha, T., Banaszkiewicz, T., Kaczmarek, Z., & Urbanek, K. (2015). *Linum usitatissimum* L. genotypes cultivated for medicinal purposes. *Herba Polonica*, 61(1), 19-30.
- Rahman, M. A. & Hasegawa, H. (2011). Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere*, 83, 633–646.http://dx.doi.org/10.1016/j.chemosphere.2011.02.045
- Salinitro, M., Mattarello, G., Guardigli, G., et al. (2021). Induction of hormesis in plants by urban trace metal pollution. *Scientific Reports*, 11, 20329.http://dx.doi.org/10.1038/s41598-021-99657-3
- Sánchez-Castro, I., Molina, L., Prieto-Fernández, M. Á., & Segura, A. (2023). Past, present and future trends in the remediation of heavy-metal contaminated soil Remediation techniques applied in real soil-contamination events. *Heliyon*, 9(6).http://dx.doi.org/10.1016/j.heliyon.2023.e16692
- Shaari, N. E. M., Tajudin, M. T. F. M., Khandaker, M. M., Majrashi, A., Alenazi, M. M., Abdullahi, U. A., & Mohd, K. S. (2022). Cadmium toxicity symptoms and uptake mechanism in plants: A review. *Brazilian Journal of Biology*, 84.http://dx.doi.org/10.1590/1519-6984.252143
- Shi, G., & Cai, Q. (2009). Cadmium tolerance and accumulation in eight potential energy crops. *Biotechnology Advances*, 27, 555–561. https://doi.org/10.1016/j.biotechadv.2009.04.006
- Singh, S., Parihar, P., Singh, R., Singh, V. P., & Prasad, S. M. (2016). Heavy metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics. *Frontiers in Plant Science*, 6, 1143.http://dx.doi.org/10.3389/fpls.2015.01143
- Smykalova, I., Vrbova, M., Tejklova, E., Vetrovcova, M., & Griga, M. (2010). Large scale screening of heavy metal tolerance in flax/linseed (*Linum usitatissimum L.*) tested in vitro. *Industrial Crops and Products*, 32(3), 527-533.https://doi.org/10.1016/j.indcrop.2010.06.027
- Socha, P., Bernstein, N., Rybanský, L., Mészáros, P., Gálusová, T., Spiess, N., Libantová, J., Moravčíková, J., & Matušíková, I. (2015). Cd accumulation potential as a marker for heavy metal tolerance in soybean. *Israel Journal of Plant Sciences*, 62(3), 160-166.http://dx.doi.org/10.1080/07929978.2015.1042307
- Thomas, G., Sheridan, C. & Holm, P. E. (2022). A critical review of phytoremediation for acid mine drainage-impacted environments. *Science of The Total Environment*, 811,
- e1552230.https://doi.org/10.1016/j.scitotenv.2021.152230
- Ueno, D., Yamaji, N., & Ma, J. F. (2011). Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of *Thlaspi caerulescens*. *The Plant Journal*, 66(5), 852-862.https://doi.org/10.1111/j.1365-313x.2011.04548.x
- Wan, Y., Liu, J., Zhuang, Z., Wang, Q., & Li, H. (2024). Heavy metals in agricultural soils: Sources, influencing factors, and remediation strategies. *Toxics*, 12(1), 63.http://dx.doi.org/10.3390/toxics12010063
- Yaşar, M. (2023). Sensitivity of different flax (*Linum usitatissimum* L.) genotypes to salinity determined by GE biplot. *Saudi Journal of Biological Sciences*, 30(4), 103592. http://dx.doi.org/10.1016/j.sjbs.2023.103592
- Yotsova, E., Dobrikova, A., Stefanov, M., Misheva, S., Bardáčová, M., Matušíková, I., Žideková, L., Blehová, A., & Apostolova, E. (2020). Effects of cadmium on two wheat cultivars depending on different nitrogen supply. *Plant Physiology and Biochemistry*, 155, 789-799.https://doi.org/10.1016/j.plaphy.2020.06.042
- You, F. M., Jia, G., Xiao, J., Duguid, S. D., Rashid, K. Y., Booker, H. M., & Cloutier, S. (2017). Genetic variability of 27 traits in a core collection of flax (*Linum usitatissimum* L.). *Frontiers in Plant Science*, 8, 1636.http://dx.doi.org/10.3389/fpls.2017.01636
- Zine, H., Midhat, L., Hakkou, R., El Adnani, M., & Ouhammou, A. (2020). Guidelines for a phytomanagement plan by the phytostabilization of mining wastes.

 Scientific African, 10, e00654.http://dx.doi.org/10.1016/j.sciaf.2020.e00654