ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

Fakulta agrobiologie, potravinových a přírodních zdrojů

Ing. Le Minh Phuong

Katedra chemie

Biotransfer of Selected Risk Metals into Plants and Their Accumulation and Distribution in Plants

Biotransfer vybraných rizikových kovů do rostlin a jejich akumulace a distribuce v rostlinách

Autoreferát doktorské disertační práce

Studijní program:	P4106 Zemědělská specializace
Studijníobor:	4106V017 Zemědělská chemie
Školitel**:**	**Prof. Ing. Jaromír Lachman, CSc.** Katedra chemie
Konzultant:	**Ing. Brigita Zámečníková, Ph.D.** Katedra chemie
Oponenti**:**	**Prof. Ing. Pavel Kalač, CSc.** **Prof. Ing. Ladislav Kokoška, Ph.D.** **Prof. RNDr.Alena Vollmannová, Ph.D.**

Obhajoba doktorské disertační práce se koná dne: ....
v ....hod. na: Fakultě agrobiologie, potravinových a přírodních zdrojů ČZU v Praze

S doktorskou disertační prací je možno se seznámit na děkanátě FAPPZ ČZU v Praze.

P r a h a 2 0 1 7

SUMMARY

Wheat (*Triticum spp*.) and potato (*Solanum tuberosum* L.*)* are popular cultivated crops in the world. These days, heavy metals are one of the most serious situations for human being and environment. Some heavy metals like cadmium, mercury, lead and zinc, when their concentrations are excessive, can cause a danger to health of human.

In the present study, the accumulation of four heavy metals (mercury, zinc, lead and cadmium) and in addition beneficial trace element selenium in different wheat and potato cultivars are reported. Atomic Absorption Spectrometry (AAS) has been used to characterize the heavy metal concentrations in wheat. For all measurements averages and standard errors were calculated in Microsoft Excel 2007. The data were processed by Excel (Microsoft, Redmond, WA, USA). Statistical evaluation was performed using the Statistica software (ver. 12; StatSoft, Inc., Tulsa, OK, USA).

In the experiment of wheat, the concentration of heavy metals decreased in the order zinc (Zn) > lead (Pb) > cadmium (Cd) > mercury (Hg) in the wheat grain. The comparison between three varieties of investigated wheat revealed that the emmer wheat was rich in zinc content (62.12 mg kg^{-1} dry matter), while the spring wheat had the lowest average concentration of zinc in the grain (40.99 mg kg^{-1} dry matter). The concentrations of mercury in four typical growth stages of wheat (boot stage, stage 10.2, leaf-stage 10.2 and stage 11 according to Feekes`scale) were also determined. Among individual varieties significant differences were determined.

Eighteen winter wheat varieties with different grain colour (purple-, blue-, yellow- and red-grained) and three spring tritordeum yellowed-grained varieties and breeding lines were assessed for grain selenium (Se) content from the crop season 2014/2015 at the site Kroměříž (Czech Republic). Se content has shown to be genotype dependent, with the highest contents in control red-grained variety Bohemia (0.235 mg kg^{-1} dry matter) and yellow-grained Bona Vita (0.229 mg kg^{-1} dry matter), and breeding lines V2-10-16 (Skorpion x Magister, blue-grained), KM 53-14 (blue-grained) and V2-15-16 (Citrus x Bona Dea, yellow-grained) winter wheats. In new spring tritordeums average Se content was comparable (0.039 mg kg^{-1} dry matter) with colour-grained winter wheats (blue aleurone

0.057 mg kg^{-1} dry matter, purple pericarp 0.042 mg kg^{-1} dry matter and yellow endosperm 0.069 mg kg^{-1} dry matter). Although in most varieties the Se contents were not statistically significant different, in colour-grained wheat statistically significant differences were determined between the Bohemia and Bona Vita varieties with the highest Se content and breeding line V2-31-16 with the lowest Se content as well as between variety Bohemia and breeding line KM 178-14. Diversity in certain wheat accessions offers the genetic potential for developing cultivars with better ability to accumulate beneficial Se micronutrient in grains.

In the grains of sixteen different wheat varieties contents of Cd, Hg and Pb in two different locations Uhříněves and Valečov in the crop years 2013 and 2014 were determined. Using statistical analysis, the results showed that there were no significant differences between two investigated groups of samples (samples from Uhříněves and Valečov in 2013 and 2014) considering either one of investigated metals (Cd, Pb, Hg). Concentrations of Cd and Pb were much higher than concentration of Hg in the same varieties. For the experiment with the effect of cooking methods on potato varieties, two different methods were used. The potatoes were boiled in water for 20 minutes at 100 °C and then analyzed for heavy metals contents. The same varieties were baked for 45 minutes at 180 °C. The results showed that contents of heavy metals in samples under cooking methods were significantly higher than in raw samples.

Plasma membrane or cell membrane is a biological active membrane separating the interior of cell from the outside environment. In our experiment, the potato plants were grown hydroponically in the Research Institute of Plant Crops Prague-Ruzyně. Protoplasts were released in the dark at 25 degrees Celsius for 18 hours. The 70-90 microns sieve was used to filter and filtrate was centrifuged for 5 minutes at 100g. All the steps were carefully carried out to prevent the breakage of protoplasts.

Keywords: spring wheat; einkorn; emmer; potato; heavy metals; atomic absorption spectrometry; Se content; purple pericarp; blue aleurone; yellow endosperm; *Triticumaestivum*; × *Tritordeummartinii* A. Pujadas nothosp. nov.; enzyme; protoplast isolation; plasma membrane

ABSTRAKT

Pšenice (*Triticum*spp.) a lilek brambor (*Solanum tuberosum* L.) jsou populární kultivované plodiny na světě. V dnešní době jsou těžké kovy jednou z nejvážnějších situací pro člověka a životní prostředí. Některé těžké kovy, jako je kadmium, rtuť, olovo a zinek, pokud jsou jejich koncentrace nadměrné, mohou ohrozit lidské zdraví.

V této studii je uvedena akumulace čtyř těžkých kovů (rtuť, zinek, olovo a kadmium) a navíc užitečné stopové prvky selenu u různých odrůd pšenice a brambor. Atomová absorpční spektrometrie (AAS) byla použita pro charakterizaci koncentrací těžkých kovů u pšenice. Pro všechna měření byla vypočítána průměry a standardní chyby v aplikaci Microsoft Excel 2007. Data byla zpracována aplikací Excel (Microsoft, Redmond, WA, USA). Statistické vyhodnocení bylo provedeno pomocí softwaru Statistica (verze 12; StatSoft, Inc., Tulsa, OK, USA).

Při experimentu s pšenicí se koncentrace těžkých kovů snížila v pořadí zinku (Zn)> olova (Pb)> kadmia (Cd)> rtuti (Hg) u pšenice. Porovnání tří odrůd zkoumané pšenice ukázalo, že pšenice dvouzrnka byla bohatá na obsah zinku (62,12 mg kg^{-1} sušiny), zatímco jarní pšenice měla nejnižší průměrnou koncentraci zinku v zrnu (40,99 mg kg^{-1} sušiny). Byly stanoveny také koncentrace rtuti ve čtyřech typických růstových stádiích pšenice (stadium metání, stupeň 10.2, listový stupeň 10.2 a stadium metání, raně mléčná zralost, stupeň 11 podle Feekesovy stupnice). Mezi jednotlivými odrůdami byly zjištěny významné rozdíly.

Osmnáct odrůd ozimé pšenice a šlechtitelskými liniemi s různou barvou zrna (purpurovou, modrou, žlutou a červenou) a třemi jarními odrůdami tritordea se žlutými obilkami bylo hodnoceno na obsah selenu (Se) v zrnech v pěstebním roce 2014/2015 z lokality Kroměříž (Česká republika). Obsah se ukázal být genotypově závislý, s nejvyšším obsahem u ozimých pšenic v kontrolní odrůdě s červeným zrnem Bohemia (0,235 mg kg^{-1} sušiny) a odrůdě se žlutým zrnem Bona Vita (0,229 mg kg^{-1} sušiny) a šlechtitelských liniích V2-10-16 (Skorpion x Magister, modrý aleuron), KM 53-14 (modrý aleuron) a V2-15-16 (Citrus x Bona Dea, žluté zrno). V nových jarních odrůdách tritordea byl průměrný obsah Se srovnatelný (0,039 mg kg^{-1} sušiny) s ozimými pšenicemi (s modrým

aleuronem 0,057 mg kg^{-1} sušiny, purpurovým perikarpem 0,042 mg kg^{-1} sušiny a žlutým endospermem 0,069 mg kg^{-1} sušiny). Ačkoli ve většině odrůd nebyl obsah Se statisticky významně odlišný, v pšenici s barevnými obilkami byly zjištěny statisticky významné rozdíly mezi odrůdami Bohemia a Bona Vita s nejvyšším obsahem Se a šlechtitelskou linií V2-31-16 s nejnižším obsahem Se stejně jako mezi odrůdou Bohemia a šlechtitelskou linií KM 178-14. Rozmanitost některých pšenic nabízí genetický potenciál pro vývoj kultivarů s lepší schopností akumulovat v zrnech prospěšný mikroprvek Se.

V zrnech šestnácti různých odrůd pšenice byly stanoveny obsahy Cd, Hg a Pb na dvou různých lokalitách Uhříněves a Valečov v létech 2013 a 2014. Pomocí statistické analýzy výsledky ukázaly, že mezi dvěma sledovanými skupinami vzorků nebyly zjištěny žádné významné rozdíly v obsahu sledovaných kovů (Cd, Pb, Hg). Koncentrace Cd a Pb byly mnohem vyšší než koncentrace Hg ve stejných odrůdách. V experimentu sledujícím vliv různých způsobů tepelné úpravy na obsah těžkých kovů v odrůdách brambor byly použity dvě různé metody. Brambory byly vařeny ve vodě po dobu 20 minut při 100 ° C a poté analyzovány na obsah těžkých kovů. Hlízy brambor stejných odrůd byly pečeny po dobu 45 minut při 180 °C. Výsledky ukázaly, že obsah těžkých kovů ve vzorcích po tepelné úpravě byl výrazně vyšší než u nezpracovaných vzorků.

Plazmová nebo buněčná membrána je biologicky aktivní membrána oddělující vnitřní buňku od vnějšího prostředí. V našem experimentu rostliny brambor rostly hydroponicky ve Výzkumném ústavu rostlinných plodin Praha-Ruzyně. Protoplasty byly uvolněny ve tmě při teplotě 25 °C po dobu 18 hodin. Síto o velikosti ok 70 až 90 mikrometrů bylo použito k filtraci a filtrát byl odstřeďován po dobu 5 minut při 100g. Všechny kroky byly pečlivě provedeny, aby se zabránilo přetržení protoplastů.

Klíčová slova: jarní pšenice; jednozrnka; dvouzrnka; brambory; těžké kovy; atomová absorpční spektrometrie; obsah Se; purpurový perikarp; modrý aleuron; žlutý endosperm; *Triticumaestivum*; × *Tritordeummartinii* A. Pujadasnotho sp. nov.; enzym; izolace protoplastu; plazmatická membrána

Content

SUMMARY 2
ABSTRAKT 4
1 INTRODUCTION 7
2 SCIENTIFIC HYPOTHESIS AND OBJECTIVE OF THESIS 8
3 MATERIALS AND METHODS 8
3.1 Plant materials and conditions of cultivation 8
3.2 Methods of chemical analyses 9
3.2.1 Determination of mercury 9
3.2.2 Determination of cadmium, lead and zinc 9
3.2.3 Determination of selenium 10
3.2.4 Replicates and statistical analysis 10
4 RESULTS AND DISCUSSION 11
5 CONCLUSIONS 15
6 REFERENCES 17
7 PERSONAL INFORMATION 20
8 LIST OF PUBLICATIONS OF AUTHOR 21

1 INTRODUCTION

Contamination of soils with heavy metals is one of the serious environmental problems threatening human being (Renella et al., 2005). In some documents, heavy metals are considered to interact with plant metabolisms, water regime and proteins (Duchovskis et al., 2006). Heavy metals are considered as the special hazard of soil pollutants because of the adverse effects on the plant growth, the amount, activity of useful microorganisms in soils and the quality of food. Regard to the persistent and toxicity, the heavy metals are toxic when we consider different kinds of pollutants in soils (Abrahams, 2002). Another source of toxic element accumulation is from industrial sludge (Jamali et al., 2009; Pandey et al., 2009). When these metals are accumulated by plants, these metals can cause damage to humans and the environment.

Wheat and potato are the main crops in the world which largely consumed by human. Jamali (2009) and Chandra (2009) found that heavy metals in many varieties of wheat grown in soils with domestic sewage sludge or irrigated with industrial effluents had the significant accumulation. Some international organization such as Food and Agriculture Organization (FAO), European Commission (EC) and World Health Organization (WHO) strictly regulate the allowable concentrations or maximum concentrations of toxic heavy metals in foods (EC Commission Regulation, 2002).

In the soil, zinc (Zn), cadmium (Cd), lead (Pb) and mercury (Hg) toxicities frequently occur than the other metals because of their precipitation and sorption by the soil. It is a very dangerous situation because when these metals are taken up by plants, they can be transported to the food web (Farmer and Farmer, 2000). Food plants which suffer the high concentrations of heavy metals can cause the serious health risk to both animal and human.

2 SCIENTIFIC HYPOTHESIS AND OBJECTIVE OF THESIS

The aim of this study is to

- Preparation of sterile plant material for the electrochemical monitoring of differences in the membranes affected by selected metals.
- Optimization and validation of the separation and detection techniques (method of atomic absorption spectrometry (AAS) for the determination of selected risk metals in different parts of the model plants.
- Determination of the content of toxic metals in different organs of plants and their bioaccumulation.
- Monitoring the effects of other present essential metalsto transport hazardous metals in the plant organs and their interaction with the aim of the minimization of adverse and toxic elements in plant.

3 MATERIALS AND METHODS

3.1 Plant materials and conditions of cultivation

3.1.1 Plant materials

The study was carried out in 2014-2017 at the Czech University of Life Science in Prague, at the Department of Chemistry.

For analytical experiments: Twenty two cultivars of potatoes (*Solanum tuberosum* L. and *Solanum phureja*) were grown in field experiments and harvested in 2013 and 2014 at Uhříněves and Valečov, Czech Republic. After harvest, potatoes were cleaned mechanically and inspected for mechanical, physiological damage as well as diseases. Standard practices in growing techniques were used.

Potato cultivars (*Solanum tuberosum* L.) for hydroponical experiments were obtained from the Department of Plant Production of the Czech University of Life Sciences in Prague and from the Potato Research Institute, Havlíčkův Brod. Heavy and essential elements were monitored in potato cultivars in the exact field experiments and in hydroponically grown plants. Hydroponical experiments were some potato samples collected. The elements were determined by methods F-AAS, ET-AAS, AMA (Advance Mercury Analysis).

The cultivars of emmer, einkorn and common spring wheat that were growing on the same environmental conditions were investigated. Total 15 samples of *Triticum* species were investigated and the used procedures and methods for all analyses were identical for all of them.

For Se experiment, a total of eighteen wheat species and three tritordeum varieties were grown in 2014/15 (harvest 2015) at the Agricultural Research Institute in Kroměříž, Czech Republic (49.2851172N, 17.3646269E). The experimental field is located 235 meters above sea level, has Luvic Chernozem (Loamic), an average annual temperature 9.2 °C, mild winters and precipitations averaging 576 mm.

3.2 Methods of chemical analyses

3.2.1 Determination of mercury

The Advanced Mercury Analyzer AMA 254 (Altec, CZ) was employed to determine for mercury determination. It is the AA-spectrometer method which determined the mercury in range of ppb without decomposition. The samples were combusted in the stream of oxygen at the temperature 850- 900 °C. After passing through the catalytic furnace at 650 °C, mercury was trapped in gold amalgamator. It was released at high temperature and the atomic absorption was measured.

3.2.2 Determination of cadmium, lead and zinc

Cadmium and lead concentrations in tubers of plant species were measured using AAS with electrothermal atomisation (ET-AAS). A spectrometer Varian SpectrA 280Z with graphite atomiser was used and programmable sample dispenser Varian 120. The concentration of Cd and Pb were determined out in argon atmosphere in a pyrolytic graphite tube with platform. The concentration of Zn was measured using AAS with air-acetylene flame technique. We used a spectrometer Varian SpectrAA 110 (Varian, Inc., Mulgrave, Australia). The chosen wavelength was 213.9 nm with deuterium background correction. Standard solution ASTASOL (Analytika, Ltd., Prague, Czech Republic) of Zn was used in the preparation of a calibration curve for the measurement. The quality of analytical data was assessed by simultaneous analysis of certified reference material SRM NIST 1567a (Nist Wheat Flour) (4 % of all the samples).

3.2.3 Determination of selenium

The content of selenium was determined in digested samples of the cereals by AAS with hydride generation technique (HGAAS). Grain samples were ground finely and microwave digested in an acid solution using MWS-3± (Berghof Products ± Instruments, Eningen, Germany). The concentration of selenium in the digests of cereals were measured by HGAAS technique using VarianAA 280Z (Varian, Mulgrave, Victoria, Australia) with vapour generation accessory VGA-76 and sample preparation system Varian SPS3. Standard solution ASTASOL (Analytika, Prague, CR) of selenium was used in the preparation of a calibration curve for the measurement. The quality of analytical data was assessed by simultaneous analysis of certified reference material BCR 281 (Rye grass) (4 % of all the samples). The accuracy for selenium with respect to the reference material was 96.5%. The background of the trace element laboratory was monitored by analysis of 17.5% blanks prepared under the same conditions, but without samples, and experimental data was corrected by mean concentration of analyte in blanks, and compared with detection limit (mean ± 3SD of blanks) (0.08 ng ml^{-1}).

3.2.4 Replicates and statistical analysis

All experiments were conducted in triplicates. For all measurements averages and standard errors were calculated in Microsoft Excel 2007. The data were processed by Chromeleon (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and Excel (Microsoft, Redmond, WA, USA). Statistical evaluation was performed using the Statistica software (ver. 12; StatSoft, Inc., Tulsa, OK, USA). Genotype differences in Se contents were evaluated by one-way ANOVA ($P \leq 0.05$). Tukey's Post Hoc HSD test was used for detailed evaluation and non-parametric Kruskal-Wallis H-test.

4 RESULTS AND DISCUSSION

The concentrations of heavy metals (cadmium, zinc, lead and mercury) in the whole grain of spring accessions of emmer, einkorn and common spring wheat cultivars were measured using the Atomic Absorption Spectrometry (AAS). Among the investigated varieties with high lead concentration, prevailing were the spring wheat varieties, less presented are einkorn and emmer wheat varieties. Between different varieties, the significant differences have been determined. In this study, the concentration of mercury was determined in four typical growth stages of wheat (boot stage, stage 10.2, leaf-stage 10.2 and stage 11 according to Feekes).

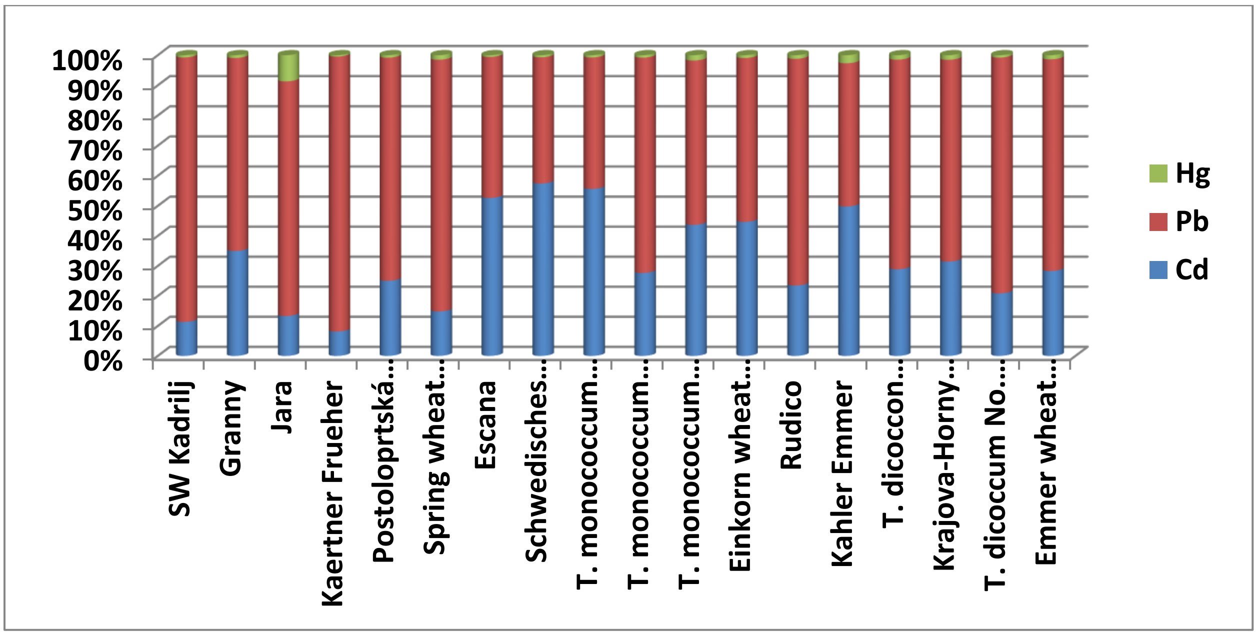

Figure 1. Content of cadmium (Cd), lead (Pb) and mercury (Hg) in spring, einkorn and emmer wheat species (mg kg^{-1} dry matter)

In our study, we also identified selenium contents in eighteen winter wheat varieties with different grain colour and three spring tritordeum yellowed-grained varieties and breeding lines. Comparison of Se contents in wheat and tritordeum grains revealed differences between some varieties and genotypes. The highest levels were determined in red-grained cv. Bohemia, yellow-grained Bona Vita, blue-grained breeding line V2 10-16 (Skorpion x Magister), KM 53-14 (Skorpion × Ludwig) and yellow-grained V2 15-16 (Citrus x Bona Dea). Diversity in certain wheat accessions offers genetic potential for developing cultivars with better ability to accumulate important micronutrients in grains. Selenium in wheat grain in the form of selenoproteins glutathione peroxidases could also contribute to antioxidant activity of wheat and tritordeum grain containing in blue and purple grain especially anthocyanins and in yellow grain carotenoids with antioxidant properties.

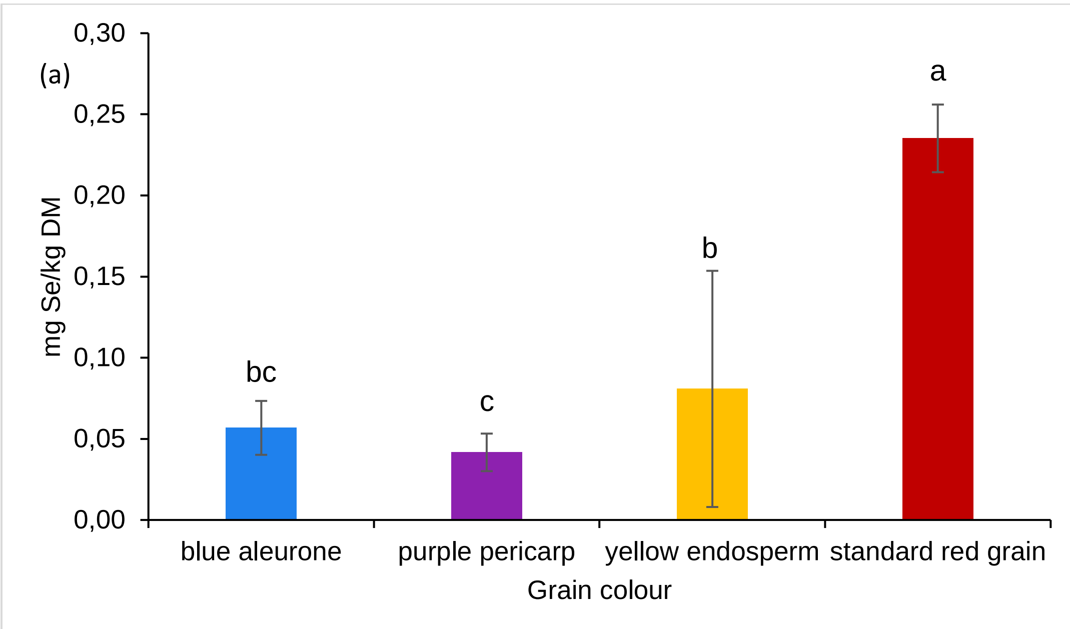

Figure 2. Effect of cereal grain colour on selenium content

In the potato experiments, 16 different varieties were used including: Agria, Blaue Anneliese, HB Red, Blaue St. Galler, Violette, Rosalinde, Valfi. Herbie 26, Blue Congo, Blaue Elise, Rotte Emma, Salad Blue, Boravaley, Königsblau, Königspurpur and Blaue de la Mancha. 3 varieties (Königsblau, Königspurpur and Blaue de la Mancha) were only had in Uhříněves 2014. Amounts of toxic and potentially toxic elements detected in investigated potato tubers are characterized by a large variability within investigated groups. Performing statistical analysis (one–way ANOVA) showed that there were no significant differences between two investigated groups of samples (samples from Uhříněves and Valečov in the year 2013 and 2014) considering either one of investigated metals. Measurable levels of mercury were found in smallest amounts in all investigated potato samples comparing to other metals (Cd, Pb).

To investigate the effect of thermal processing on concentration of heavy metals on analyzed potato tubers, two different cooking methods were used (boiling in water for 20 minutes at 100 °C and baking them for 45 minutes at 180 °C) to compared with the raw samples. The results showed the significant increase the concentration of Hg, Cd and Hg in cooking methods. Cadmium and lead had higher values comparing to mercury on the same varieties. According to Seregin and Kozhevnikova (2008), some plants can accumulate higher amount of heavy metals than other plants. The absorption of heavy metals can effect on the growth, development of plants and when are consumed by human, it causes the serious problems to human health. A lot of researchers have focused on the heavy metal in *Triticum* plants because of their potential values in the future (Jingh et al., 2007; Gajewska and Sklodowska, 2008).

The research of cadmium concentration in wheat variety can be described in the documents of Kusa (2005), Matsumoto (2007) and Romkens (2009). They found that the accumulation of lead in wheat grain was approximately 2.5 times higher than in our research, while the obtained results for cadmium were the same. Comparing to our study, the lead accumulation in grain wheat was lower than those found by other authors (Lavado et al., 2007). In addition, compared with the recent research of Bermudez et al. (2011), the cadmium, lead and zinc concentrations in wheat were higher (0.017 mg kg^{-1} dry matter; 0.088 mg kg^{-1} dry matter and 29.20 mg kg^{-1} dry matter, respectively).

There are many factors affecting the uptake of heavy metals such as varieties and characteristics of plants or soil characteristics (pH, cation exchange capacity or organic matters) (Gupta et al., 2007). Durum wheat is considered as more sensitive to zinc deficient soils than other varieties. The increase of cadmium and lead concentrations can be explained by the influence of soil characteristics (cation exchange capacity, the concentration of available metals and organic matter content in soils).

Several researches on the effects of lead on plants have been reported (Seregin, 2008). When the concentration of lead is high, it can reduce the development of root hair and significantly affect to the plant growth (Lin et al., 2007). Kikuchi (2007) reported the uptake of cadmium concentration in wheat is higher than in rice when growing on the same conditions. The accumulation of cadmium in grain wheat also depends on the genetic variation of wheat.

The uptake of Se from soils into plants depends on several parameters such as bio-available Se concentration, soil characteristics, Se speciation, plant species and concentration of competing ions (Hegedüsová et al., 2012). The decrease of selenium from plants can only occur through the volatilization (Whanger, 2004). The soil in the experimental location of our study had pseudo total (Aqua Regia soluble) Se average content 1.179 ± 0.077 mg kg^{-1} dry matter; this corresponds to the range of Se concentrations between 0.2-1.4 mg kg^{-1} dry matter in Czech soils (Száková et al., 2015).

Cereals were reported poor in bioavailability and concentration of microelements such as Zn, Fe, and Se in the seeds (Cakmak, 2008). However, cereals play an essential and invaluable role in human diet of which wheat is the third most produced staple cereal on earth. Currently around 758 million tons of wheat is produced in the world and its global consumption is 67 kg/capita/year. Zn and Se concentrations in grains exhibit 2- and 1.5-fold difference between wheat accessions (Souza et al., 2014). Se income in Slovakia from cereals was estimated as 14 % of total (Tóth et al., 2012). However, some wheat species like the diploid wheat *Aegilopstauschii* was 42% higher in grain Se concentration than commercial bread and durum wheat (Lyons et al., 2005). One of the promising solutions for reducing malnutrition is developing cereals that are genetically enriched in micronutrients and proteins (Lyons, 2010).

5 CONCLUSIONS

The concentrations of heavy metals (cadmium, zinc, lead and mercury) in the whole grain of spring accessions of emmer, einkorn and common spring wheat cultivars were measured using the Atomic Absorption Spectrometry (AAS). Among the investigated varieties with high lead concentration, prevailing were the spring wheat varieties, less presented are einkorn and emmer wheat varieties. Between different varieties, the significant differences have been determined. Einkorn wheat accessions have been shown 2.0 times higher and 1.7 times higher than emmer wheat and spring wheat varieties in the concentration of cadmium in grains. Jara has the lowest content of cadmium (0.013 ± 0.001 mg kg^{-1} dry matter) and the highest value stands for *T. monococcum* 2101 (0.058 ± 0.001 mg kg^{-1} dry matter). Wheat variety of high mercury content was represented mainly by spring wheat (Jara variety 0.009 ± 0.001 mg kg^{-1} dry matter). Otherwise, the concentration of zinc was higher than other investigated heavy metals, ranging from 35.19 ± 2.733 mg kg^{-1} dry matter to 67.41 ± 1.990 mg kg^{-1} dry matter. Low level of zinc was found almost exclusively in spring wheat (40.99 mg kg^{-1} dry matter). In this study, the concentration of mercury was determined in four typical growth stages of wheat (boot stage, stage 10.2, leaf-stage 10.2 and stage 11 according to Feekes). Stage 10.2 and leaf-stage 10.2 showed high mercury content. Additionally, it has been showed that wheat varieties absorbed a wide range of mercury (Hg) in different growth stages, where different concentrations were determined. For example, in the boot growth stage and leaf-stage 10.2, Schwedisches Einkorn contained the highest content of mercury, while in stage 11, *T. monococcum* 2103 absorbed the highest mercury amount.

In our study, we also identified selenium contents in eighteen winter wheat varieties with different grain colour and three spring tritordeum yellowed-grained varieties and breeding lines. Comparison of Se contents in wheat and tritordeum grains revealed differences between some varieties and genotypes. The highest levels were determined in red-grained cv. Bohemia, yellow-grained Bona Vita, blue-grained breeding line V2 10-16 (Skorpion x Magister), KM 53-14 (Skorpion × Ludwig) and yellow-grained V2 15-16 (Citrus x Bona Dea). Diversity in certain wheat accessions offers genetic potential for developing cultivars with better ability to accumulate important micronutrients in grains. Selenium in wheat grain in the form of selenoproteins glutathione peroxidases could also contribute to antioxidant activity of wheat and tritordeum grain containing in blue and purple grain especially anthocyanins and in yellow grain carotenoids with antioxidant properties.

In the potato experiments, 16 different varieties were used including: Agria, Blaue Anneliese, HB Red, Blaue St. Galler, Violette, Rosalinde, Valfi. Herbie 26, Blue Congo, Blaue Elise, Rotte Emma, Salad Blue, Boravaley, Königsblau, Königspurpur and Blaue de la Mancha. 3 varieties (Königsblau, Königspurpur and Blaue de la Mancha) were only had in Uhříněves 2014. Amounts of toxic and potentially toxic elements detected in investigated potato tubers are characterized by a large variability within investigated groups. Performing statistical analysis (one–way ANOVA) showed that there were no significant differences between two investigated groups of samples (samples from Uhříněves and Valečov in the year 2013 and 2014) considering either one of investigated metals. Measurable levels of mercury were found in smallest amounts in all investigated potato samples comparing to other metals (Cd, Pb).

To investigate the effect of thermal processing on concentration of heavy metals on analyzed potato tubers, two different cooking methods were used (boiling in water for 20 minutes at 100 °C and baking them for 45 minutes at 180 °C) to compared with the raw samples. The results showed the significant increase the concentration of Hg, Cd and Hg in cooking methods. Cadmium and lead had higher values comparing to mercury on the same varieties.

Different plant tissues are accumulated in different concentrations. In most studies, the highest contents of heavy metals are absorbed in roots. The lower contents are accumulated in stems and leaves. The lowest parts of plants absorbing metals are organs and fruits. In research of Stefanovic (1999), the concentration of cadmium in potato tips and tubers were (0.58, 3.46, and 7.35 mg kg^{-1}) and (0.18, 0.89, and 1.06 mg kg^{-1}) according to the cadmium introduction (800, 1600, and 3200 mg kg^{-1}).

6 REFERENCES

Abrahams, P.W. 2002. Soils: their implications to human health, Science of Total Environment, 291, 1-32.

Bermudez, G.M.A, Jasan, R., Pla, R., Pignata, M.R. 2011. Heavy metal and trace element concentrations in wheat grains: Assessment of potential non- carcinogenic health hazard through their consumption. Journal of Hazards Materials, 193, 264-271.

Cakmak I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 302, 1-17.

Chandra, R., Gharagava, R., M., Yadav, S., Mohan, D. 2009 Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents, Journal of Hazard, Master, 162, 1514- 1521.

Dukhovskis, P., Brazaityte, A., Juknys, R., Ianuskaitiene, I., Sliesravicius, A., Ramaskeviciene, A., Burbulis, N., Skisnianiene, J.B., Baranauskis, K., Duchovskiene, L., Stanys, V., Bobinas, C. 2006. Changes of physiological and genetics indices of *Lycopersicon esculentum* Mill by cadmium under different acidicity and nutrition. Polish Journal of Environmental Studies, 15 (2), 235-242.

European Commission, Commission Regulation (EC) No. 466/2001. 2002. Setting Maximum Levels for Certain Contaminants in Foodstuffs.

Farmer, A.A., Farmer, A.M. 2000. Concentration of cadmium, lead and zinc in livestock feed and organs around a metal production center in eastern Kazakhstan. Science of the Total Environment, 257, 53-60.

Gajewska, E., Sklodowska, M. 2008. Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. Plant Growth Regulation, 54, 179- 188.

Gupta, A.K., Sinha, S. 2007. Phytoextraction capacity of the plants growing on tannery sludge dumping sites. Bioresource Technology, 98, 1788-794.

Hegedüsová A., Hegedüs O., Vollmannová A., Mezeyová, I., Andrejiová, A. 2016. The selenium transfer from the soil into the agricultural plants in Nitra region of Slovakia. In SGEM 2016. Sofia: STEP92 Technology: 425--431. ISBN 978-619-7105-62-9.

Jamali, M.K., Kazi, T.G., Arain, M. B., Afridi, H.I., Jalbani, N.J., Kandhro, G.A., Shah, A.Q., Baig, J.A. 2009. Heavy metal accumulation in different varieties of wheat (*Triticum aestivum* L.) grown in soil amended with domestic sewage sludge, Journal of Hazard. Mater, 164, 1368-1391.

Jingh, D., Nath, K., Sharma, Y.K. 2007. Response of wheat seed germination and seedling growth under copper stress. Journal of Environmental Biology, 28 (2), 409- 414.

Kikuchi, T., Obazaki, M., Toyota, K., Motobayashi, T., Kato, M. 2007. The input-output balance of cadmium in a paddy field of Tokyo. Chemosphere, 68, 920- 927.

Kusa, K., Hatta, K., Hara, Y., Tsuchiya, K. 2005. Varietal difference in concentration and location of cadmium in wheat and barley grain. Report of Kyushu Agriculture, 67, 46.

Lavado, R.S., Rodriguez, M.M., Alvarez, R., Taboada, M.A., Zubillaga, M.S. 2007. Transfer of potentially toxic elements from biosolid-treated soils to maize and wheat crops. Agriculture, Ecosystems and Environment, 118, 312-318.

Lin, R., Wang, X., Luo, Y., Du, W., Guo, H., Yin, D. 2007. Effect of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings *(Triticum aestivum* L.). Chemosphere, 69, 89-98.

Lyons G.H., Ortiz-Monasterio I., Stangoulis J.C.R., Graham, L. 2005. Selenium concentration in wheat grain: is there sufficient genotypic variation to use in breeding? Plant Soil, 269, 369-380.

Lyons G.H. 2010. Selenium in cereals: improving the efficiency of agronomic biofortification in the UK. Plant Soil, 332, 1-4.

Matsumoto, T., Kara, H., Higasa, Y. 2007. Evaluation of pollution risk of cadmium in crops by chemical characteristics of arable soils as an indicator. Report Sophisticated Project of Ministry of Agriculture, Forestry and Fisheries, 3.

Pandey, J., Pandey, R., Shubhashish, K. 2009. Air- borne heavy metal contamination to dietary vegetables: a case study from India. Bulletin of Environmental Contamination and Toxicology*,* 83, 931-936.

Renella, G., Mench, M., Gelsomino, A., Landi, L., Nannipieri, P. 2005. Functional activity and microbial community structure in soils amended with bimetallic sludge. Soil Biology and Biochemistry, 37, 1498-1506.

Romkens, M., Guo, H.Y., Chu, C.L., Liu, T.S., Chiang, C.F., Koopmans, G.F. 2009. Prediction of cadmium uptake by brown rice and derivation of soil- plant transfer models to improve soil protection guideline. Environmental Pollution, 157, 2435-2444.

Seregin, I.V., Kozhevnikova, A.D. 2008. Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel and strontium. Russian Journal of Plant Physiology, 55, 1-22.

Souza, G.A., Hart, J.J., Carvalho, J.G., Rutzke, M.A., Albrecht, J.C., Guilherme, L.R.G., Kochian, L.V., Li, L. 2014. Genotypic variation of zinc and selenium concentration in grains of Brazilian wheat lines. Plant Science, 224, 27-35.

Száková J., Tremlová, J., Pegová, K., Najmanová, J., Tlustoš, P. 2015. Soil-to-plant transfer of native selenium for wild vegetation cover at selected locations of the Czech Republic. Environmental Monitoring and Assessment, 187, 358-366.

Tóth T., Urminská D., Miššík J., Vollmannová A., Árvay J. 2012. Selenium sources in human nutrition. Proceedings of 1. Conference of Centrum of Excellence for White-green Biotechnology, 218-222. ISBN 978-80-971156-1-6.

Whanger P.D. 2004. Selenium and its relationship to cancer: an update. British Journal of Nutrition, 91, 11–28.

7 PERSONAL INFORMATION

Name: Ing. Le Minh Phuong

Date, place of birth: 14-03-1987, Vietnam

Phone: +420 602 844 568

Email: phuongleminh87@gmail.com ; le_minh@czu.cz

Education

2014- 2017 PhD. studies: Czech University of Life Science (CULS), Faculty of Agrobiology, Food and Natural Resources, Department of Chemistry

2011- 2013 MSc. Studies: Czech University of Life Science (CULS), Faculty of Agrobiology, Food and Natural Resources

2011 Working at Institute of Agro-Biology, Hanoi University of Agriculture, Ha Noi City, VN.

2006-2011 BSc, Studies: Advanced Training Education – Ha Noi University of Agriculture, Ha Noi City, VN

8 LIST OF PUBLICATIONS OF AUTHOR

Impaktované časopisy

LE, MINH PHUONG, LACHMAN, J., KOTÍKOVÁ, Z. ORSÁK, M., MARTINEK, P. Selenium in colour-grained winter wheat and spring tritordeum. Plant, Soil and Environment, 2017, 63, (7), in print.

NOVÁKOVÁ, K. – NAVRÁTIL, T. – ŠESTÁKOVÁ, I. – **LE MINH, P**. – VODIČKOVÁ, H. – ZÁMEČNÍKOVÁ, B. – SOKOLOVÁ, R. – BULÍČKOVÁ, J. – GÁL, M. Characterization of cadmium ion transport across model and real biomembranes and indication of induced damage of plant tissues. Monatshefte für Chemie, 2015, 146, 819-829.

Recenzované časopisy

LE MINH, P. – VODIČKOVÁ, H. – ZÁMEČNÍKOVÁ, B. – LACHMAN, J. Optimization the Cell Wall Degrading Enzymes and Technique for Isolation of Protoplasts in Potato. 2016, Journal of Pharmacy and Pharmacology, 2016, 4, (4), 191-194.

Sborníky z konferencí

LE MINH, P. – VODIČKOVÁ, H. – ZÁMEČNÍKOVÁ, B. – LACHMAN, J. Methods for studying of plant membrane transport. In Modern Electrochemical Methods XXXV, Collection of Conference Proceedings 18.05.2015, Jetřichovice. Ústí nad Labem: Best servis Ústí nad Labem, 2015. s. 128-132.

LE MINH, P. – ZÁMEČNÍKOVÁ, B. – VODIČKOVÁ, H. – LACHMAN, J. Podtyp: Příspěvekvesborníku (mimokategorie RIV); Preparation of plant material for the study of transport across membranes. 2015, Applied Natural Sciences. Book of Abstracts, 2015, September 30-October 2, Jasná, s. 117. ISBN 978-80-8105-723-6.

ZÁMEČNÍKOVÁ, B. – VODIČKOVÁ, H. – **LE MINH, P**. – LACHMAN, J. Methods of water status measurement in plants. 2015, Applied Natural Sciences. Book of Abstracts, 2015, September 30-October 2, Jasná, s. 157. ISBN 978-80-8105-723-6.

PAZNOCHT, L. – VODIČKOVÁ, H. – **LE MINH, P.** – NOVÁKOVÁ, K. – ZÁMEČNÍKOVÁ, B. – KOTÍKOVÁ, Z. – MIHOLOVÁ, D. – NAVRÁTIL, T. Influence of Cadmium on its Metabolism and Changes of Content of Nutritionally Important Compounds in Spring Barley. In: Modern Electrochemical Methods XXXV, Collection of Conference Proceedings Jetřichovice. Praha, BEST SERVIS, ÚSTÍ NAD LABEM, 2015. s. 166-169.

LE MINH, P. – VODIČKOVÁ, H. – ZÁMEČNÍKOVÁ, B. – LACHMAN, J. Optimization the cell wall degrading enzymes and technique for isolation of protoplasts in potato. 2014, 47th Heyrovsky Discussion on Electrochemistry of Organic and Bioactive Compounds. Book of Abstracts, 2014, May 25th-29th, Třešť, s. 49. ISBN: 978-80-87351-29-1.

LE MINH, P. – ZÁMEČNÍKOVÁ, B. – VODIČKOVÁ, H. – LACHMAN, J. Preparation of plant material for the study of membranes by electrochemical methods. 2014, 47th Heyrovsky Discussion on Electrochemistry of Organic and Bioactive Compounds. Book of Abstracts, 2014, May 25th-29th, Třešť, s. 50. ISBN: 978-80-87351-29-1.