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

PHD. DISSERTATION

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DECLARATION

I declare that I have written my diploma thesis "Biotransfer of Selected Risk Metals into Plants and Their Accumulation and Distribution in plants" on my own with the help of literature listed in References and on the basis of consultations and recommendations of supervisor. I agree with the dissertation publication pursuant to Act No. 111/1998 Coll. on Higher Education Institutions as amended, regardless of the outcome of their defense.

In Prague 30th April 2017 Le Minh Phuong

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Student

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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

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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 recognized 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 heavy metals are accumulated by plants, they 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 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 metals to transport hazardous metals in the plant organs and their interaction with the aim of the minimization of adverse and toxic elements in plant.

3 LITERATURE OVERVIEW

3.1 Introduction to wheat (*Triticum spp.*L.)

Wheat (*Triticum spp*. L.) is one of the most important cereal crops in the world, which is harvested annually over 600 million tonnes (FAO, 2010). In 2010, wheat was the third-most produced crop in the world, accounting for 651 million tonnes, after rice (672 million tonnes) and maize (844 million tonnes). Wheat can be cultivated over a wide range of climatic conditions, from 67^0N in Scandinavia and Russia to 45^0S in Argentina, including the regions in the sub- tropics and tropics of the world (Feldman, 1995).

Most people consume wheat rather than other kinds of cereal grain (Singh et al., 2007). In 2010, the world's main wheat producing countries were China, India, Russian Federation, United States of America, France, Canada, Germany, Pakistan, Australia, Turkey (FAO, 2010). They predicted the global demand of wheat from 840 million tonnes to 1050 million tonnes in 2020. From the current production yield, the global production needs to be increased by 1% per year to reach this target in the future.

There were many research reports on the origin and domestication of wheat (Salamini et al., 2002). Wheat was one of the first grains to domesticate. It started about 9,000- 11,000 years ago in the Middle East, when human changed from gathering and hunting to cultivation as agriculture (Shewry, 2009). 4,000 years ago, bread wheat became a common staple crop growing from England to China. In some documents, the earliest cultivated varieties of wheat were einkorn and emmer wheats from the south- eastern of Turkey (Nesbitt, 1998).

Wheat belonged to the tribe *Triticeae* in a subdivision of *Panicoideae*, a member of the family of grass *Poaceae* (*Graminiceae*). Wheat can be classified by season of planting, by the colour, by chromosomes or hardness of grain. For example, wheat can be divided into diploid, tetraploid and hexaploid species (Breiman et al., 1995).

The popular currently cultivated variety of wheat is hexaploid wheat (*Triticum aestivum* L.) which is used for making bread, durum wheat (*Triticum durum*) or spelt wheat (*Triticum spelta*). The key important of wheat is the nutritional value. Wheat provides more nutrients and calories to human diet than other cereal crops (55% of carbohydrates, 8-15% protein, 1.5-2% fats, 1.5-2% minerals, 2.2% crude fibers and 20% of calories). (Abdel-Aal et

al., 1998; Breiman and Graur, 1995). Wheat can be consumed as pasta, bread, noodles or other products (Kumar et al., 2011).

3.1.1 Spring wheat *(Triticum aestivum* L.*)*

Spring wheat (*Triticum aestivum* L.) is very popular planted wheat, also known as the bread wheat. Spring wheat is planted for grain.

Spring wheat was first domesticated in the western Asia, after that spreading to Africa, East Asia and Europe. In the mid 1980s, the spring wheat was grown for more than 20% of the total wheat area. However, in 1990 due to the expanding of winter wheat, the area of spring wheat decreased to 15%. This situation has changed nowadays when people realize the profit from growing spring wheat. Today, it is the best known and most widely grown in the world.

Spring wheat (*Triticum aestivum* L.) belongs to the sub-tribe *Tritiinae* in a tribe *Triticeae*, a member of the family of grass *Poaceae*. Spring wheat is the hexaploid, which has six sets of chromosomes. This is the annual grass with 20- 38 cm long, and about 1-3 cm broad.

The main use of spring wheat is daily bread making. The grain is also the source of alcoholic beverages (beer). Other parts of the plant like the bran can be used for feeding livestock or the straw can be supplied to handcraft industry. Other purposes are paper making, pastes or textiles.

3.1.2 Emmer wheat *(Triticum dicoccum* Schrank*)*

Emmer wheat (*Triticum dicoccum* Schrank) is one of the first crops domesticated in the Near East, which is known as *T. dicoccon* Schrank. It is considered to be similar as the origin site of einkorn wheat. Traditionally, it is grown in the arid.

The wild emmer wheat was cultivated about 10,000 to 12,000 years ago (Nesbitt and Samuel, 1996). In the 19th century, Aaronsohn et al. (1906) discovered the geographic distribution and natural habitat of wild emmer wheat. It has contributed greatly to the knowledge of wheat history and domestication. According to Feldman and Kislev (2007), Ozbek (2007), the emmer wheat had been changed in morphology, biochemistry and molecular variation through the hybridization.

Emmer wheat is an annual crop, which belongs to the glumeous variety of wheat. It has two homologous sets of chromosomes (Kilian et al., 2007). If cultivating in unfavourable growing season, yield of emmer wheat can exceed the yield of oat or barley. In contrast, when growing in favourable conditions for cereal, it shows the lower yield than other wheat cultivars. Emmer wheat provides high nutrients with crude protein content and more than 60% of calories (Gill et al., 2004).

3.1.3 Einkorn wheat *(Triticum monococcum* L.*)*

Einkorn (*Triticum monococcum* L.) literally means the single grain. The name einkorn is a modern name which came from botanical classification. Einkorn can be classified as the domesticated wheat variety (*Triticum monococcum* L.) or as the wild wheat variety (*Triticum boeoticum*). The cultivated wheat has the similar form as the wild wheat. The two kinds (domesticated and wild wheat variety) are considered as the subspecies of *Triticum monococcum*.

Einkorn wheat was also one of earliest wheat varieties that were cultivated. Historically, it recorded that einkorn was first domesticated as early as 7500 BC (Heun et al., 1997). It was cultivated originally in southeast Turkey, and then spreading through Mid-East and South-western Europe.

During the Bronze Age, the area of growing einkorn wheat was decreased. In the 20th century, it was mainly grown in European countries like France, Morocco, and Turkey etc. Nowadays, although einkorn is a health food, it is rarely planted.

Einkorn is diploid wheat. It has lower yield comparing to other kinds of wheat varieties. However, when growing under adverse conditions for example cool environment, it can show equal yield to barley or oat. Einkorn is not suitable for making bread even though it contains 50-70% protein content in grain. When baking products with einkorn, it tastes a light and rich flavour. Some places, einkorn wheat is using as livestock food.

3.2 Introduction to potato

3.2.1 Origin and classification

The potato was first cultivated in South America between three and seven thousand years ago, though scientists believe they may have grown wild in the region as long as 13,000 years ago. The genetic patterns of potato distribution indicate that the potato probably originated in the mountainous west-central region of the continent. There are many expressions of the extended use of the potato in the pre-Inca cultures from the Peruvian Andes. The crop diffused from Peru to the rest of the Andes and beyond. The Spanish conquistadors first encountered the potato when they arrived in Peru in 1532 in search of gold, and noted Inca miners eating chuñu. From Spain, potatoes had slowly spread to Italy and other European countries during the late 1500s. By 1600, the potato had entered Spain, Italy, Austria, Belgium, Holland, France, Switzerland, England, Germany, Portugal and Ireland.

The potato diffused widely after 1600, becoming a major food resource in Europe and East Asia. Following its introduction into China toward the end of the Ming dynasty, the potato immediately became a delicacy of the imperial family. After the middle period of the Qianlong reign (1735–96), population increased and a subsequent needed to increase grain yields coupled with greater peasant geographic mobility led to the rapid spread of potato cultivation throughout China, and it was acclimated to local natural condition

The potato is a starchy, tuberous crop from the perennial *Solanum tuberosum* of the *Solanaceae* family (also known as the nightshades). The word potato may refer to the plant itself as well as the edible tuber. *Solanum tuberosum* is divided into two subspecies. They are *tuberosum* and *andigena.* The first subspecies *tuberosum* is the widely cultivated potato in Europe and North America. The other subspecies *andigena* is popular mostly in Central and North America (Hanneman, 1994).

Table 1. Taxonomic position of *Solanum tuberosum* subspecies *tuberosum.*

Taxonomic rank	Latin name
Kingdom	Plantae
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Section	*petota*
Subsection	*potatoe*
Series	*tuberosa*
Species	*Solanum tuberosum*
Subspecies	*tuberosum*

3.2.2 Morphology of the potato

The potato is an herbaceous plant. The potato is perennial but as a crop it is treated as an annual.

Roots: Occasionally, roots may also grow on stolons. Root growth is usually restricted to top layers at a depth 20 – 25 cm. In rich soils, roots of some varieties may reach up to 90 – 100 cm.

Stems: Stems are round to angular in cross section. At the angulke margins wings or ribs are often formed. Wings could be straight, undulate, or dentate. Stem color is generally green; sometimes it may be red- brown, or purple. Stems may be solid or partly hollow due to disintegration of the pith cell.

Leaves: leaves are arranged spirally on the stem. Normally leaves are compound that is they consist of a midrib and several leaflets.

Flower: Numerous, arranged in compound terminal cymes, with long peduncles. Each flower has 5 stamens, 5 sepals and 5 petals united for almost all their length. Most varieties bear infertile pollens hence fruit or berries are not formed. The inflorescence is a compact racemose type born on the apex.

Berry: Perfectly globose, smooth, under an inch in diameter

Tuber: It is a thickened stem having the cells mostly filled with starch as a reserve food for the new plants. The eyes are the promise of the future branches.

3.3 Protoplasts

Plasma membrane of plant cells is surrounded by cellulose wall and adjacent cells are joined together by a thick pectin rich matrix. So, the protoplast contains the plasmalemma and all things included in, for example the entire cell without its cellulosic cell wall.

Separation of plant cells and removal of the cell wall experimentally, by either a mechanical or an enzymatic process, results in the production of protoplasts.

The two methods for protoplast isolation are mechanical method and enzymatic method.

- Mechanical isolation: The isolation of protoplasts was firstly performed by Klercker (1892) in *Stratiates aloides*. Later experiments were carried on tissues of onion bulbs. After immersing the scales in sucrose, the protoplasts were shrunk from enclosing walls. The next step was using the sharp knife to cut the plasmolysed tissues. Without damaging the protoplasts, the cell walls were cut only.

- Enzymatic isolation: The enzymes were used to dissolve the cell walls for releasing protoplasts. Enzymatic isolation was initially carried out on the yeast cells. The cell wall was digested using the gastric juice from the snail- *Haliz poneatia*. Remarkably, Cocking (1960) isolated the higher plants to obtain high yield protoplasts. The material in this experiment was tomato roots and the enzyme cellulase was prepared from the fungus *Myrothecium verrucaria*. Later, with the modification of enzymes, this method was developed by a lot of scientists. This method can be used in both two ways. The first way is that isolation of protoplasts using the mixture of two enzymes: cellulase and pectinase. The second way is isolation the cells from tissues by pectinase and later adding cellulase for digestion cell wall. The protoplasts are released after that.

Protoplasts are useful tools to study the uptake and transport of macromolecules and production of somatic hybrids. Several researchers have achieved the isolation of potato protoplasts and their subsequent regeneration. Considering the importance of protoplasts in potato, in the present study we propose the following objectives: to prepare

plant material for the study of membranes and to optimize the cell wall degrading enzymes and technique for isolation of protoplasts in potato (*Solanum tuberosum* L.).

The biochemical and physiological properties between cells and their protoplasts looks similar in the reports of some scientists. However, during the protoplast isolation, the ion uptake, protein synthesis and intracellular transport are changed. The reasons may be due to the osmotic stresses during the isolation of protoplasts.

3.4 Heavy metals

3.4.1 Overview of heavy metals

According to Sanita di Toppi and Gabbrielli (1999), he defined the heavy metals were a group of metals which have the density higher than 5.0 g cm^{-3}. The European Community has reported the heavy metals that need the highest concern are cadmium, mercury, lead, arsenic, nickel, manganese, zinc etc.

Some heavy metals have significant role for plants when they participate in enzymatic redox reactions. Other effects of metals are stability of lysosome membrane, protein denaturation, mitochondrial membrane permeability, nucleic acids and enzymes inhibition. Even though the plants need a small amount of these elements, they are important for growth and development of plants (Ivanova et al., 2010). The metals are absorbed from the soil to the root surface of plants, the roots continuously transferred to the shoot. So, the concentration of metals strongly influences to the amount of absorbing metals.

Schutzendubel and Polle (2002) said that there were two main natural sources of heavy metals in the terrestrial ecosystems. They were in the atmosphere and in the soil. The origin of heavy metals in the soil mainly came from human activities, while continental volcanoes dusts were the main origin from the atmosphere.

There were many research reports on the ways heavy metals enter the soil by the influenced of human activities (Schutzendubel and Polle, 2002). Other researchers like Schuhmacher (2009), Bermudez (2010) and Fabietti (2010) reported the source of heavy metals from industrial activities. Surprisingly, agricultural activities were also one of the sources of heavy metal contamination (Dragovic, 2008). For example, cadmium and lead

may come from waste water irrigation or overuse of agrochemical products. The processes such as burned fossil fuel are responsible for the increasing the heavy metals releasing to the atmosphere, while other processes such as precipitation and adsorption are responsible for the transport of heavy metals to other places in the environment.

Some metals are biologically essential. They are used in industrial processes or consumed in some products. But in the case when using them too much in dosage, they become toxic to health (Jarup, 1998). Because of the adverse effects on human and animal health, the contamination of toxic heavy metals is very importance issue. Consequences of heavy metal contamination in human are bone disorders, neurological or impaired kidney functions (Dyer, 2007).

According to scientists, some metals might be suspected to cause the carcinogenic diseases to human life. So, the important issue for scientists is to recognize in which case can cause the adverse effects. In recent decades, with the dramatically increasing the number of exposure living organisms to heavy metals, there are more and more researches on the effect of heavy metals on cellular systems in the environment (Wang et al., 2009).

There is no doubt that plants are important components in the ecosystems. They have the ability to transfer to elements from abiotic environment to biotic environment (Forsberg and Ledin, 2006). According to Seregin and Kozhevnikova (2008), some plants can accumulate higher amount of heavy metals than other plants. The toxic heavy metals can effect on the growth and development of plants. When are consumed by human, it might cause the serious problems to human health. So the scientists need to pay more attention to the plants which are grown or consumed in the areas having the toxic metals.

The plants which are growing on the metal- contaminated areas also develop the tolerant characteristics to survive. As a result, today there are a lot of studies on the crops grown in the surrounding of industrial areas or in the big cities. The changes in soil properties (both physical and chemical properties of soil) strongly influence on the bioavailability and solubility of metals. When considering the properties of soil, it includes the organic matter contents of soil, pH and dissolved organic matter.

Kisku (2000) reported on the accumulation of heavy metals in crop plants and how heavy metals can transfer to the systems. There are many factors affecting the uptake of heavy metals by roots in plants. They are plant species, plant characteristics (physiology of

plant etc.) or the soil conditions during growing. Soil properties (pH, cation exchange capacity or organic matters) can influence the accumulation and uptake of heavy metals (Gupta et al., 2007). The activity and availability of micro- organisms and macro-organisms in soil are also affected by plant- uptake metal (Yang et al., 2007).

Heavy metals in the soils can bound to clay or organic matter or sometimes they can also bound to hydrous oxides of Al, Mn and Fe. Heavy metals can also act in the soil as the inorganic components. To evaluate the potential effects of heavy metals, Adriano (2004) suggested using the regulatory limits for heavy metals both in total amount and bioavailable concentration. Castaldi (2005) and Tandy (2009) investigated the influence of addition amendments or sorbent on the immobilization of heavy metals in the soils. As a result, it can reduce the ability to uptake heavy metals by plants, the groundwater contamination and effects on animal and human health.

3.4.2 Mercury (Hg)

Mercury is not an abundant element. The presence of this element in soil causes the potential risk to health. The ways which mercury is taken into the human body, through the skin, eating food and breathing. When the concentration of mercury is high enough, it can damage the kidney and the brain. The consequences of exposure to high level of mercury are the memory loss, changes in both hearing and vision abilities in human. According to Environmental Agency (2009), the mean daily intake of mercury for adult inhalation is 0.05 $\mu g\ day^{-1}$. Generally, mercury is widely distributed pollutant. Therefore, mercury can cause the toxicity for higher plants in the ecosystem.

Mercury is a volatile metal. The reasons of harmful effects by mercury are the mobility and bioaccumulation in the atmosphere (Rodriguez et al., 2003). In the research of Engle (2005), he found that the accumulation of mercury in soils often associated with the atmospheric deposition. It can remain from half year to two years in the atmosphere before depositing in the soil. Steinnes (1995) reported the major source of mercury emission is the anthropogenic activities (burning fossil fuel, mining and smelting, waste incineration etc).

Mercury in the environment combines with other element to form inorganic mercury compounds. Organic mercury compounds are formed when mercury combines

with carbon, for example methylmercury. In the environment, the common forms of mercury are mercuric chloride, methylmercury and metallic mercury etc. Among them, methylmercury is the most concern. The reason is the bioaccumulation of methylmercury in the food chain. The scientists have reported the amount of methylmercury that a person (has 150 pound weight) can ingest safely everyday is 0.001 mg.

The amount of absorbing mercury is different among the plant varieties. Similarly to other heavy metals, mercury is mainly accumulated in roots. Several researches on the accumulation of mercury in roots are Patra (2000) and Kabata-Pendias and Pendias (1999).

McLaughlin (1996) investigated the importance interaction between mercury and plant systems. The reason is that mercury is applied in the fertilizer, herbicide, and fungicide and in the seed disinfectants. When mercury is applied as the fungicide on plant, it can be translocated and redistributed in plant. The form of mercury and its sorption affect the toxicity and phytoavailability of mercury. The plants which are grown in the mercury-enriched system can develop the ability to adapt to the environment.

3.4.3 Cadmium (Cd)

Since 1980, the accumulation of cadmium in agricultural soils has been discussed. Cadmium is a toxic element (Dahmani-Muller et al., 2000). The contamination of cadmium is a dangerous situation for both animal and human health. Cadmium can significantly influence on the food supply chains and on the ecosystems. It is in active enzymes and affects to the plant cells (Stroinski, 1999). The symptoms of cadmium toxicity are chlorosis, influencing the photosynthesis, transpiration, changing in morphology and physiological properties of plants and inhibition of plant growth, nutrient accumulation. The effects of cadmium on human health can be kidney damage, inhibition of vitamin activation etc (Jarup et al., 1998; Larsson et al., 1998).

FAO/WHO recommends the maximum intake of cadmium is 70 μg day^{-1} for human (Vasilev and Yordanova., 1997). According to European Commission in 2001, the maximum permissible cadmium concentration is 0.1 μg g^{-1} wet mass for cereals. The maximum cadmium concentration for rice and wheat grain is 0.2 μg g^{-1} wet mass.

Industrial effluents are considered as the main source of cadmium contamination. In arable soil, Das (1997) reported that phosphorous fertilizers are other source of cadmium. Cadmium is accumulated in leaves from the dust deposition. The form of dissolved cadmium is $Cd^{2\pm}$. Cadmium also presents in the complex forms such as CdCl, $CdCl_2$ or $CdSO_4$ (Singh et al., 1999).

Cadmium is unessential element to plant. Greger and Landberg (1996) reported the transport of cadmium occurs in the xylem of the plants. About the uptake of cadmium, most hypotheses said that uptake of cadmium is passive, some considers uptake is active (Greger and Landberg, 1996). The higher the concentration of cadmium, the more cadmium is uptake by plants. More than 50% of absorbing cadmium is retained in the roots of plants (Koeppe, 1997). After that, cadmium is taken up by vegetables and crops which are consumed by human. So, the source of cadmium in taken by human mostly comes from food.

According to Grant (1998), cadmium is taken up and transported to plants in the similar way as zinc. Das (1997) also reported zinc and cadmium have similar properties of environment and geochemistry. Kabata-Pendias (1999) reported the presence of calcium can limit the uptake of cadmium by plants.

The relationship between cadmium concentration in soil solution and properties of soils has been studied. The Langmuir isotherm can be used to describe the relationship between the concentration of cadmium in the solution and the absorbed amount of cadmium, the adsorption increases if the concentration of cadmium in the solution increases. Eriksson (1996) investigated that the solubility of cadmium is influenced by pH, organic matter and clay content of soil. Other factors of soil properties such as cation exchange capacity, forms of metals and concentration of metals are also related to the phytoavailability of cadmium (Sayyad et al., 2009).

3.4.4 Lead (Pb)

The contamination of heavy metals in agricultural soils has increased. Heavy metal contamination can affect both on the productivity of plant and the health of human and animal. The increased of heavy metals concentrations in the agricultural soils can be

resulted naturally or by human activities (such as application of manure and fertilizers, sewage sludge, mining and smelting, battery manufacturing etc). With the rapid urbanization and industrialization, lead becomes one of major environmental contaminants and of challenging issues (Watanabe, 1997).

In the past, lead was also used in the petrol, paint or in water pipe making. Since 1970, the controlling measures on the concentration of lead presenting in the petrol, paint, water pipe and food cans had been informed. From the combustion of petrol containing lead or from coal burning and smelting, lead can be released to the atmosphere. Lead can present both in organic forms or inorganic forms but inorganic forms are more common. Lead is often presented in the forms of lead- sulfide, lead- nitrate, and lead- acetate. These forms are readily available for plant absorbing (Lopez et al., 2009).

Plants are absorbed lead through water, air or soil. Lead tends to stay in the top layer of agricultural soils than other layers. Ryan (2004) reported the potential risk from lead exposure in the ecosystem because lead can remain in the near surface of soil. Lead is also present in the components of lead batteries, rubber or some metal products. Mostly lead is orally taken to the human body. Besides, the lead from the plant roots or leaves comes to the food; it can also come from the food storage such as food containers or from food processing.

The impacts of metals to human health have been reported on many documents. Copper (Cu), manganese (Mn) or zinc (Zn) are harmful when the ingestion rates of such elements are too high while other trace elements such as lead (Pb) and cadmium (Cd) are toxic when their intakes are excessive.

Lead is a physiological and neurological toxin. Lead is primarily accumulated in the skeleton of human body. It can remain in the bones from 10 to 30 year. When entering the human body, the central nervous system is the main target of lead contamination. It also affects in other organs of human (such as kidney) and some biochemical processes (Tong et al., 2000). The serious health effect when absorbing high concentration of lead is the neurological impairment and hypertension. If the woman is pregnant, the problem will be more serious. It can damage to the fetus or cause the abortion.

Lead is not an essential for plants. However, if lead is present in the environment such as in the polluted area, plant can absorb. The largest amount of lead is accumulated in

the roots of plants. Rantalainen (2006) reported the translocation of lead from roots to shoots is relatively poor. Because the plant can absorb and retain the high concentration of lead than animals, the concentration of lead in plant foods is higher than the concentration of lead in animal foods. Species of plants and concentration or types of salts are the main factors affecting on lead.

Several researches on the effects of lead on plants have been reported (Sharma, 2005; Seregin, 2008). When the concentration of lead is high, it can reduce the development of root hairs and significantly affects to the plant growth (Iqbal and Shazia, 2004; Lin et al., 2007). Other symptom of lead toxicity is the impairment of plant metabolism. However, the transportation of lead from soil to roots of plants is quite small amounts comparing to other transportation ways.

Eun et al. (2002) investigated the exposure of lead can decrease the amount of canxi, zinc and iron in the root tips. Lead can have effect on the CO_2 assimilation, the mineral nutrition, chlorophyll and carotenoid contents (Lamhamdi et al., 2011). Other effects on lead toxicity are alternation in structure, physiology of plant cells and protein denaturation (Akinci et al., 2010). When the concentration of lead is increased, the synthesis of protein and nucleic acids are decreased. It also reduces the germination of seedlings. Root elongation, transpiration and photosynthesis are also being influenced if the concentration of lead is high (Pinero et al., 2002; Kaznina et al., 2005). As a result, it can change the mitotic activity and the transcriptional process in the plants.

3.5 Selenium (Se)

Selenium (Se) occurs in two distinctly different forms -inorganic and organic (Whanger 2002). Inorganic forms -selenites (IV) and selenates (VI) occur only in soils. These forms are assimilated by plants and converted to L-selenomethionine (Pyrzyńska 2009). While selenate is taken up in plant roots by sulphate transporters (Sors et al. 2005), selenite is believed to be taken up into plants passively and/or by phosphate transporters (Li et al. 2008). Li et al. (2008) also found that selenate is the major species in neutral to alkaline soils and selenite is the major inorganic species in acidic to neutral ones. Selenite is

less bioavailable than selenate in soils because iron oxides and/ or hydroxides strongly absorbed selenite.

Se has a crucial antioxidant role as part of the enzyme glutathione peroxidase also known as selenoproteins, which are a family of antioxidant enzymes that speed the reaction between glutathione and toxic free radicals. Because the organic forms of selenium act as antioxidants, these help to prevent DNA damage and heavy metal toxicity. Thereby, selenium organic forms can prevent cancer and degenerative diseases (Finley et al. 2001). Under oxidative stress-related conditions, a low dietary selenium intake leads to immune dysfunctions, senility, and the development of Alzheimer's diseases (Fordyce 2013). According to World Health Organization (1996), the narrowest range of selenium between dietary deficiencies is lesser than 40 g/day and toxicity is greater than 400 g/day.

Plants are readily taken up selenium in the form of selenite, selenate (Hawrylak-Nowak 2013). Excessive selenium in plants can induce pathological effects, like chlorosis, stunted root growth, reduced photosynthetic efficiency and biomass (Van Hoewyk 2013).

In soils, there are frequently low amounts of available selenium; hence wheat is an important dietary source for this element (Rayman 2002). In wheat grain, the concentration of selenium is highly variable. The crustal abundance of Se is 0.050 mg kg^{-1}. Selenium-rich soils or crop produced selenium are the main sources to provide selenium. In addition, the genetic breeding of new varieties which can accumulate more selenium in grain is also other source (Ducsay et al. 2007). The direct source of selenium to crops is probable atmospheric deposition of selenium on crops. To enrich the Se status of plants, foliar application of selenite or selenate is a good way. In low Se- soil, soil applications of commercial fertilizer which are enriched with Se are a safe method. In the research of Curtin et al. (2006), foliar application of Se was found more effective than soil fertilization in increasing growth and yield of wheat plant. Se concentration in wheat varies between regions and wheat species (Zhu et al. 2009). Among field crops, wheat is the most important accumulator of Se (De Temmerman et al. 2014).

4 MATERIALS AND METHODS

4.1 Plant materials and conditions of cultivation

4.1.1 Plant materials

The study was carried out in 2014-2017 at the Czech University of Life Science 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. The experimental site Uhříněves lies in Central Bohemia, with the elevation of 295 m a.s.l., average annual temperature of 8.4°C and average annual precipitation of 575 mm. The soil is a clay-loam brown soil with good reserve of all essential nutrients and with the depth of arable land of 25–30 cm. The experimental site, Valečov, lies at 49°38'40" N, 14°30'25" E and 461 m a.s.l. near Havlíčkův Brod town in the Bohemo-Moravian highland, Czech Republic. The soil type is deep Stagnosol (IUSS Working Group WRB 2007) on weathered paragneiss. The topsoil, about 25 to 30 cm thick, is quite fertile, due to a long history of previous intensive cultivation. The subsoil is acid, dense and less favourable to root growth. The soil is fairly heterogeneous due to heterogeneity of the parent rock (Table 13, 14).

After harvest, potatoes were cleaned mechanically and inspected for mechanical, physiological damage as well as diseases. Standard practices in growing techniques were used. Detailed information of characteristics of potato cultivars (origin, maturity, skin and flesh colours and shapes of tubers) can be found in Table 2.

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. Their major characteristics were

described in the Table 3 below. Total 15 samples of *Triticum* species were investigated (Table 3) 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). Their major characteristics are described in Table 4. 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.

4.1.2 Plant sampling

After harvest, mechanically and physiologically undamaged healthy tubes which weighed from 20 to 80 g were used. The tubers were randomly chosen from the field sample and then later were reduced in laboratory to the subsample of tubers. The plant materials were stored for 5 months in a forced- air cooling storage room in the dark at 85-90% relative humidity and 4 °C.

4.1.3 Sample preparation for cooking experiments

The potato tubers were subjected to culinary treatments including baking or boiling and also comparing to the analyzed raw. Firstly, tubers were washed with water and then paper dried and left unpeeled and quartered. One quarter was removed to constitute for raw analyzed batch, one quarter for boiled and one for baked batch in each tuber. The remaining quarter was discarded.

- The raw ones were put in plastic bag. They were kept in frozen at -22 ^{0}C
- The boiled batches were put in boiling water in 20 minutes. After that, they were put out, left for cooling and put in plastic bag. They were kept in frozen at -22 ^{0}C
- The baked batches were placed on baking sheet. They were baked in a forced-air oven Venticell 111 (BMT, Medical Technology, Ltd., Brno, Czech Republic) at 180 ^{0}C for 45 minutes. After that, they were put out, left for cooling and put in plastic bag. They were kept in frozen at -22 ^{0}C

All the frozen batches were freeze-dried (Lyovac GT2, Steris, Hurth, Germany) in the dark. They were ground on a laboratory mill HR 2185 (Philips, Amsterdam, Netherlands) and homogenized. In the end, they were analyzed.

Table 2. Characteristics of potato cultivars

Cultivar	Origin of tubers	Maturity	Skin colour	Flesh colour	Shape of tubers
Agria	Holland	medium-early to medium-late	yellow	yellow	oval
Russet Burbank	Czech (Gene Bank)	late	yellow	white	long
Valy	Czech	early	yellow	pale-yellow	oval
Salome	Germany	very early	yellow	pale-yellow	round to oval
Bohemia	Czech	early	yellow	yellow	oval
Axa	Czech	early	yellow with red spots	yellow	oval
Jelly	Germany	medium-late	yellow	yellow	oval
Ditta	Austria	medium-early	yellow	yellow	long-oval
Bionta	Austria	medium-late	yellow	dark-yellow	round to oval
Keřkovský rohlíček	Czech	medium-early	yellow	dark-yellow	long
Rosara	Germany	very early	red	yellow	oval
Dali	Holland	early	yellow	pale-yellow	oval
Mayan Gold (*Solanum phureja*)	Germany	late	yellow	deep yellow	long
Valfi	Czech	medium-early to medium-late	purple	purple partially coloured	round to oval
Violette	Germany	medium-early	purple	purple	long
Blaue Anneliese	Germany	medium-early	purple	purple	oval
Rosemarie	Germany	medium-early	red	red	long oval
Vitelotte	France	late	purple	deep purple with bright spots	long oval
Königspurpur	Germany	medium-early	red	red	round to oval
Highland Burgundy Red	Germany	medium-early	red	red with white borders	long
Herbie 26	Czech (Gene Bank)	early to medium early	red	red	long oval
Red Emmalie	Germany	early to medium-early	red	red	long

Table 3. Characteristics of analyzed wheat sample ([1] spring wheat, [2] einkorn wheat, [3] emmer wheat); ECN–identification number of gene bank, BCHAR–taxonomical code–botanical characteristics

Wheat sample	*Sample No.*	*ECN*	*BCHAR*	*Name of the variety*
1	2353	01C0204877	635090	SW Kadrilj [1]
2	2354	01C0204799	635001	Granny [1]
3	2355	01C0200100	635090	Jara [1]
4	2356	01C0203840	635104	Kaerntner Frueher [1]
5	2357	01C0200043	635090	Postoloprstska presivka 6 [1]
6	2358	01C0201503	242008	Escana [2]
7	2359	01C0204053	635019	Schwedisches Einkorn [2]
8	2360	01C0204039	242007	*T. monococcum* 2101 [2]
9	2361	01C0204040	242007	*T. monococcum* 2102 [2]
10	2362	01C0204044	242019	*T. monococcum* 2103 [2]
11	2363	01C0200948	412048	Rudico [3]
12	2364	01C0203989	412013	Kahler Emmer [3]
13	2365	01C0201282	412048	*T. dicoccon* (Tapioszele) [3]
14	2366	01C0200117	412013	Krajova-Horny Tisovnik (Malov) [3]
15	2367	01C0204501	412013	*T. dicoccon* No 8909 [3]

Table 4. Analyzed grain-colored wheat and tritordeum samples and their characteristics.

Field No. 2016	Official name	Grain colour	Origin	State of origin	Variety status
Wheat (*Triticum aestivum* L.), winter forms					
V2 3-16		Ba	BAUB 2786.2 × Skorpion	CZE	breeding line
V2 9-16	KM 53-14*)	Ba	Skorpion × Ludwig	CZE	breeding line
V2 10-16		Ba	Skorpion × Magister	CZE	breeding line
V2 13-16	Skorpion**)	Ba	Line 5 × Versailles**)	CZE	released variety
V2 14-16		Ba	KM 824-1-01 × RU 440-5	CZE	breeding line
V2 15-16		Ye	Citrus × Bona Dea	CZE	breeding line
V2 16-16	Bona Vita	Ye	(SO-690 × Arida) × Arida	SVK	released variety
V2 17-16	Citrus	Ye	(Sunnan × Monopol) × Stamm GI 912	GER	released variety
V2 18-16		Pp	Purple grain line from Slovakia × Akteur	CZE	breeding line
V2 22-16	KM 178-14*)	Pp	Meritto × ANK-28A	CZE	breeding line
V2 28-16	PS Karkulka	Pp	ANK-28A × PS 11	SVK	released variety
V2 31-16		Pp	(Indigo × Akteur) × (Skorpion × Bohemia)	CZE	breeding line
V2 32-16		Pp	Blaucorn × Zappa	CZE	breeding line
V2 33-16		Pp	Purple grain line from Slovakia × Akteur	CZE	breeding line
SU 5-16	Bohemia	R	(540i × U6192) × (540i × Kontrast)	CZE	released variety
V1 47-16		Ba	(Skorpion × V1-702) × (Citrus × Bona Dea)	CZE	breeding line
V1 48-16		Ba	Skorpion × UC 66049	CZE	breeding line
V1 50-16		Pp	Indigo × Mironovskaya 808	CZE	breeding line
Tritordeum (× *Tritordeum martinii* A. Pujadas nothosp. nov.), spring forms					
1m2-81-16	HT 439*)	Ye	*Triticum turgidum* x *Hordeum chilense*	ESP	breeding line
1m2-88-16	JB 1*)	Ye	*Triticum turgidum* x *Hordeum chilense*	ESP	released variety
1m2-89-16	JB 3*)	Ye	*Triticum turgidum* x *Hordeum chilense*	ESP	released variety

Ba-blue aleurone; Pp-purple pericarp; Ye-yellow endosperm; R-standard red grain; *) Breeding line (KM-lines are tested in the Official State Tests in the Czech Republic); **) Origin of „Line 5“: (Barevná5 × Brigand) × </(Brimstone × Židlochovickáosinatka) × Hana/ × Hana>; „Barevná 5“isa donor of blue aleurone from heritage of Erich von Tschermak-Seysenegg. Lines without official name are breeding lines or genetic resources. **) Variety Skorpion was developed in the Czech Republic and in 2011 registered in Austria. Countries of origin: CZE – Czech Republic; ESP – Spain; GER – Germany; SVK – Slovak Republic

4.2 Chemical and laboratory materials and equipments

The laboratory tools and chemicals during the experiments were listed below:

Laboratory tools:

- Advanced Mercury Analyzer AMA 254 (Altec, CZ)
- Teflon digestion vessel DAP-60S spectrometer Varian SpectrA 280Z with graphite atomiser and programmable sample dispenser Varian 120
- Laboratory balance
- Laboratory spoon
- Silica beaker
- Laboratory mill
- Cuvettes
- Centrifugal machine

Chemicals and reagents:

- MWS-3 ± Berghof Products
- Nitric acid 65%, p.a. ISO (Merck)
- H_2O_2 30%, TraceSelect (Fluka)
- 1.5% HNO_3
- Wash-bottle with distilled water

4.3 Methods of chemical analyses

4.3.1 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.

4.3.2 Determination of cadmium, lead and zinc

The concentrations of lead (Pb) and cadmium (Cd) in plant species have been determined by using the Atomic Absorption Spectrometry.

Preparation of samples: Firstly, the samples of grain were finely ground and digested in acid solution using MWS-3 ± Berghof Products ± Instruments, Germany. 350 mg of the samples were weighed. After that, they were put into the Teflon digestion vessel DAP-60S and adding 2 ml of nitric acid 65%, p.a. ISO (Merck) and 3ml H_2O_2 30%, TraceSelect (Fluka). The mixtures were shaken carefully.

After one hour closing the vessel and heating in the microwave oven, the decomposition proceeded for 1 hour in the temperature from 100 to 190 °C. The digest obtained was transferred into the 50ml silica beaker. The wet residue was obtained after evaporation and dissolved in 1.5% HNO_3. The dissolving was accelerated by sonication. Then digests were transferred to probes and adjusted with 1.5% HNO_3 to 12 ml.

Measurement of cadmium and lead concentrations in the digests: 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. Detailed parameters of the measurement are given in the Table 5.

Table 5. The parameters of measurement of Cd and Pb in plant species using Varian AA 280Z spectrometer.

Parameters	Cadmium	Lead
Calibration	standard addition method	standard addition method
Wavelength (nm)	228.8 (0.5)	283.3 (0.5)
Background correction	Zeeman	Zeeman
Evaluation	peak area	peak area

Modifier	$(NH_4)_2HPO_4$	$(NH_4)_2HPO_4$
Pyrolysis temperature	650 °C	850 °C
Atomization temperature	2150 °C	2400 °C
Bulk concentration	3 µg l^{-1}	30 µg l^{-1}
Sample volume on platform	30 µl	30 µl

Measurement of Zn concentration in the digests: 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.

All samples of plant species were analyzed in three replicates. 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).

All data obtained were in the confidence intervals given by CRM producer. 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 were corrected by mean concentration of analytes in blanks, and compared with detection limits (mean ± 3SD of blanks) which were 0.07 ng ml^{-1} for cadmium, and 0.21 ng ml^{-1} for lead.

4.3.3 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). Four hundred mg of the sample was weighted into the Teflon digestion vessel DAP-60S and 2 ml of nitric acid 65% Suprapur®, p.a. ISO (EMD Millipore Merck, KGaA, Darmstadt, Germany) and 3ml H_2O_2 30%, TraceSELECT® Ultra (Sigma-Aldrich, Pty. Ltd., NSW Australia) were added. The mixture was shaken carefully and vessel was after half hour waiting closed and heated in the microwave oven. The decomposition proceeded within 1 hour in the temperature range 100 -190 °C.

The digest obtained was transferred into the 50ml silica beaker and evaporated to wet residue, then diluted with minimum of 10% hydrochloric acid prepared from HCl 37%, p.a. ± (Analytika, Co., Ltd., Prague, CR) and deionised water (Barnstead, Dubuque, Iowa). Formicacid 98%, puriss. p. a (Sigma-Aldrich, St. Louis, MO) in the volume of 1 ml was added for the reduction of nitrogen oxides from reaction mixture. To reduce all selenium compounds in the digest to Se^{4+} 5 ml of hydrochloric acid diluted with deionised water 1:1 (V/V) was added and the solution was heated at 90 °C for half of hour. Then digests were transferred to probes and adjusted with 10% HCl to 10 ml.

The concentration of selenium in the digests of cereals were measured by HGAAS technique using Varian AA 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. Samples of the cereals was analysed in three replicates.

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}).

4.3.4 Hydroponical experiments

Plants were grown hydroponically in culture vessels of 550 ml volume in the Research Institute of Plant Crops Prague-Ruzyně. For the experiment, different potato cultivars were used. Plants were replanted into culture containers, and the roots were immersed in the nutrient solution. Plants were grown in air- conditioned rooms with artificial lighting. Plant roots were immersed in the Knop- nutrient solution. As the light sources pressure sodium lamps, light bulbs and energy saving lamps were used. Exposure time periods of light and darkness were set as 14/10 hours and temperature valued 22/16 ^{0}C. Irradiance during cultivation was averaged 400 μmol m^2s^{-1}. Humidity of air was adjusted using a special mist maker and a ventilator supplying the same

moisture condition throughout the growth chamber. These materials were consequently used for spectroscopic measurements.

Protoplasts can be obtained from all types of actively growing young and healthy tissues. The most convenient and widely used source of plant protoplasts is the leaf. Juvenile seedling tissues, cotyledons are other alternative tissues most frequently used for protoplasts isolation. Protoplasts can be isolated by two methods, mechanical and enzymatic. The enzyme mixture solution of cellulose/ macerozyme was used to digest the cell wall.

4.3.5 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.

5 RESULTS

5.1 Levels of mercury (Hg), cadmium (Cd), lead (Pb) and zinc (Zn) in the analyzed grain wheat species (mg kg^{-1} dry matter)

The above procedures were employed to describe the 15 samples of *Triticum* species. The 15 samples of *Triticum* species belonged to three groups: spring wheat, emmer wheat and einkorn wheat. The amount of cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) was obtained from the average of three determinations.

The amount of mercury was obtained from the Advanced Mercury Analyzer AMA 254, while the amount of other heavy metals (lead, cadmium and zinc) was obtained from the Atomic Absorption Spectrometry (AAS). Cadmium and lead were determined using the electrothermal atomization (ET-AAS).

In the experiments, the wheat varieties belong to three main groups, each group includes 5 varieties. Spring wheat contains SW Kadrilj, Granny, Jara, Kaerntner Frueher and Postoloprtská přesívka. The second group is einkorn wheat with Escana, Schwedisches Einkorn, and *T. monococcum* 2101**,** *T. monococcum* 2102, and *T. monococcum* 2103. Rudico, Kahler Emmer, *T. dicoccon* (Tapioszele), Krajova-Horny Tisovnik (Malov) and *T. dicoccon* No. 8909 belong to the emmer wheat.

Table 6 reports the most significant results of the analysis of the investigated samples. All heavy metal concentration measurements were based on the dry weight basis (mg kg^{-1}). The results were the average of three replicated samples, expressed to one standard deviation. The reliability of our methods was shown by the low standard deviation.

Table 6. Content of mercury (Hg), cadmium (Cd), lead (Pb) and zinc (Zn) in the analyzed grain wheat species (mg kg^{-1} dry matter).

Wheat variety	Hg	Cd	Pb	Zn
SW Kadrilj [1]	0.002 ± 0.000 [d]	0.036 ± 0.003 [bc]	0.278 ± 0.027 [b]	36.98 ± 1.336 [ab]
Granny [1]	0.001 ± 0.000 [ab]	0.034 ± 0.001 [bc]	0.062 ± 0.013 [a]	35.19 ± 2.733 [a]
Jara [1]	0.009 ± 0.001 [e]	0.013 ± 0.001 [a]	0.078 ± 0.002 [a]	48.92 ± 0.601 [cd]
Kaerntner Frueher [1]	0.002 ± 0.000 [abcd]	0.026 ± 0.005 [ab]	0.295 ± 0.175 [b]	46.40 ± 2.273 [bc]
Postoloprstska presivka 6 [1]	0.001 ± 0.000 [abcd]	0.0357 ± 0.0018 [bc]	0.106 ± 0.031 [a]	37.44 ± 1.373 [ab]
Escana [2]	0.001 ± 0.000 [a]	0.054 ± 0.001 [d]	0.048 ± 0.014 [a]	40.24 ± 10.986 [abc]
Schwedisches Einkorn [2]	0.001 ± 0.000 [a]	0.057 ± 0.002 [d]	0.042 ± 0.019 [a]	35.34 ± 0.239 [a]
***T. monococcum* 2101** [2]	0.001 ± 0.000 [ab]	0.058 ± 0.001 [d]	0.046 ± 0.002 [a]	36.69 ± 2.056 [abc]
***T. monococcum* 2102** [2]	0.002 ± 0.000 [abcd]	0.051 ± 0.008 [cd]	0.131 ± 0.098 [ab]	57.00 ± 1.133 [de]
***T. monococcum* 2103** [2]	0.002 ± 0.000 [cd]	0.054 ± 0.002 [d]	0.068 ± 0.026 [a]	35.19 ± 2.733 [g]
Rudico [3]	0.001 ± 0.000 [abc]	0.023 ± 0.002 [ab]	0.075 ± 0.005 [a]	67.41 ± 1.990 [fg]
Kahler Emmer [3]	0.002 ± 0.000 [bcd]	0.033 ± 0.002 [bc]	0.032 ± 0.005 [a]	66.70 ± 0.558 [efg]
***T. dicoccon* (Tapioszele)** [3]	0.001 ± 0.000 [abcd]	0.027 ± 0.005 [ab]	0.066 ± 0.040 [a]	67.12 ± 2.807 [fg]
Krajova-Horny Tisovnik (Malov) [3]	0.001 ± 0.000 [ab]	0.019 ± 0.005 [ab]	0.040 ± 0.023 [a]	61.50 ± 1.280 [ef]
***T. dicoccon* No 8909** [3]	0.001 ± 0.000 [abcd]	0.034 ± 0.007 [bc]	0.127 ± 0.044 [ab]	47.90 ± 1.991 [cd]

[1] spring wheat, [2] einkorn wheat, [3] emmer wheat

Values followed by the same letter in the same column are not significantly different. Different small letters indicate significant differences ($p < 0.05$) among analyzed wheat varieties in the same column.

5.1.1 Content of mercury (Hg) in the analyzed grain wheat species (mg kg^{-1}dry matter)

The concentration of mercury in 15 samples of wheat varieties was shown in the Table 6 and Figure 1. The concentration of mercury in the grain wheat was present in very small quantity. The concentration ranged between 0.001 ± 0.000 mg kg^{-1} dry matter and 0.087 ± 0.001 mg kg^{-1} dry matter. When considering on different varieties, the highest value was characteristic for the Jara variety (0.009 ± 0.001 mg kg^{-1} dry matter). On the other hand, the lowest values were measured in Escana (0.001 ± 0.000 mg kg^{-1} dry matter) and Schwedisches Einkorn (0.001 ± 0.000 mg kg^{-1} dry matter). Among three types of investigated wheat varieties, the spring wheat prevailed absorbing the highest concentration of mercury, which was less presented in the emmer and einkorn wheat. Jara showed the most statistically significant difference between other varieties ($p < 0.05$).

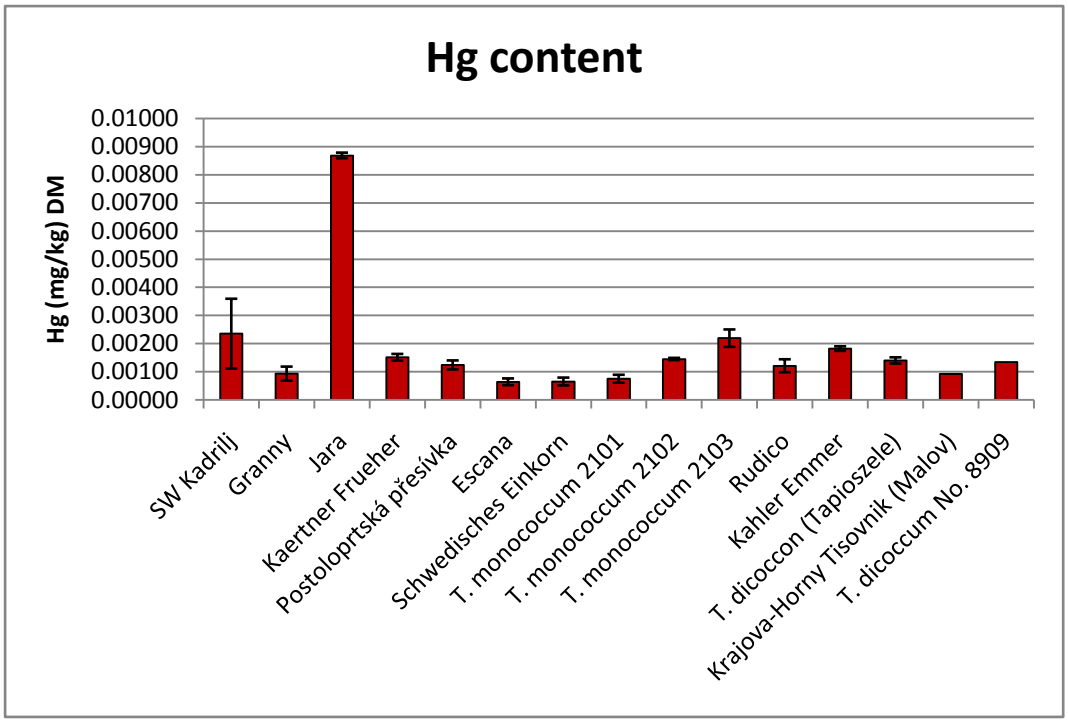

Figure 1. Content of mercury (Hg) in spring, einkorn and emmer wheat species (mg kg^{-1} dry matter)

5.1.2 Content of cadmium (Cd) in the analyzed grain wheat species (mg kg^{-1} dry matter)

The highest cadmium concentration was found for all analyzed einkorn varieties (Table 6, Figure 2). The most distinctive varieties were Jara, Escana, Schwedisches Einkorn, *T. monococcum* 2101 with average absorbed cadmium amounts 0.013 ± 0.001, 0.054 ± 0.001, 0.057 ± 0.002, 0.058 ± 0.001 mg kg^{-1} dry matter, respectively. Spring wheat and emmer varieties had the lower concentration of cadmium than einkorn wheat. The lowest value was determined in the grain of the Jara variety (0.013 ± 0.001 mg kg^{-1} dry matter) and the highest was found in *T. monococcum* 2101 (0.058 ± 0.001 mg kg^{-1} dry matter).

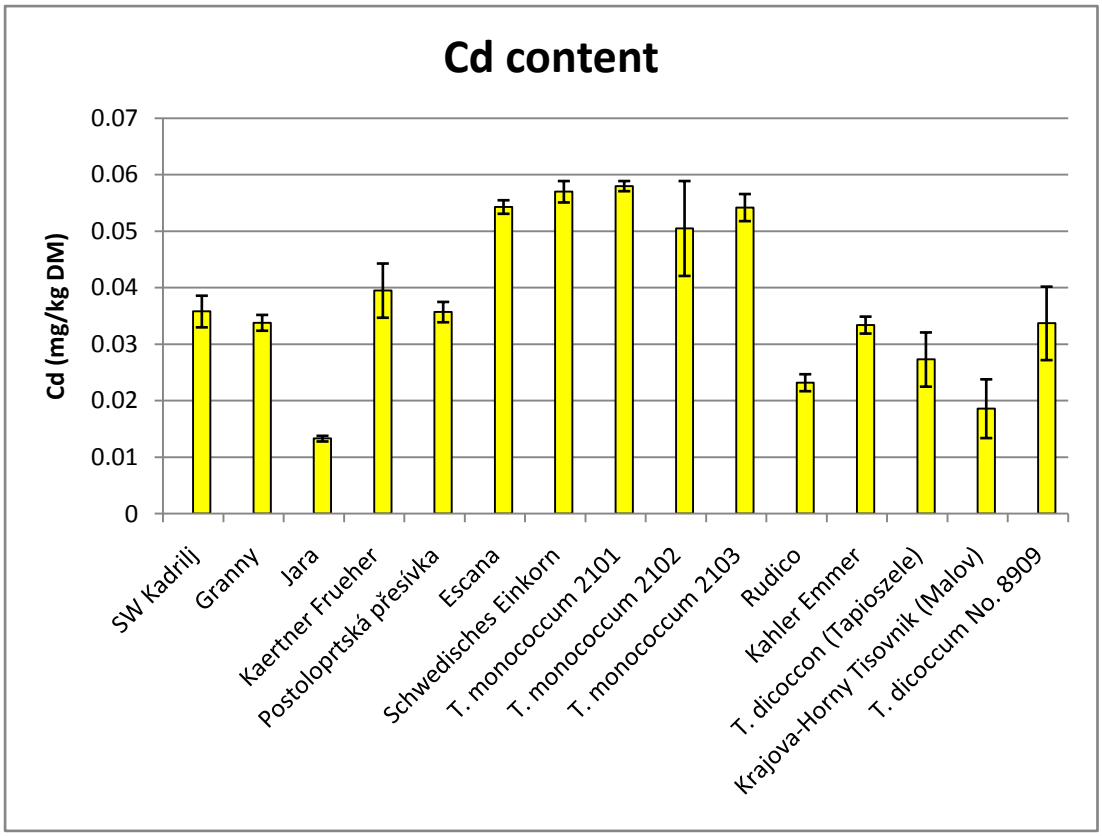

Figure 2. Content of cadmium (Cd) in spring, einkorn and emmer wheat species (mg kg^{-1} dry matter)

5.1.3 Content of lead (Pb) in the analyzed grain wheat species (mg kg^{-1}dry matter)

Generally, the values of lead concentration in grain wheat varieties were low (Table 6, Figure 3). High lead contents were typical for Kaerntner Frueher, SW Kadrilj and *T. dicoccon* No 8909 varieties (0.295 ± 0.175, 0.278 ± 0.027 and 0.127 ± 0.044 mg kg^{-1} dry matter, respectively). Other varieties did not show statistically significant differences in lead content in grain wheat ($p < 0.05$).

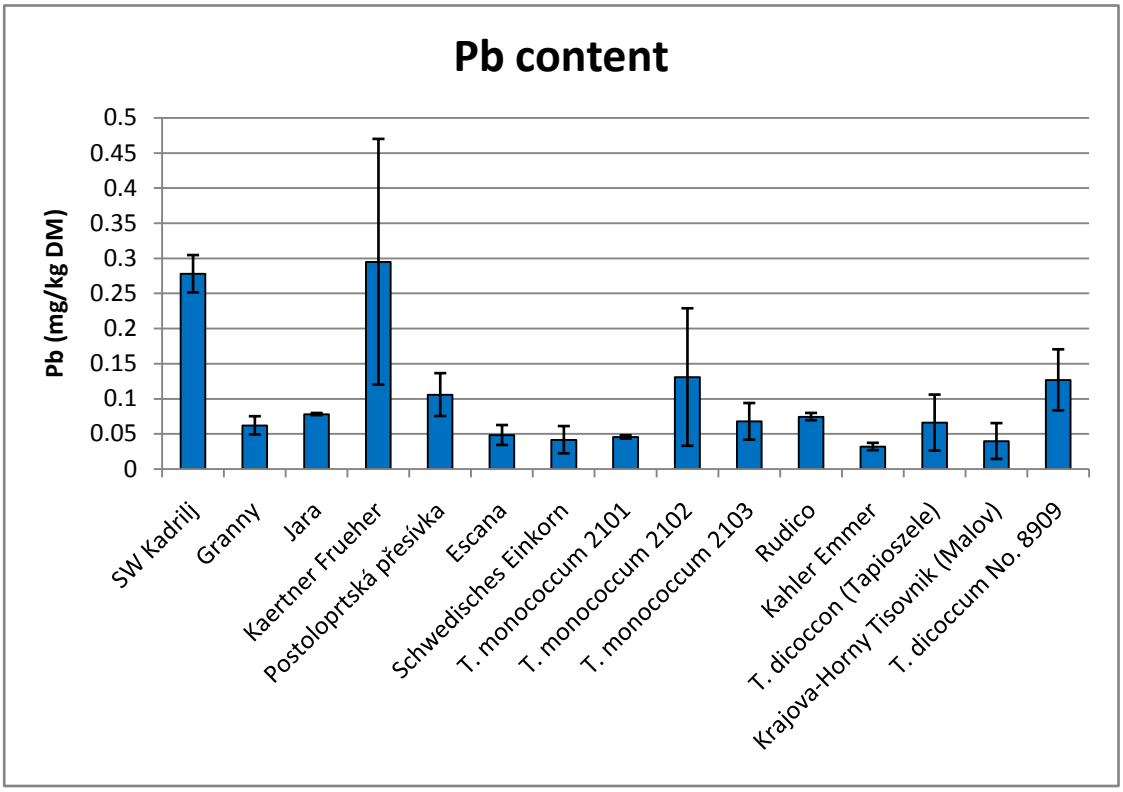

Figure 3. Content of lead (Pb) in spring, einkorn and emmer wheat species (mg kg^{-1} dry matter)

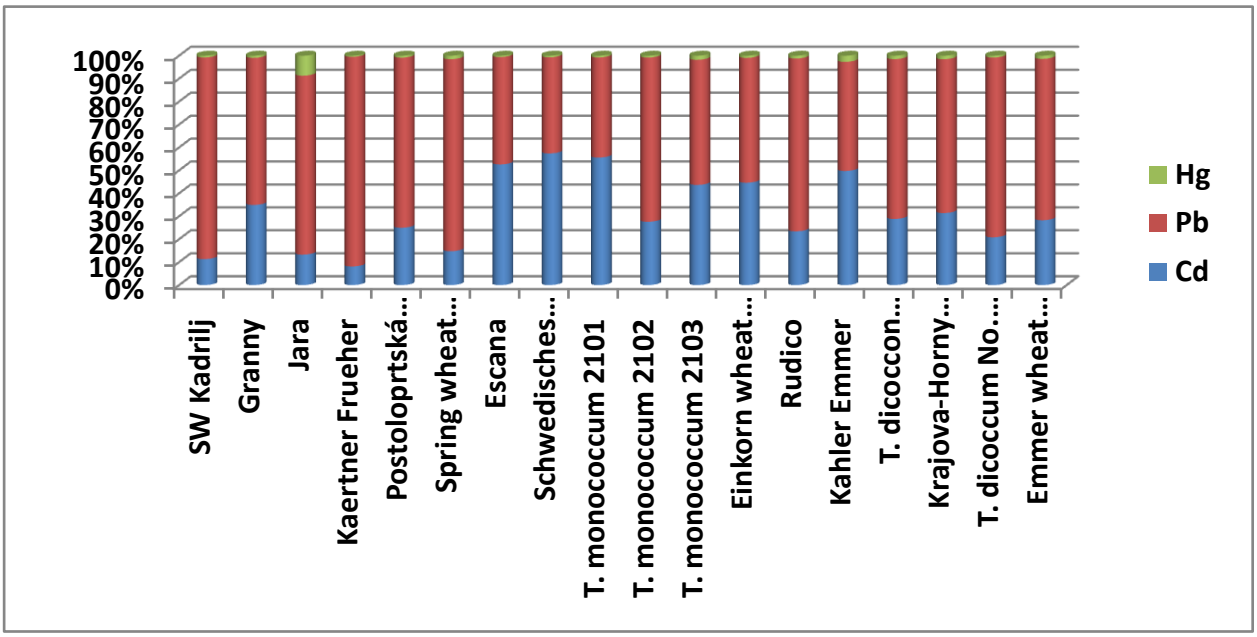

Figure 4. Content of cadmium (Cd), lead (Pb) and mercury (Hg) in spring, einkorn and emmer wheat species (mg kg^{-1} dry matter)

5.1.4 Content of zinc (Zn) in the analyzed grain wheat species (mg kg^{-1}dry matter)

In case of zinc determination, the content in the wheat grain under investigation ranged between 35.19 ± 2.732 and 67.40 ± 1.989 mg kg^{-1} dry matter. The most distinctive species and varieties were Granny (35.19 ± 2.732 mg kg^{-1} dry matter), Schwedisches Einkorn (35.343 ± 0.239 mg kg^{-1} dry matter) and *T. monococcum* 2103 (35.19 ± 2.732 mg kg^{-1} dry matter).

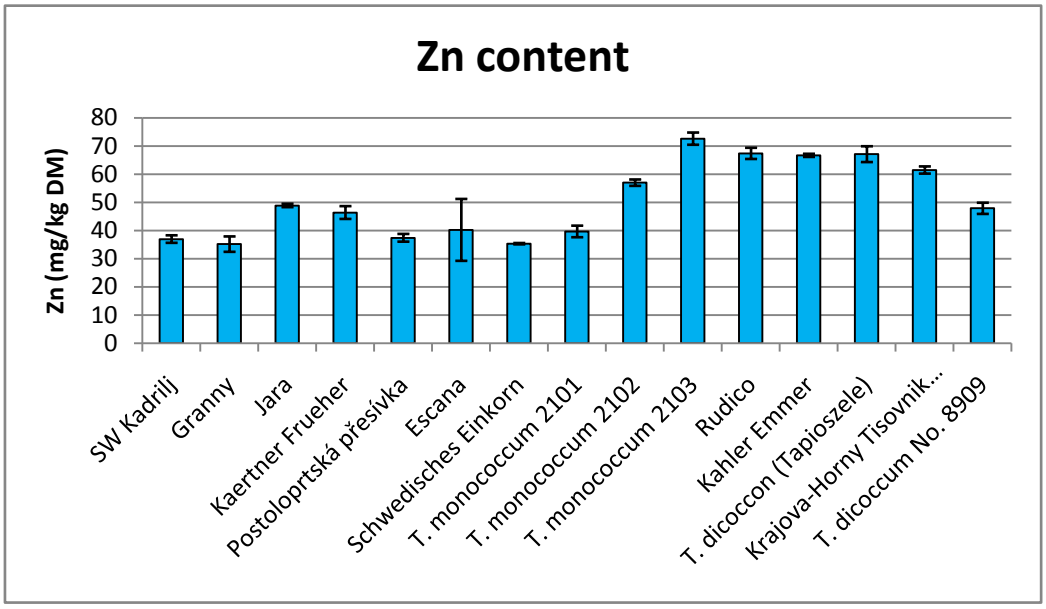

Figure 5. Content of zinc (Zn) in grains of spring, einkorn and emmer wheat species (mg kg^{-1}dry matter)

Comparing between three varieties of investigated wheat, the emmer wheat was rich in zinc content with an average 62.12 mg kg^{-1} dry matter (Figure 5). Among the emmer wheat species, *T. dicoccon* No 8909 distinguished with lower Zn content (47.90 ± 1.991 mg kg^{-1} dry matter). Spring wheat had the lowest average concentration of zinc in the grain (40.99 mg kg^{-1} dry matter).

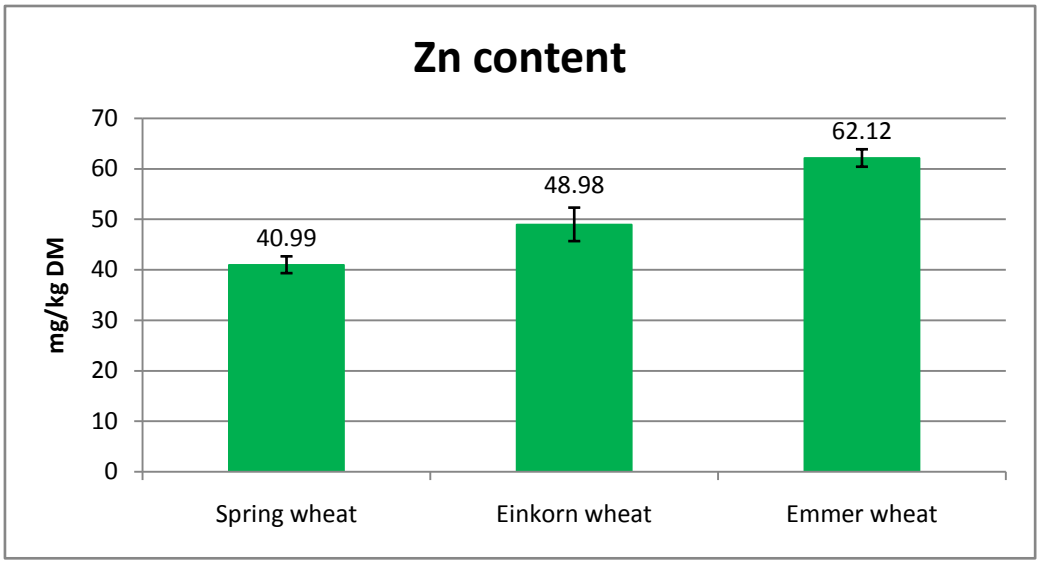

Figure 6. Average zinc (Zn) content in grains of spring, einkorn and emmer wheat species (mg kg^{-1} dry matter)

Moreover, different wheat varieties exercise differently in accumulation of heavy metals (cadmium, lead and mercury) in grains (Figure 7). In present study, spring wheat has shown the maximum accumulation of lead (average 0.164 mg kg^{-1} dry matter), followed by cadmium (average 0.032 mg kg^{-1} dry matter) and mercury (0.003 mg kg^{-1} dry matter). On the other hand, einkorn variety has shown the maximum accumulation of cadmium among 3 wheat species (average 0.055 mg kg^{-1} dry matter).

The average amount of lead in einkorn and emmer wheat was quite small, only 0.067 and 0.068 mg kg^{-1} dry matter in the order. The absorption of mercury in three groups of wheat varieties is the lowest comparing to other investigated heavy metals (less than 0.007 mg kg^{-1} dry matter).

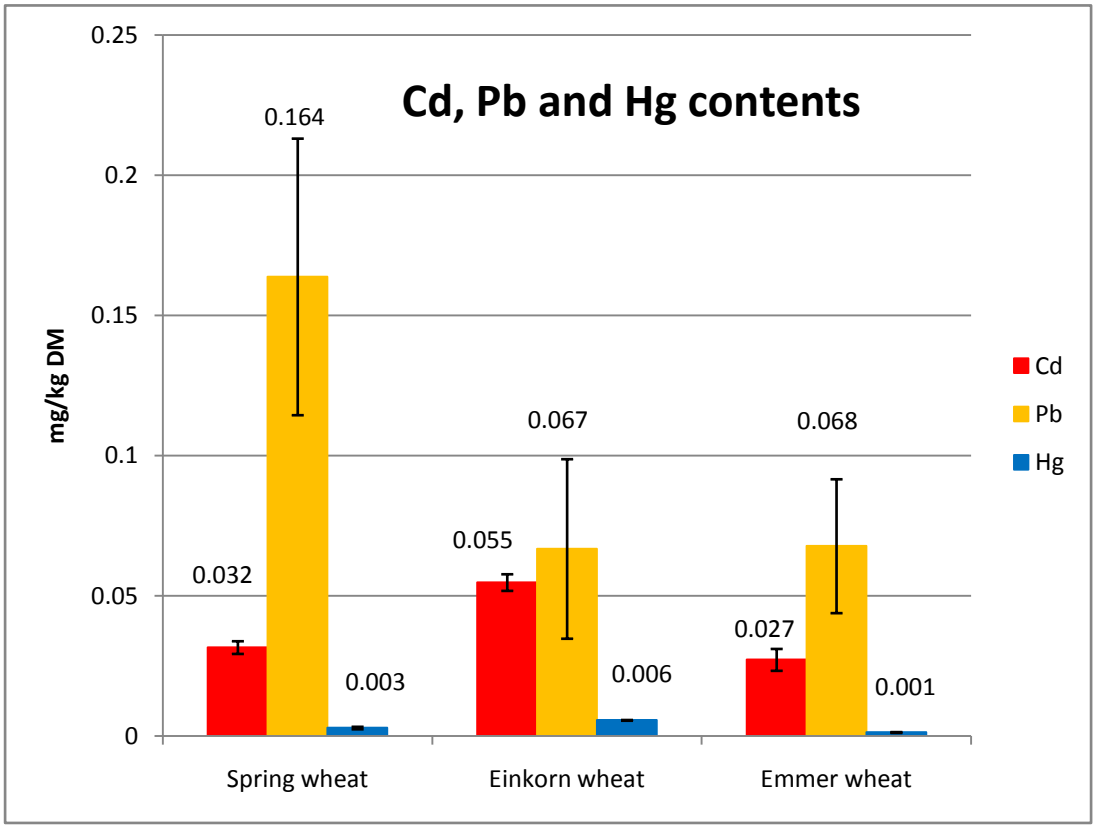

Figure 7. Average cadmium (Cd), lead (Pb) and mercury (Hg) contents in grains of spring, einkorn and emmer wheat species (mg kg^{-1} dry matter)

5.2 Effect of different growth stages (boot growth, stage 11, stage 10.2 and leaf-stage 10.2) on the accumulation of mercury (Hg) (mg kg^{-1} dry matter)

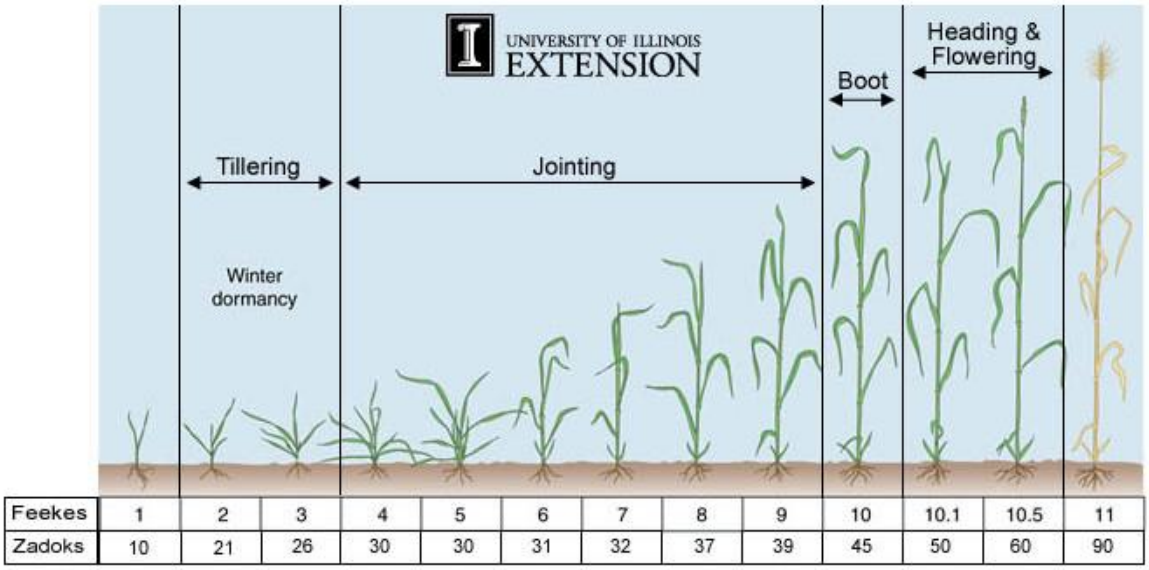

Figure 8. Wheat growth development according to Feekes

(Source: http://weedsoft.unl.edu/documents/growthstagesmodule/wheat/wheat.htm)

Figure 8 shows the stages in the growth and development of wheat according to Feekes. The boot stage is the stage when the heads are developed. They can be seed in the swollen part of the sheath of the flag leaf. The stage 10.2 is the stage when the awns are visible. In the 10.2 stage, through the slit of sheath of flag leaf, the heads are emerging about 50 %. Stage 11 is the stage when the plant has the physiological maturity. At this stage, the kernel is ripened and the grain is dried.

Table 7. Effect of different growth stages (boot growth, stage 11, stage 10.2 and leaf stage 10.2) on the accumulation of mercury (mg kg^{-1}dry matter).

Stage of growth	Hg (mg kg^{-1} dry matter)
Boot growth stage	0.003 [a]
Stage 11	0.003 [a]
Stage 10.2	0.015 [b]
Leaf-stage 10.2	0.021 [c]

The content of mercury in 4 growth stages of investigated wheat was described in the Table 7. The maximum mercury content was of 0.021 mg kg^{-1} dry matter for the leaf-stage 10.2 and of 0.015 mg kg^{-1} dry matter in the stage 10.2, respectively, while in the boot growth stage, it was lowest (0.003 mg kg^{-1} dry matter). Experimental plants of the stage 10.2 and leaf-stage 10.2 significantly differ with the plants in the boot and stage 11.

5.3 Content of mercury (Hg) in the analyzed grain wheat species in different wheat growth stages (mg kg^{-1}dry matter)

From the study it is apparent that the investigated wheat plant absorbed a wide range of mercury (Hg) in different growth stages, in different concentrations. The mercury concentration was measured based on dry weight basis (mg kg^{-1} dry matter) and expressed to one standard deviation. All varieties showed statistically differences ($p < 0.05$) (Table 8).

The higher mercury content was found in Schwedisches Einkorn in boot growth stage and leaf-stage 10.2, with the concentration 0.021 ± 0.002 and 0.028 ± 0.007 mg kg^{-1} dry matter, respectively, in comparison with the stage 11 when the lowest mercury level was recorded (0.002 ± 0.000 mg kg^{-1} dry matter). The varieties of spring wheat (SW Kadrilj, Granny, Jara, and Kaerntner Frueher) did not differ in concentration of mercury.

In the boot growth stage, the content of mercury in Schwedisches Einkorn was 2 times higher than in *T. monococcum* 2103. In the stage 11, the concentration of mercury in analyzed wheat varied widely, in range from 0.002 ± 0.000 mg kg^{-1} dry matter to 0.004 ± 0.001 mg kg^{-1} dry matter. The mercury content reached the maximum level in *T. monococcum* 2103**.**

Likewise, in the stage 10.2, in the case of Jara and Granny varieties, the levels of mercury are almost the same and they are situated around 0.005 mg kg^{-1} dry matter. In contrast, *T. monococcum* 2101 had lower concentration of mercury, only 0.002 ± 0.000 mg kg^{-1} dry matter.

Table 8. Content of mercury (Hg) in the analyzed wheat species (in the boot growth stage, stage 11, stage 10.2 and leaf-stage 10.2 according to Feekes scale (mg kg^{-1} dry matter).

WHEAT VARIETY	BOOT GROWTH STAGE	STAGE 11	STAGE 10.2	LEAF-STAGE 10.2
SW KADRILJ [1]	0.019 ± 0.003 [b]	0.002 ± 0.000 [a]	0.002 ± 0.000 [ab]	0.0163 ± 0.0003 [a]
GRANNY [1]	0.014 ± 0.000 [a]	0.003 ± 0.000 [abc]	0.005 ± 0.000 [c]	0.0160 ± 0.0002 [a]
JARA [1]	0.020 ± 0.001 [b]	0.002 ± 0.000 [ab]	0.005 ± 0.000 [c]	0.0246 ± 0.0002 [bc]
KAERNTNER FRUEHER [1]	0.014 ± 0.000 [a]	0.003 ± 0.001 [abcd]	0.003 ± 0.000 [ab]	0.0210 ± 0.0007 [ab]
SCHWEDISCHES EINKORN [2]	0.021 ± 0.002 [b]	0.002 ± 0.000 [a]	0.003 ± 0.001 [a]	0.0280 ± 0.0068 [c]
***T. MONOCOCCUM* 2101** [2]	0.014 ± 0.000 [a]	0.002 ± 0.000 [ab]	0.002 ± 0.000 [b]	0.0265 ± 0.0003 [bc]
***T. MONOCOCCUM* 2102** [2]	0.011 ± 0.000 [ac]	0.004 ± 0.000 [cd]	0.003 ± 0.000 [a]	0.0159 ± 0.0005 [a]
***T. MONOCOCCUM* 2103** [2]	0.010 ± 0.000 [c]	0.004 ± 0.001 [d]	0.003 ± 0.001 [ab]	0.0217 ± 0.0007 [abc]
KRAJOVA-HORNY TISOVNIK (MALOV) [3]	0.015 ± 0.001 [a]	0.004 ± 0.000 [bcd]	0.002 ± 0.000 [ab]	0.0229 ± 0.0001 [bc]

[1] spring wheat, [2] einkorn wheat, [3] emmer wheat

Values followed by the same letter in the same column are not significantly different. Different small letters indicate significant differences ($P < 0.05$) among analyzed wheat varieties in the same column

5.4 Contents of selenium in color-grain

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Se concentration measurements were based on the dry mater basis (mg kg-^{1} dry matter). The results were the average of three replicated samples, expressed to one standard deviation. The reliability of our methods was shown by the low standard deviation.

Large variations were observed in investigated grain Se concentrations among investigated wheat and tritordeum species (Table 9). The grain Se concentrations ranged from 0.022 to 0.235 mg kg^{-1}dry matter, with an average of 0.067 mg kg^{-1} dry matter. **The variety with the highest grain Se concentrations was control** red-grained wheat variety Bohemia (0.235 mg kg^{-1} dry matter) and yellow-grained variety Bona Vita (0.229 mg kg^{-1} dry matter).

Average Se content in wheat varieties with blue aleurone was 0.057 mg kg^{-1} dry matter, with purple pericarp 0.042 mg kg^{-1} dry matter, and with yellow pericarp 0.069 mg kg^{-1} dry matter (except cv. Bona Vita with high Se content). Se content in blue-grained wheats ranged between 0.042-0.083 mg kg^{-1} dry matter, purple-grained wheats 0.022-0.053 mg kg^{-1} dry matter and yellow-grained wheats 0.058-0.079 mg kg^{-1} dry matter, respectively. According to the study of Lachman et al. (2011) comparable data were also determined in einkorn (0.050-0.055 mg kg^{-1} dry matter), emmer wheat (0.059-0.065 mg kg^{-1} dry matter) and spring wheat (0.030-0.068 mg kg^{-1} dry matter). 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 and between variety Bohemia and breeding line KM 178-14.

Table 9. Total content of selenium in wheat and tritordeum grain (mg Se kg^{-1} DM ± SD) and selenium yield in grain (g ha^{-1}).

Cereal type	Field Nos. 2016	Official name	Grain colour	Total Se content mg kg^{-1} DM ± SD)	Yield (t ha^{-1})	Se in grain (g ha^{-1})
Winter wheat	V2 3-16		blue aleurone	0.049±0.001bc	9.92	0.486
	V2 9-16	KM 53-14	blue aleurone	0.073±0.021de	10.93	0.798
	V2 10-16		blue aleurone	0.083±0.011e	7.97	0.661
	V2 13-16	Skorpion	blue aleurone	0.042±0.002bc	9.99	0.419
	V2 14-16		blue aleurone	0.047±0.007bc	7.21	0.339
	V2 15-16		yellow endosperm	0.079±0.004e	10.31	0.815
	V2 16-16	Bona Vita	yellow endosperm	0.229±0.042f	9.27	2.123
	V2 17-16	Citrus	yellow endosperm	0.058±0.006cd	9.66	0.560
	V2 18-16		purple pericarp	0.045±0.005bc	9.02	0.406
	V2 22-16	KM 178-14	purple pericarp	0.032±0.003ab	11.49	0.368
	V2 28-16	PS Karkulka	purple pericarp	0.050±0.001bc	9.75	0.488
	V2 31-16		purple pericarp	0.022±0.000a	9.60	0.211
	V232-16		purple pericarp	0.052±0.002cd	8.56	0.445
	V2 33-16		purple pericarp	0.038±0.003abc	9.27	0.352
	SU 5-16	Bohemia	standard red grain	0.235±0.021f	10.87	2.224
	V1 47-16		blue aleurone	0.055±0.002cd	8.58	0.472
	V1 48-16		blue aleurone	0.048±0.002bc	6.76	0.324
	V1 50-16		purple pericarp	0.053±0.007cd	10.68	0.566
Spring tritordeum	1m2-81-16	HT 439	yellow endosperm	0.037±0.001abc	2.26	0.084
	1m2-88-16	JB 1	yellow endosperm	0.041±0.002abc	2.03	0.083
	1m2-89-16	JB 3	yellow endosperm	0.040±0.005abc	2.38	0.095

Different letters in the Se concentration column indicate significant difference ($P \leq 0.05$).

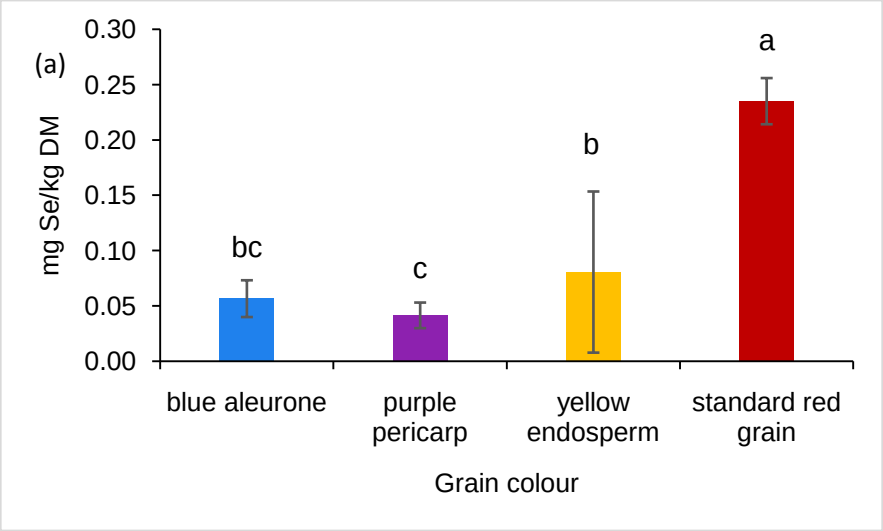

Figure 9. Effect of cereal grain colour on selenium content

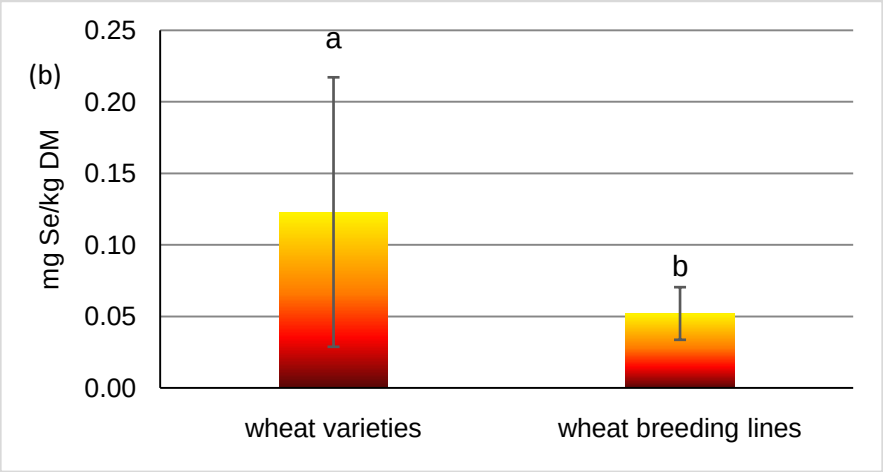

Figure 10. Effect of wheat varieties and breeding lines on selenium content

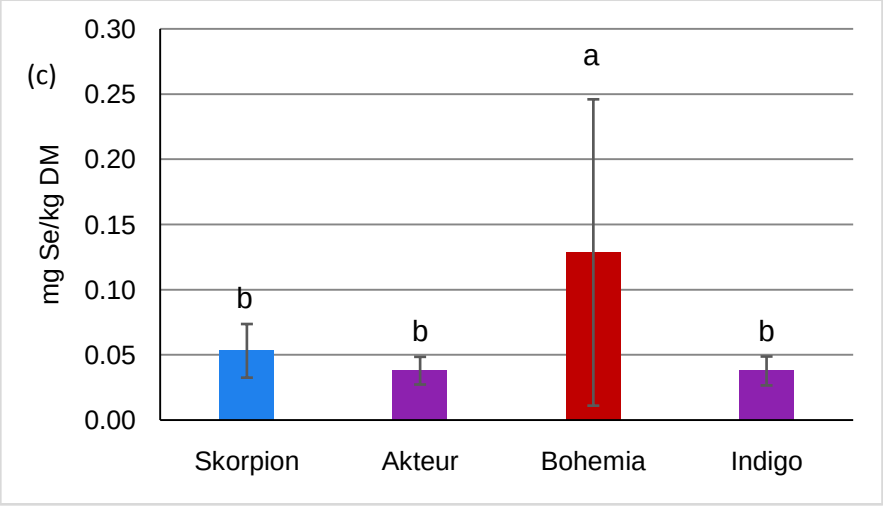

Figure 11. Genetic wheat resources with a higher average contribution to selenium content

5.5 Content of mercury (Hg), cadmium (Cd) and lead (Pb) in the analyzed potato varieties from two different locations: Uhříněves (in the years 2013 and 2014) and Valečov in the year 2013 (mg kg^{-1} dry matter)

The above procedures were employed to describe 13 samples of potato varieties in two different locations: Uhříněves and Valečov in the year 2013 and 2014. The amount of cadmium (Cd), lead (Pb) and mercury (Hg) were obtained from the average of three determinations.

In the experiments, 16 different varieties of potato 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.

The amount of mercury was obtained from the Advanced Mercury Analyzer AMA 254, while the amount of other heavy metals (lead and cadmium) was obtained from the Atomic Absorption Spectrometry (AAS). Cadmium and lead were determined using the electrothermal atomization (ET- AAS).

Table 10 reports the most significant results of the analysis of the investigated samples. All heavy metal concentration measurements were based on the dry weight basis (mg kg^{-1}). The results were the average of three replicated samples, expressed to one standard deviation. The reliability of our methods was shown by the low standard deviation.

Table 10. Content of cadmium (Cd), lead (Pb) and mercury (Hg) in tubers of different potato cultivars from two locations: Uhříněves (2013 and 2014) and Valečov in the year 2013 (mg kg^{-1} dry matter).

Potato cultivar	Uhříněves 2013			Valečov 2013			Uhříněves 2014		
	Cd	Pb	Hg	Cd	Pb	Hg	Cd	Pb	Hg
Agria	0.090±0.044	0.358±0.069	0.0020±0.0003	0.155±0.008	0.172±0.052	0.002±0.001	0.065±0.026	0.066±0.079	0.0019±0.0010
Blaue Anneliese	0.043±0.001	0.404±0.245	0.0014±0.0001	0.116±0.004	0.166±0.048	0.002±0.000			
HB Red	0.053±0.002	0.171±0.085	0.0011±0.0001	0.176±0.004	0.108±0.034	0.002±0.000	0.046±0.015	0.036±0.015	0.002±0.001
Blaue St. Galler	0.105±0.003	0.489±0.249	0.0017±0.0001	0.362±0.007	0.219±0.185	0.001±0.000	0.149±0.066	0.060±0.066	0.002±0.001
Violette	0.036±0.002	0.229±0.043	0.0014±0.0002	0.146±0.009	0.159±0.046	0.002±0.000	0.038±0.004	0.758±0.645	0.001±0.000
Rosalinde	0.201±0.260	0.841±0.824	0.0009±0.0001	0.220±0.057	0.288±0.137	0.001±0.000	0.115±0.011	0.189±0.236	0.001±0.000
Valfi	0.048±0.001	0.174±0.058	0.0007±0.0002	0.286±0.003	0.258±0.128	0.001±0.0000	0.087±0.005	0.071±0.034	0.001±0.000
Herbie 26	0.108±0.011	0.312±0.143	0.0026±0.0005	0.177±0.023	0.268±0.072	0.002±0.000	0.065±0.010	0.105±0.091	0.001±0.000
Blue Congo	0.067±0.003	0.173±0.095	0.0033±0.0010	0.173±0.002	0.398±0.208	0.002±0.000	0.079±0.002	0.111±0.120	0.001±0.000
Blaue Elise	0.065±0.002	0.142±0.013	0.0032±0.0005	0.279±0.014	0.322±0.192	0.001±0.000	0.044±0.005	0.090±0.061	0.001±0.000
Rotte Emma	0.079±0.008	0.405±0.241	0.0035±0.0002	0.210±0.004	0.260±0.020	0.001±0.000	0.053±0.003	0.151±0.142	0.001±0.000
Salad Blue	0.073±0.001	0.114±0.028	0.0032±0.0002	0.194±0.003	0.360±0.082	0.001±0.0000	0.074±0.004	0.106±0.083	0.001±0.000
Boravaley	0.292±0.363	0.901±1.119	0.0039±0.0001	0.204±0.001	0.450±0.101	0.001±0.000	0.067±0.003	0.094±0.087	0.001±0.000
Königsblau							0.080±0.005	0.133±0.145	0.001±0.000
Königspurpur							0.061±0.002	0.254±0.164	0.001±0.000
Blaue de la Mancha							0.083±0.003	0.112±0.088	0.001±0.000

(results are expressed in mean ± standard deviation)

Amounts of toxic and potentially toxic elements detected in investigated potato tubers are shown in Table 10, and 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 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).

Generally, Uhříněves 2013 had the highest concentration of Hg and Pb while Cd was found biggest amount in Valečov 2013. A one-way analysis of variance was conducted. The conclusion is that there was a significant difference in concentrations of of Cd, Pb and Hg in different varieties in Uhříněves and Valečov in 2013. But in Uhříněves 2014 there was no significant difference between 3 heavy metal concentrations in different potato varieties.

In Uhříněves 2013, the mean contents of Cd, Pb and Hg in investigated varieties of potato were ranging from 0.043-0.292; 0.114-0.901; 0.001-0.004 (mg kg^{-1}). In 2014, content of Cd was found at 0.038-0.149; for Pb was 0.036-0.254; and Hg was at 0.001-0.002 (mg kg^{-1}). There was no data for concentrations of Cd, Pb, Hg in 3 varieties (Königsblau, Königspurpur, Blaue de la Mancha) in 2 years in 2 different locations.

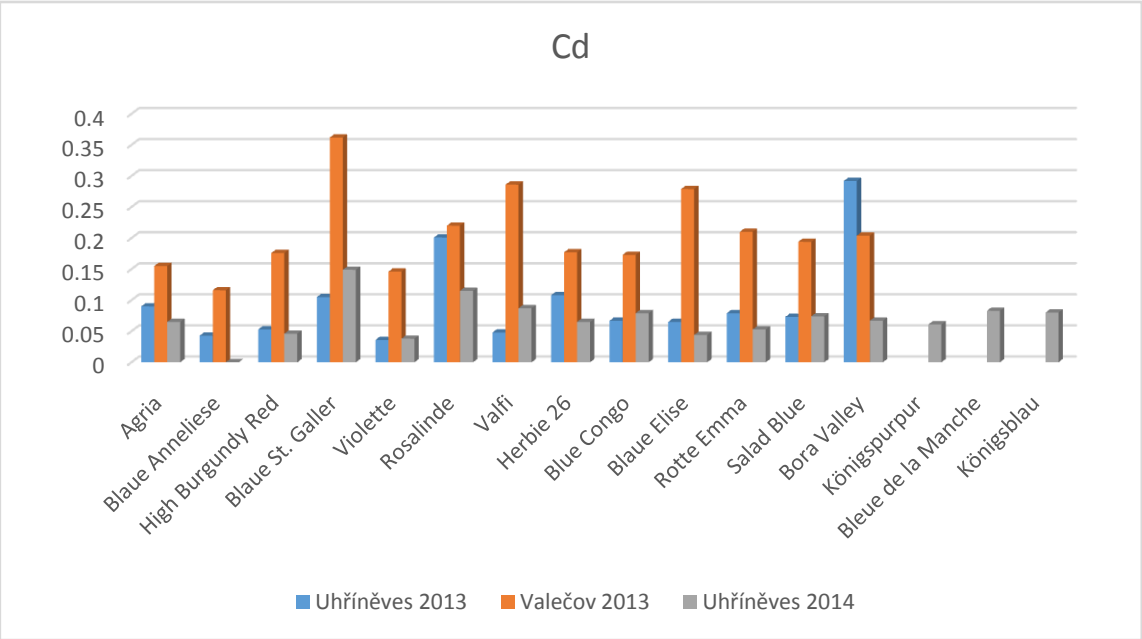

Figure 12. Concentration of Cd in the tubers of different potato cultivars in Uhříněves 2013, Valečov 2013 and Uhříněves 2014 (mg kg^{-1} dry matter)

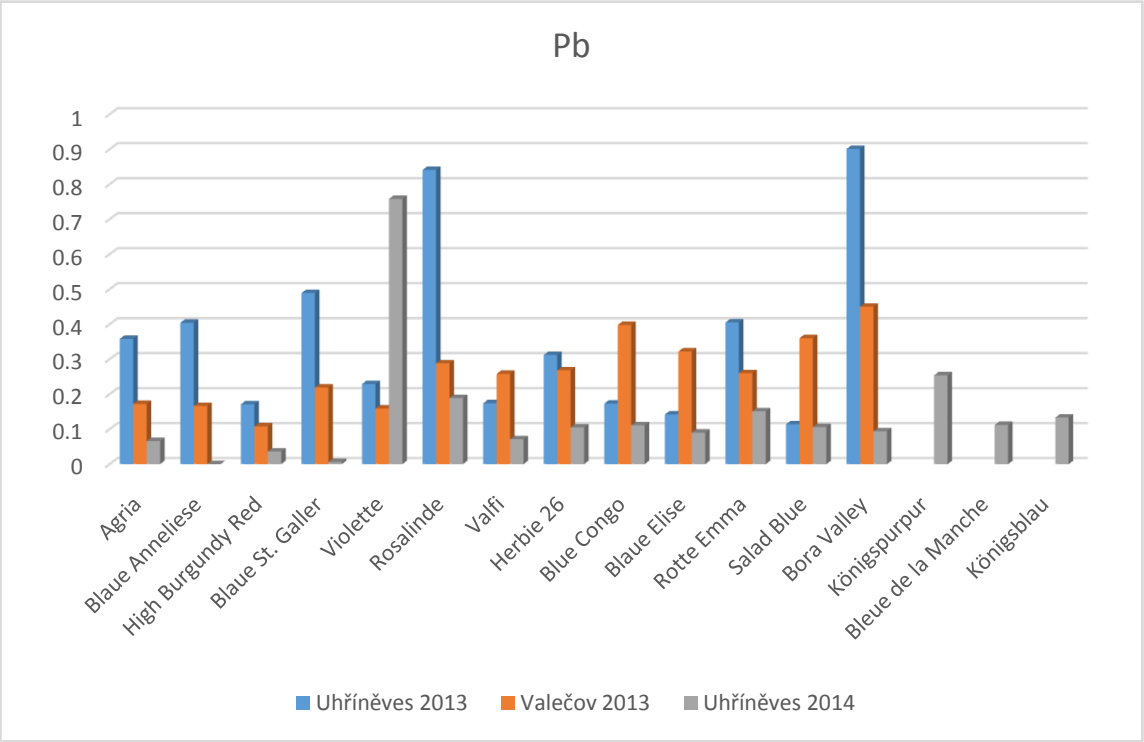

Figure 13. Concentration of Pb in the tubers of different potato cultivars in Uhříněves 2013, Valečov 2013 and Uhříněves 2014 (mg kg^{-1} dry matter)

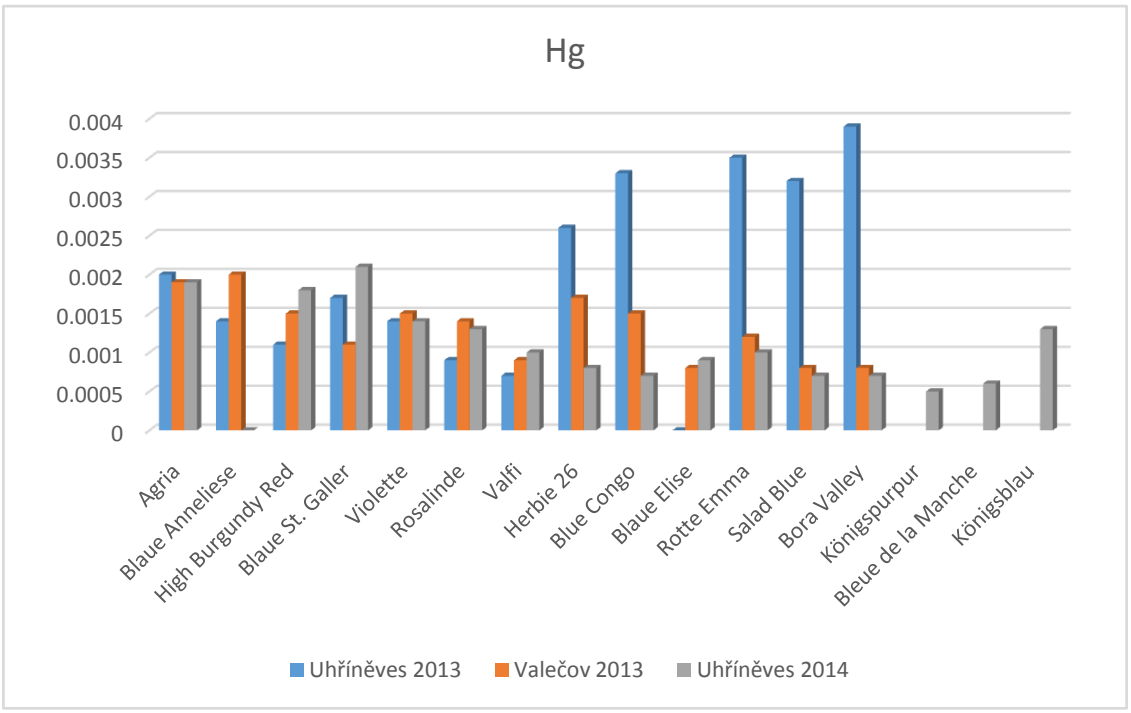

Figure 14. Concentration of Hg in the tubers of different potato cultivars in Uhříněves 2013, Valečov 2013 and Uhříněves 2014 (mg kg^{-1} dry matter)

Using two-way Anova (with alfa = 0.05) (Post-Hoc: Tukey's test) with Factor I (independent variable)- Variety, Factor II (Independent variable) - Year, dependent variable−Cd, Pb, Hg (mg kg^{-1}), we found:

- The significant effect of varieties and year on the concentration of Cd (p=0.188 and 0.199, respectively).

For lead, there is significant effect of varieties on the concentration of Pb while year did not show the significant effect (p=0.275 and 0.020, respectively). The same results were found on Hg (p=0.110 and 0.000, respectively).

Using two-way Anova (with alfa=0.05) (Post-Hoc: Tukey's test) with Factor I (independent variable) − Variety, Factor II (Independent variable) − Locality, dependent variable − Cd, Pb, Hg (mg kg^{-1}), we found:

- The significant effect of varieties on the concentration of Cd while locality did not show the significant effect (p=0.057 and 0.000, respectively).

For lead, there is no significant effect of varieties on the concentration of Pb while locality showed the significant effect (p=0.039 and 0.216, respectively). There is no significant effect of varieties and locality on the concentration of Hg (p=0.004 and 0.000, respectively).

5.6 Contents of mercury (Hg), cadmium (Cd) and lead (Pb) in the analyzed potato tubers treated with two different cooking methods

In the Czech Republic, potatoes are typically cooked by boiling the tubers (with or without skin) or baked. Having considered the types of potatoes in our study and the size of tubers, boiling in water for 20 minutes at 100 °C or baking them for 45 minutes at 180 °C was chosen. From the results shown in Table 11 and Figures 15, 16 and 17, it is evident that boiling and baking resulted in significant increase the concentration of Hg, Cd and Hg in analyzed potato tubers. The concentration of cadmium and lead were much higher than concentration of mercury on the same varieties. The highest concentrations were found in Axa and Blaue Anneliese while the smallest values were identified on Vitelotte and HB Red. Using two-way Anova (with alfa=0.05) (Post-Hoc: Tukey's test) with Factor I (independent variable) − Variety, Factor II (Independent variable) − Cooking methods, dependent variable- Cd, Pb, Hg (mg kg^{-1}), we found: there is no significant effect of varieties and cooking methods on the concentration of Cd, Pb, Hg.

Table 11. Content of cadmium (Cd), lead (Pb) and mercury (Hg) in analyzed potato tubers treated with cooking methods (mg kg^{-1} dry matter)

POTATO CULTIVAR	BOILING			BAKING			RAW		
	Cd	Pb	Hg	Cd	Pb	Hg	Cd	Pb	Hg
AGRIA	0.093±0.003	0.330±0.113	0.002±0.001	0.057±0.026	0.118±0.076	0.006±0.001	0.050±0.058	0.025±0.048	0.002±0.001
RUSSET BURBANK	0.093±0.004	0.176±0.125	0.002±0.001	0.067±0.005	0.151±0.040	0.003±0.001	0.073±0.052	0.047±0.069	0.003±0.000
VALY	0.034±0.004	0.210±0.109	0.003±0.001	0.019±0.007	0.193±0.006	0.003±0.000	0.023±0.010	0.061±0.006	0.003±0.000
SALOME	0.121±0.027	0.030±0.009	0.002±0.001	0.114±0.004	0.223±0.052	0.006±0.003	0.108±0.038	0.020±0.003	0.0025±0.000
BOHEMIA	0.097±0.018	0.016±0.018	0.002±0.001	0.061±0.007	0.255±0.071	0.005±0.000	0.057±0.002	0.024±0.004	0.003±0.000
AXA	**0.315±0.018**	**0.035±0.029**	**0.003±0.001**	**0.182±0.015**	**0.148±0.118**	**0.006±0.001**	**0.238±0.042**	**0.011±0.007**	**0.005±0.000**
JELLY	0.137±0.022	0.135±0.001	0.002±0.001	0.088±0.007	0.089±0.112	0.005±0.000	0.119±0.006	0.004±0.003	0.003±0.000
DITTA	0.091±0.012	0.016±0.019	0.002±0.001	0.075±0.008	0.180±0.048	0.003±0.000	0.073±0.007	0.021±0.006	0.003±0.000
BIOTA	0.104±0.012	0.032±0.046	0.002±0.001	0.069±0.010	0.191±0.078	0.004±0.000	0.069±0.007	0.011±0.001	0.002±0.000
KEŘKOVSKÝ ROHLÍČEK	0.231±0.027	0.034±0.009	0.002±0.001	0.122±0.004	0.092±0.151	0.003±0.000	0.198±0.004	0.002±0.003	0.003±0.000
ROSARA	0.113±0.009	0.082±0.068	0.002±0.001	0.100±0.002	0.101±0.081	0.005±0.000	0.115±0.005	0.016±0.003	0.002±0.000
DALI	0.130±0.014	0.038±0.000	0.003±0.002	0.089±0.004	0.048±0.019	0.006±0.001	0.124±0.007	0.004±0.003	0.002±0.000
MAYAN GOLD	0.086±0.002	0.330±0.101	0.002±0.001	0.085±0.003	0.373±0.082	0.005±0.001	0.074±0.002	0.016±0.013	0.003±0.000
VALFI	0.087±0.003	0.373±0.0079	0.002±0.001	0.078±0.002	0.336±0.010	0.003±0.001	0.067±0.002	0.092±0.021	0.002±0.000
BLAUE ELISE	0.042±0.005	0.373±0.002	0.002±0.001	0.040±0.004	0.033±0.004	0.004±0.000	0.027±0.013	0.068±0.026	0.002±0.000
BLAUE ANNELIESE	**0.033±0.002**	**0.042±0.000**	**0.002±0.001**	**0.030±0.003**	**0.090±0.060**	**0.004±0.001**	**0.018±0.002**	**0.104±0.029**	**0.002±0.000**
ROSALINDE	0.098±0.015	0.090±0.070	0.002±0.001	0.108±0.006	0.167±0.033	0.007±0.000	0.083±0.005	0.014±0.004	0.002±0.000
VITELOTTE	**0.022±0.010**	**0.070±0.028**	**0.002±0.001**	**0.017±0.009**	**0.118±0.046**	**0.006±0.001**	**0.015±0.002**	**0.065±0.020**	**0.003±0.000**
KÖNIGS PURPUR	0.044±0.003	0.268±0.002	0.001±0.001	0.047±0.005	0.053±0.050	0.006±0.001	0.044±0.004	0.008±0.006	0.002±0.000
HB RED	**0.029±0.024**	**0.252±0.027**	**0.002±0.001**	**0.037±0.005**	**0.029±0.005**	**0.005±0.001**	**0.049±0.010**	**0.028±0.004**	**0.002±0.000**
HERBIE 26	0.060±0.004	0.118±0.001	0.002±0.001	0.036±0.002	0.009±0.003	0.004±0.001	0.050±0.004	0.006±0.010	0.002±0.000
ROTTE EMMA	0.073±0.001	0.196±0.067	0.002±0.001	0.092±0.016	0.140±0.002	0.005±0.001	0.281±0.131	0.028±0.010	0.002±0.000

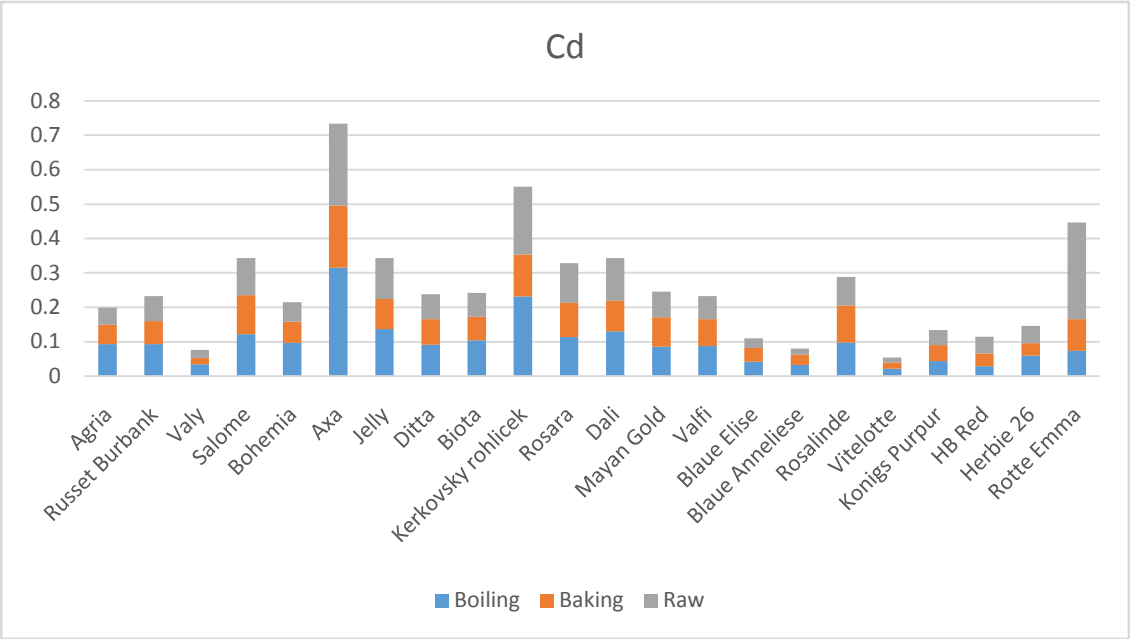

Figure 15. Content of Cd in the tubers of different potato cultivars in 2 different cooking methods: boiling and baking and raw tubers (mg kg^{-1} dry matter)

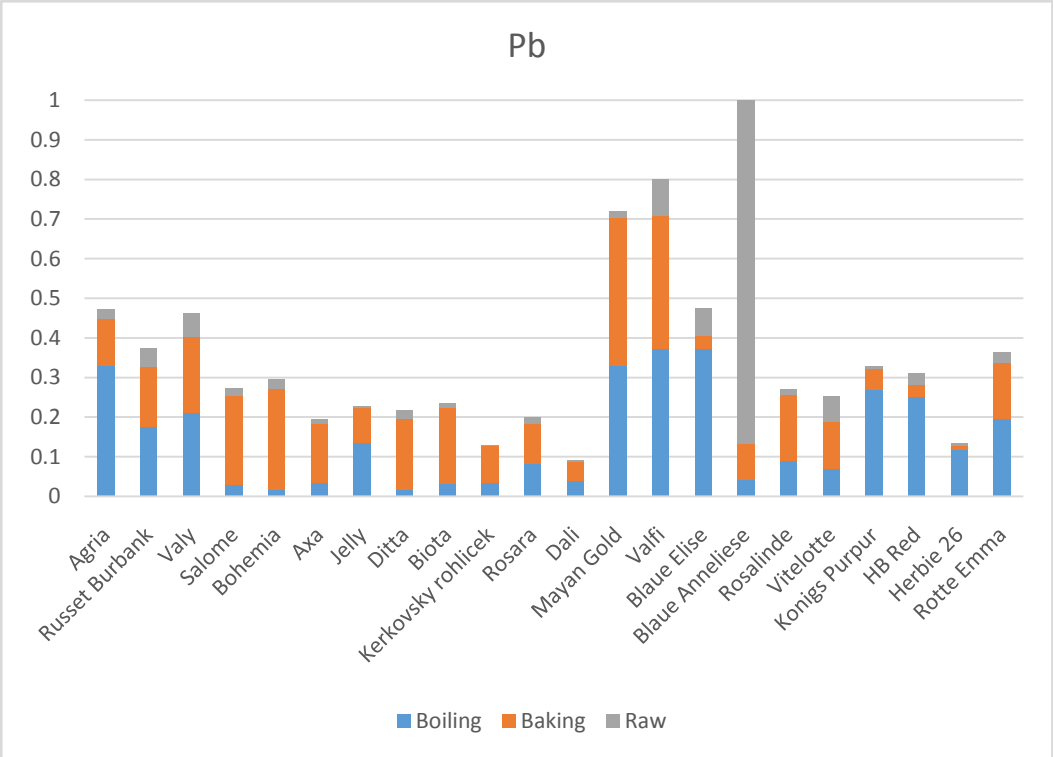

Figure 16. Content of Pb in the tubers of different potato cultivars in 2 different cooking methods: boiling and baking and raw tubers (mg kg^{-1} dry matter)

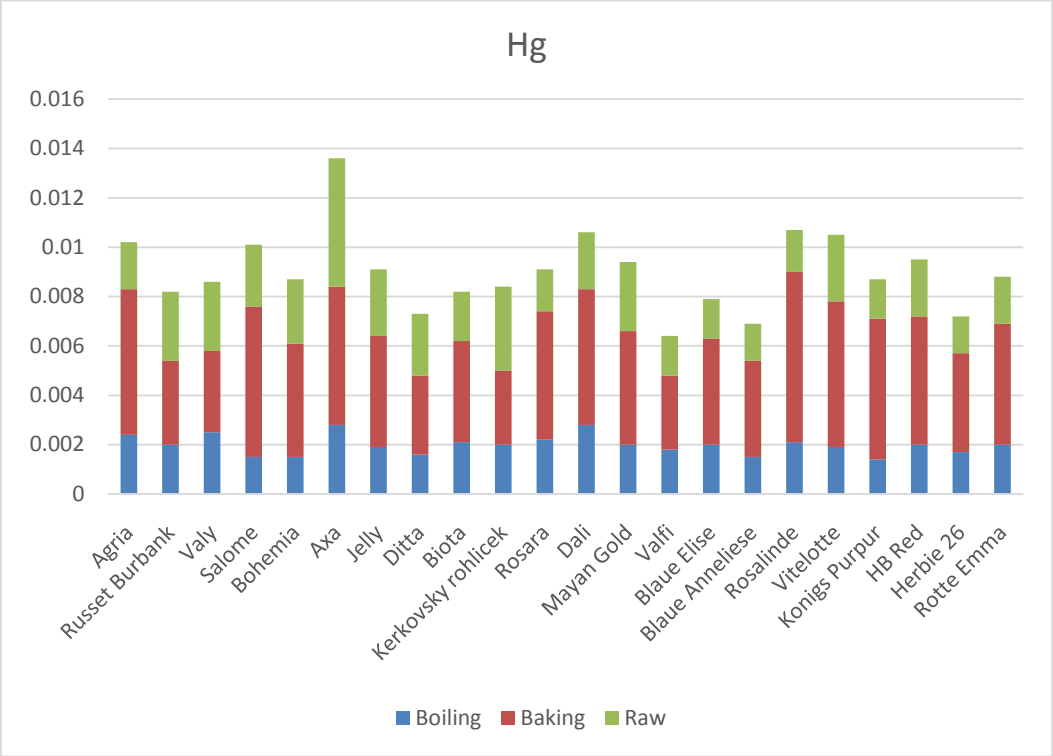

Figure 17. Content of Hg in the tubers of different potato cultivars in 2 different cooking methods: boiling and baking and raw tubers (mg kg^{-1} dry matter)

5.7 Optimization the cell wall degrading enzymes and technique for isolation of protoplasts in potato

Plant cells compared to animal cells are characterized by the formation of cell walls. If we examine the plant membrane, one option is to remove the cell wall by means of special enzymes. When we intend to define the membrane, it is best to work all the time in sterile conditions. This will ensure that the membranes of undesirable organisms such as fungi and bacteria, are not part of the material obtained.

In our experiments we used a potato plant material grown under sterile in vitro conditions in special growing chambers. The leaves of potato plants grown in vitro, we cut into small segments, strips, which are stored in an enzyme solution mixture of 1% cellulasa Onozuka R10 and 0,25% macerozyme R10 (6 ml) dissolved in W5 ($CaCl_2$, glucose, KCl,2-(N-

morpholino) ethanesulfonicacid (MES), NaCl, and pH (5.8) was adjusted by KOH; all from Sigma-Aldrich Czech Republic) in Petri dishes (diameter 55 mm). Release of protoplasts is carried out in the dark at 25 degrees of Celsius for 18 hours. Mixture of enzyme solution is filtered through a sieve 70-90 microns and then pipette into a centrifuge tube, centrifuged for 5 minutes at 100g 800 rpm. Supernatant poured away and the sediment is resuspended in W5 solution (volume of 5 ml). We can once again centrifuged for 5 minutes at 100g 800 rpm. Supernatant poured away and the sediment is resuspended in 4 ml 20% sucrose and overlaying 2 ml of W5 (not mixed and cured). Centrifuged for 5 minutes at 100 g, with the micropipette remove the floating protoplasts into a clean centrifuge tube and resuspended them in W5 solution (volume 4 ml). Throughout we work very carefully without shock and sudden movements, because there is breakage of protoplasts not protected by the cell wall.

The isolation procedure proposed, based on preliminary experiments,was effective in the releasing of protoplasts. In our experiment, sieve 70- 90 micron was used for filtration. Mixture of cellulase/macerozyme disolving in W5 is effective for isolation of protoplast. Using the fluorescence method, the living protoplasts are recognized.

The material thus obtained is vital, can be used for other processes in biology for protoplast fusion, in biophysics for separation bio-membranes by ultracentrifugation or used to work with micropipettes or microelectrodes.

All the environmental and genotypic factors, which affect the cell wall thickenings and compactness indirectly, influence the number of protoplasts recovered. The critical factors affecting the obtaining of protoplasts are the kinds of cell wall degrading enzymes, the physiological state of plant leaves, the type of osmotic stabilizers and the composition of reaction solution (Karp et al., 1991). When the concentration of macerozyme was low, the toxic effect of long exposure to this enzyme was avoided. With the improvement of technique and enzyme combination rate, the yield of collected protoplasts will be increased higher. Power and colleagues (2004) have discovered that using the slicing source (preplasmolysed) tissues in suitable osmotic solution can enhance the protoplast yield.

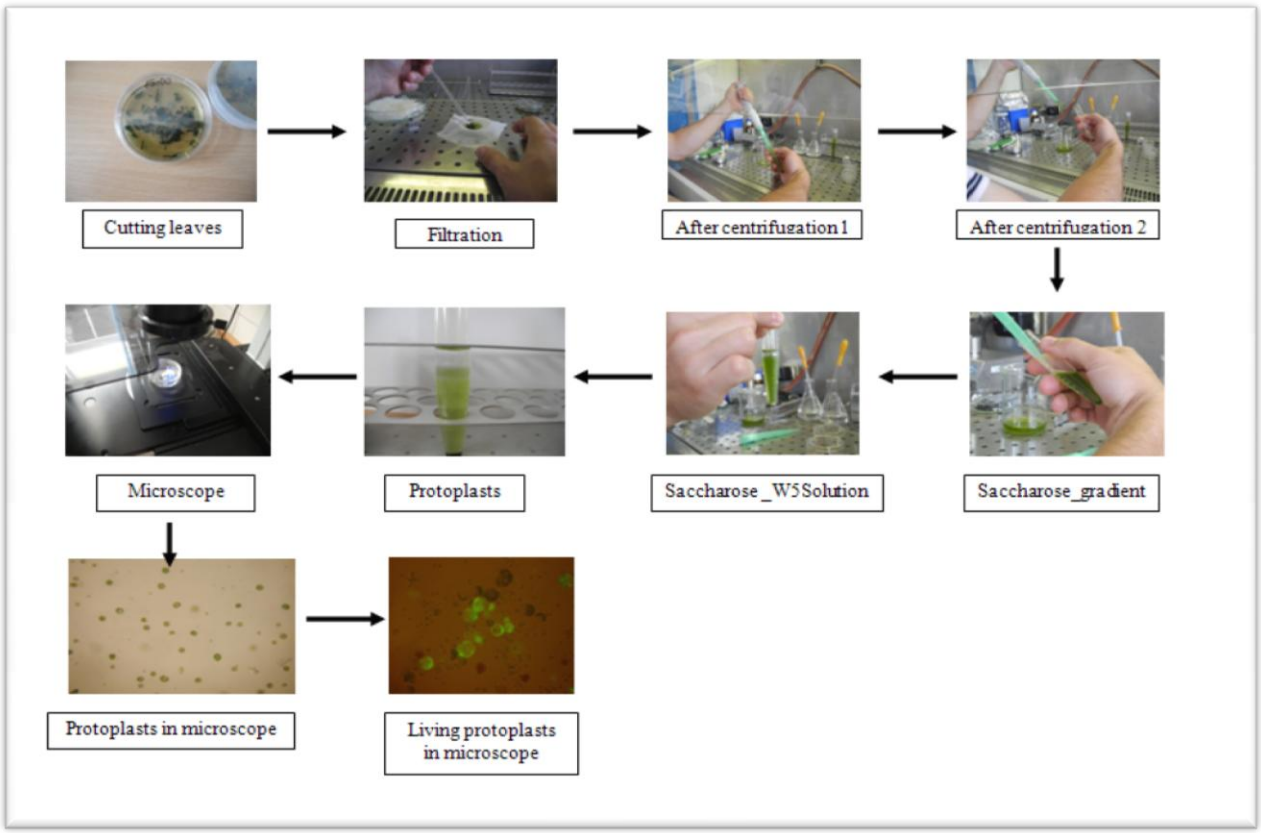

Figure 15. Protoplast isolation procedure

Photos by Ing. Miroslav Klíma, Ph.D. - Crop Research Institute in Prague

6. DISCUSSION

The study, performed on 15 plant samples, determined the content of four heavy metals (mercury, lead, cadmium, zinc) in wheat plant samples collected in the Czech Republic. The thesis was aimed to compare the concentration of cadmium, zinc, mercury and lead in the whole grains of emmer, einkorn and common spring wheat cultivars. According to the FAO/WHO and European Commission (2001), the concentrations of investigated heavy metals were below the maximum permissible concentrations in wheat.

As far as we know, plants are important components in the ecosystems and the absorption of heavy metals is an important issue to concern by scientists. 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). Grujic (1996) discovered that cadmium in plants had 40-70 wt % from the soils and 30-60 wt % from the atmosphere. Angelova et al. (2010) measured the content of lead, zinc and copper in potato tubers, they were 0.38; 3.7; 2.7 mg kg^{-1}, respectively.

Cereals contain around 75% of cadmium, especially in spring wheat (*Triticum aestivum*) and durum wheat. Adam et al. (2004) reported that the cadmium concentration in wheat grain can be predicted using soil properties.

The research of cadmium concentration in wheat variety can be described in the documents of Kusa (2005), Matsumoto (2007) and Romkens (2009). Lavado et al. (2001) determined the nutrient and heavy metal concentration and distribution in wheat, corn and soybean. 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. Other research of Mench et al. (1996) showed that the cadmium content in grain varied from 0.015 to 0.146 mg kg^{-1} dry matter. The values ranged because of the changes in soil characteristics and plant mineral composition.

Comparing to our study, the lead accumulation in grain wheat was lower than those found by other authors (Lavado et al., 2007; Duoay et al., 2008; Chandra et al., 2009). 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 29.2 mg kg^{-1} dry matter, respectively).

Kisku (2000) reported the accumulation of heavy metals in plants and how heavy metals can transfer to the systems. 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.

Furthermore, in study of Chandra et al. (2009), the amounts of cadmium and lead accumulated in grain wheat were significantly higher than our measured concentrations (1.06 ± 0.03 mg kg^{-1}dry matter and 80.6 ± 1.16 mg kg^{-1} dry matter, respectively), whereas the zinc concentration was smaller (28.26 ± 3.18 mg kg^{-1} dry matter). 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).

Chandra (2009) and Jamali (2009) reported the accumulation and distribution of heavy metals on different varieties of wheat grown on soils that were amended with sewage sludge, while Castaldi (2005) and Tandy (2009) investigated the influence of addition amendments on the immobilization of heavy metals in soils. It can be shown in the

cadmium concentration values reported from Karavoltsos et al. (2002), as well as Harcz et al. (2007).

In another report of Heather et al. (2000), the concentrations of lead and zinc surpassed the values of our metal concentrations. Because of high concentration of heavy metals in the contaminated sewage sludge, the measured concentrations were 20.36 ± 29.71 mg kg^{-1} dry matter for lead and 234.2 ± 79.88 mg kg^{-1} dry matter for zinc. All the concentration measurements in this study were the averages of five replicates. The values were also expressed to one standard deviation. During the growth stage, the concentration of lead significantly increased during the experiment.

In the report of Kim et al. (2003), they discovered the concentration of heavy metals in wheat plants. They found out that the concentration of heavy metals in different parts of plants widely varied. The shoot contained significantly higher metal accumulations than they were determined in grain wheat. The compartmentalization and translocation may be the reasons to the variability of heavy metals in wheat plant parts. The lower cadmium levels in the study of Kim et al. (2003) may be linked to the environmental conditions during growing periods of wheat. They affect not only the accumulation of heavy metals by plants, but also the mobilization of metals. In the research of Suptapa Bose and Bhattacharyya (2008), the concentration of cadmium increased in different growth stages of wheat.

In grain wheat, the level of cadmium was 0.74 mg kg^{-1} dry matter, around 1.4 times higher than the measured cadmium concentration in our study. The increased concentration of cadmium may come from the application of waste in soils. Other factor affecting the uptake of cadmium in this research is pH.

Several researches on the effects of lead on plants have been reported (Sharma, 2005; 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.

According to Grant (1998), cadmium is taken up and transported to plants in similar way as zinc. Das (1997) reported zinc and cadmium have similar properties of environment and geochemistry. Eriksson (1996) found that that the solubility of cadmium is influenced by pH, organic matter of soil. Other factors of soil properties such as cation exchange capacity and concentration of metals are also related to the phytoavailability of cadmium (Sayyad et al., 2009).

The concentration of heavy metals in wheat in our research decreased in the order of zinc (Zn) > lead (Pb) > cadmium (Cd) > mercury (Hg), similar to the results of Mingh Huang et al. (2008) in Kunshan, China. The amount of absorbing mercury is different among the plant varieties. Compared with the results of Mingh Huang et al. (2008), the concentrations of zinc, lead and cadmium in wheat grain were higher, whereas the mercury concentration was similar (around 0.003 to 0.006 mg kg^{-1} dry matter). The content of zinc ranged from 12.06 to 80.33 mg kg^{-1} dry matter, while the amounts of lead and cadmium were 0.017 – 1.158 mg kg^{-1} dry matter; 0.006-0.179 mg kg^{-1} dry matter, respectively. Zinc contained in the wheat grain represented the most abundant metal.

In the wheat production, the deficiency in zinc is a critical problem that needs a lot of efforts from scientists to research. Graham and Welch (1996) investigated that nearly half of areas growing cereals had the low concentrations of available zinc in the soil. The cereals that were grown in such conditions would suffer the deficiency of zinc (Cakmak, 1999).

During the vegetative growth stage, the zinc concentrations on leaves or shoots are about 15-17 ppm. The concentration of zinc in grain of zinc deficiency plant is from 15 to 20 ppm. Heavy metals in the soils can bound to clay or organic matter or sometimes they can also bound to hydrous oxides of Al, Mn and Fe. Heavy metals can also act in the soil as the inorganic components.

To evaluate the potential effects of heavy metals, Adriano (2004) suggested using the regulatory limits for heavy metals both in total amount and bioavailable concentration. In the wheat grain, the concentration of zinc was high which may be related to bioconcentration factor. The bioconcentration factor of zinc was high in wheat. In their

research, they measured the bioconcentration factor of zinc as high as 0.278, comparing to 0.007 for lead and 0.016 for mercury.

The concentration of heavy metals in plants is directly associated with their concentration containing in soil (Kabata-Pendias and Pendias, 2001). The difference of contents also comes from the difference in plant varieties or different genotypes in the same species.

In research of Orzturk et al (2011), the concentration of lead (Pb) in potato ranged from 0.77 mg kg^{-1} (cultivar Binella) to 0.51 mg kg^{-1} (cultivar Agria). The contents of cadmium (Cd) were lower than lead. Marene had the lowest content of cadmium (0.08 mg kg^{-1}), while Santa had the highest concentration (0.32 mg kg^{-1}). All the varieties of potato showed the difference in accumulating the heavy metals but the difference is not consistent. Measurement sixteen potato varieties, the accumulation of heavy metals were following these orders: Fe> Mn> Cu> Ni> Pb> Cd. Mendil et al (2005) also observed the same trends of these heavy metals. Different cultivars had the different concentration of heavy metals.

The absorption of cadmium, lead, manganese and copper are mostly affected by the genotypes of cultivars (Prosba- Bialczyk and Mydlarski, 2000). The concentration of metals maybe influenced by other factors, for example soil and climate properties, location (White and Zasoski, 1999; Caussy et al., 2003).

The absorption of heavy metals is different between potato cultivars, even though the difference is not consistent. McLaughlin et al (1997) found the same results about the difference of tuber cadmium concentration in different grown commercially cultivars.

Effect of different factors on Se content. Humans and animals commonly obtain selenium from cereals, grains, and vegetables grown on seleniferous soils and from animal products such as meat, milk, fish, and eggs (Fairweather-Tait et al., 2010). This element enters the food chain through plants and, consequently, it is highly dependent upon its bioavailability in soils (Ducsay and Ložek 2006). Se foliar application effectively increases its content in cereal grain, as was reported in barley (Ducsay et al., 2009) and winter wheat (Ducsay et al., 2007)

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). Soil pH can influence on the selenium content of the plants. It has been proven that chemical oxidation in alkaline soils produces selenate which is available for plants, but pH value of the soil in our experimental field was 5.75 (acidic soil). The decrease of selenium from plants can only occur through the volatilization (Whanger, 2004). The soil in the experimental location of our study had pseudototal (Aqua Regia soluble) Se average content 1.179 ± 0.077 mg kg^{-1} dry matter; this corresponds to the range of Se levels between 0.2–1.4 mg kg^{-1} in the Czech soils (Száková et al., 2015). Selenium is a rare element on our planet, with the average concentration in igneous bedrock being only 0.05 mg kg^{-1}, which is less than for any other nutrient element. Selenium is unevenly distributed over the surface of the Earth, ranging from near zero to 1250 mg kg^{-1}. In many parts of Europe, soil Se concentrations are relatively high because of high deposition either naturally from the sea (e.g. Ireland, England, Scotland and the Netherlands) or from polluted rains (e.g. Germany, the Czech Republic, Slovakia and Poland) (Haug et al., 2007). Our results indicate that only a little portion of selenium is accumulated in cereal grain (from 1.87% in V2 31-16 to 19.93% of Se content in soil in cv. Bohemia and that there are significant differences between varieties and breeding lines. Therefore, exploiting the genetic variability in crop plants for micronutrient density may be an effective method to improve Se intake in human nutrition, and use of plants that naturally contain more Se than others, or breeding plant and crop varieties with enhanced Se-accumulation characteristics, may be plausible approaches to increase the Se concentration of the human diet.

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 are produced in the world and its global consumption is 67 kg/capita/year (FAO, 2017). 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). Grain Se concentrations in divers wheat germplasm may be found in the range 0.005 - 0.720 mg kg^{-1}dry matter, but much of this variation is associated with spatial variation in soil selenium. When they are grown in microelement deficient soils, this situation is more serious. In wheat meal, white bread and raw bread in Slovakia average Se content has been evaluated to 0.025, 0.018 and 0.017 mg kg^{-1} dry matter with ranges 0.015 −0.032, 0.013−0.022 and 0.016-0.019 mg kg^{-1} dry matter, respectively (Töth et al., 2012). However, some wheat species like the diploid wheat *Aegilops tauschii* 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).

The **normative requirement estimates of dietary Se are 0.04 and 0.03 mg day^{-1} for man and woman, respectively (World Health Organization, 1996). European and USA recommended dietary allowance for selenium 0.055** mg kg^{-1} dry matter (Hawkesford and Zhao, 2007), while in **New Zealand and Australia are recommended 0.07 and 0.06 mg day^{-1} for men and women, respectively** (National Health and Medical Research Council, 2005). **Because of its high consumption, wheat is one of the primary sources of dietary selenium, with the major available form found in grains being selenomethionine. Comparing to fish, selenium from** wheat grain is highly bioavailable - 81.0 ± 3.0 % (Fox et al. 2005). This is the reason in recent years, why more and more researchers have focused on exploiting the genetic resources and developing of genetically selenium-enriched and protein-Se-enriched wheat using genomics tools (Lyons et al., 2005).

A relationship between of colour grain and Se content was statistically by one way ANOVA assessed. The highest Se content was found in standard bread red grain cv. Bohemia, which differed from other wheats with coloured grain. Wheats with yellow endosperm differed significantly from wheats with purple pericarp. Comparison of wheat with coloured grain revealed that Se content decreases in order yellow endosperm > blue aleurone > purple pericarp. The effect of wheat varieties on selenium content was higher as compared with breeding lines and varieties differed significantly from breeding lines. Accordingly, for Se crossing appear better genetic resources with a higher average contribution to Se content some wheat varieties. Between analysed wheats as suitable

genetic resources may be recommended the varieties Bohemia, Skorpion, Indigo and Akteur. The variety Bohemia differed significantly from other varieties with higher Se content.

7 CONCLUSION

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 (0.015 mg kg^{-1} dry matter and 0.021 mg kg^{-1} dry matter, respectively). 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 sa,mples (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.

The information about the absorption of heavy metals on crops is important to select plant cultivars and improve healthy nutrition. Plant varieties are the most important factor affecting the amount of absorbed metals. 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}).

Other factors influencing the uptake and mobility of metals are soil types, soil temperature, and soil composition and fertilization methods. Using excessive fertilization and polluted irrigation water can affect the accumulation of metals on potato tubers. In general, the toxicology of heavy metals following these orders: Cd> Cu> Co ≈ Ni> As ≈ Cr> Mn≈ Fe ≈ Pb. The toxicity of heavy metals depends firstly on the valence number of metals in the salt. Secondly, it depends on the salt types of metals in the soil. Finally, the toxicity is also influenced by plant species.

The interactions between ions and heavy metals are really complex. For example, on the addition of the content of cadmium increased the concentration of zinc in plant tips, however, when the amount of cadmium was too high, it leaded to the decrease of uptake zinc. Other metals (iron, nickel, selenium, and magnesium) were induced by the uptake of cadmium. In the case of lead, when both toxic heavy metals (lead and cadmium) presented together, the effect is more serious.

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