Czech University of Life Sciences Prague



Faculty of Agrobiology, Food and Natural Resources Department of Chemistry

A Comparison of Selected Heavy Metals Concentrations in the Whole Grain of *Triticum* Species

DIPLOMA THESIS

Supervisor of Diploma Thesis:	Prof. Ing. Jaromír Lachman, CSc.	
Consultant of Diploma Thesis:	Ing. Matyáš Orsák, Ph.D.	
	Ing. Jaroslav Přibyl	
Author of Diploma Thesis:	Le Minh Phuong	

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DECLARATION

I declare that I have written my diploma thesis "A comparison of selected heavy metals concentrations in the whole grain of *Triticum* species" on my own with the help of literature listed in References.

In Prague 10th April 2013

Le Minh Phuong

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Le Minh Phuong

SUMMARY

Wheat (*Triticum spp.*) is popular cultivated crop in the world. Wheat is also one of the major food items, accounting for 651 million tonnes (FAO, 2010). 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) in the whole grain of spring accessions of emmer, einkorn and common spring wheat cultivars is reported. Atomic Absorption Spectrometry (AAS) has been used to characterize the heavy metal concentrations in wheat. Statistical analyses were performed using SPSS 9.0 with the Tukey's HSD (Honestly Significant Difference) test ($\alpha = 0.05$).

In our study, 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). Generally, the values of lead concentration in grain wheat varieties were low (ranging from 0.1268 ± 0.0435 mg kg⁻¹ dry matter to 0.2950 ± 0.1749 mg kg⁻¹ dry matter).

High mercury content in grain was found in spring wheat (Jara variety 0.0087 ± 0.0012 mg kg⁻¹ dry matter), while this variety had the lowest content of zinc (40.99 mg kg⁻¹ dry matter) among three groups of wheat varieties. Among the varieties with high cadmium content, einkorn varieties prevailed (*T. monococcum* 2101 had the content of cadmium 0.0580 ± 0.0009 mg kg⁻¹ dry matter), less presented was emmer wheat (0.0186 ± 0.0052 mg kg⁻¹ dry matter) and spring wheat (0.0133 ± 0.0005 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) were also determined. It has been shown that the concentrations of mercury in different wheat varieties were absorbed differently at different growth stages of plant. Stage 10.2 and leaf-stage 10.2 showed the high mercury content (0.0152 mg kg⁻¹dry matter and 0.0214 mg kg⁻¹dry matter, respectively). Among individual varieties significant differences were determined.

Keywords: spring wheat, einkorn, emmer, heavy metals, atomic absorption spectrometry

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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 (Dukovskis et al., 2003; Duchovskis et al., 2006). Heavy metals are considered as the special hazard of soil pollutants because of the adverse effects on the plant growth, the amount, activity of useful microorganisms in soils and the quality of food. Regard to the persistent and toxicity, the heavy metals are toxic when we consider different kinds of pollutants in soils (Abrahams, 2002). Another source of toxic element accumulation is from industrial sludge (Duoay et al., 2008; Jamali et al., 2009; Pandey et al., 2009). When these metals are accumulated by plants, these metals can cause damage to humans and the environment (Bose and Bhattacharyya, 2008).

Wheat is one of the main cereal 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; Codex Alimentarius, 1984; US EPA- Risk Assessment Guidance, 1989).

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). Jung-Thorntorn (1997) and Devkota (2000) reported the transport of heavy metals (lead, mercury and arsenic) from plants to the food chains. Food plants which suffer the high concentrations of heavy metals can cause the serious health risk to both animal and human (Wierzbicka, 1993; Carbonell-Barrachina et al., 1999a, 1999b).

2 OBJECTIVE OF THESIS

The aim of this study is to compare the concentrations of heavy metals (Cd, Zn, Hg, Pb) in the whole grain of spring accessions of emmer, einkorn and common spring wheat cultivars which were all grown under the identical environmental conditions.

3 LITERATURE OVERVIEW

3.1 Introduction to wheat (*Triticum spp.*)

Wheat (*Triticum spp.*) 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^{0} N in Scandinavia and Russia to 45^{0} S 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 (Kronstad, 1998). 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 (Heun et al., 1997; Nesbitt, 1998).

According to Briggle and Reitz (1963), 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*)

Emmer wheat (*Triticum dicoccum*) is one of the first crops domesticated in the Near East, which is known as *T. dicoccon* Schrank (Dorofeev et al., 1979). It is considered to be similar as the origin site of einkorn wheat (Nevo, 1988). 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 1873, Kornicke first described the wild emmer wheat (Kornicke, 1889). 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 Southwestern Europe.

During the Bronze Age, the area of growing einkorn wheat was decreased. In the 20th century, it is 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 (Vallega, 1979). 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 Heavy metals

3.2.1 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. Consequents 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 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).

According to Hodgson (1963), 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.

A lot of researchers have focused on the heavy metal in *Triticum* plants because of their values at present and also in the future (Jingh et al., 2007; Gajewska and Sklodowska, 2008). 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.

3.2.2 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 μ g day⁻¹. Generally, mercury is widely distributed pollutant. Hitchcock (1957), Waldron (1975) and Goren (1976) investigated the absorbing mercury from the atmosphere by plants. 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.

Lucena (1993) reported the mercury availability in soil for plants is low. 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.

3.2.3 Cadmium (Cd)

Since 1980, the accumulation of cadmium in agricultural soils has been discussed. Kobayashi (1978) studied the effect of cadmium on the human beings. 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 μ g day⁻¹ for human (Vasilev and Yordanova., 1997). According to European Commission in 2001, the maximum permissible cadmium concentration is 0.1 μ g g⁻¹ wet mass for cereals. The maximum cadmium concentration for rice and wheat grain is 0.2 μ g g⁻¹ wet mass. Kikuchi (2007) reported the uptake of cadmium concentration in wheat is higher than in rice when growing on the same conditions.

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+} . Cadmium also presents in the complex forms such as CdCl, CdCl₂ or Cd So₄ (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 (1990 and 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).

Cereals and vegetables contain 75% of cadmium, especially in spring wheat (*Triticum aestivum*) and durum wheat. For the wheat, European Community sets 0.2 mg kg⁻¹ is the limit value of cadmium in the grain. In the wheat grain, the soil chemical characteristics strongly affect the accumulation of cadmium. Adam et al. (2004) reported the cadmium concentration in wheat and barley grains can be predicted using the soil properties.

The research of cadmium concentration in wheat variety can be described in the documents of Kusa (2005), Matsumoto (2007) and Romkens (2009). Generally, cadmium is considered as a toxic element for growth and development of plants. However, in the hydroponic experiments by some scientists, they also found the positive effect on plant growth if the concentration of cadmium uptake is low on rice (Aina et al., 2007), barley (Wu et al., 2003) and

soybean (Sobkowiak, 2003). The accumulation of cadmium in grain wheat also depends on the genetic variation of wheat.

3.2.4 Zinc (Zn)

Zinc is a minor nutrient. There are some reports on the forms of zinc in soil solution and plant living tissues. Norvell (1993) found zinc presented as an organic ligand. When an excessive amount of zinc accumulates in the living tissues, it can cause the toxicity to health.

Zinc (Zn) is an important constituent for the normal growth and development of plants. According to Huskisson (2007), more than one hundred enzyme structures contain zinc. Other functions of zinc are the insulin secretion, glucose use and the synthesis of proteins and nucleic acids (Martinez-Ballesta, 2009). Moreover, zinc is essential for respiration, biosynthesis of hormones or photosynthesis of plants (Broadley, 2007). Interestingly, Upadhyaya (2010) investigated the application of zinc can help to reverse the phytotoxicity of some heavy metals such as cadmium or zinc.

When the concentration of zinc is excessive, it causes the inhibition of photosynthesis, imbalance of nutrients or the chlorosis of leaves (Cherif, 2010). It leads to the inhibition of plant growth and reducing the agricultural products (Todeschini, 2011). Clarkson (1989) studied two main factors controlling the zinc concentration in living tissues of plants. The first is the membrane potential which allows the accumulation of high cations. The second factor is the low solubility of zinc phosphates.

The contamination of heavy metals becomes a widespread phenomenon in the world now. One of the main reasons of this phenomenon is the increasing of industrial and urban activities. Zinc is a toxic element in high concentrations (Dahmani-Muller et al, 2000). Plant species affect the rate of zinc absorption. According to Haslett (2001) reported that in the wheat plant, zinc is easily transported in the phloem. So, the distribution of zinc largely depends on the age of plants and on the content of this metal on the organs of plants.

The deficiencies of micronutrients are an important issue. It associates with the decrease of the human work productivity and affects to the gross national products. Among the micronutrient deficiencies, the deficiency of zinc is the most commonly situation (Alloway, 2004; Hotz and Brown, 2004). It occurs frequently in the arid and semi areas. The consequences of this situation are the decrease in growth and development of plants. It leads to the reduction in

yield and quality of grain, losses to economy. The visible symptoms to the deficiency in zinc are the decrease in height of plant and the size of leaves. Other symptoms are the necrotic spots on the leaves which have the whitish- brown or mottle rosette.

In human, the deficiency of zinc affects the immune system, increases the disease infection and damages DNA (Hotz and Brown, 2004). Zinc deficiency also influences on the physical growth and the learning ability in human (Cunningham-Rundles et al., 2005). Ho (2004) reported the increase risk of cancer in the zinc deficiency situation.

People reported the region where the zinc deficiency in humans is common is the region has the zinc deficiency in soils. Leaves of zinc- deficient plant have the concentration of zinc about 10- 12 ppm. If the plant is grown in the water- deficient conditions, the symptoms of plants to the deficiency in zinc are more serious. There are many factors affecting the availability of zinc to plant roots: pH, organic matter, soil moisture and so on. The growth stage, season and method of planting and the application of different fertilizers also affect the zinc deficiency.

In developing countries, cereals such as rice, maize and wheat are the main source of calories, energy and protein for human diet. In the daily intake food, 60% of calories come from wheat varieties. Although cereal consumption is high in developing countries, the diversity of cereal varieties is low. This is the reason why the deficiency of zinc is so common in developing countries. The low concentration of zinc in the cereals significantly affects the health of human. Many authors suggested the optimum amount of zinc in crops that requires for the human daily intake (Rengel, 1999; Grusak and Cakmak, 2005). In the wheat production, the deficiency in zinc is a critical problem. 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 concentration on leaves or shoots is about 15- 17 ppm. The concentration of zinc in grain of zinc- deficiency plant is from 15 to 20 ppm. There are many reasons why the amount of zinc is low in cereal- based food products. One of the main reasons is the milling process. During this process, the large amount of zinc is lost through the brans of seeds (Welch and Graham, 1999).

Besides the wheat varieties, the durum wheat is considered as the most sensitive plant to the deficiency in zinc. So, the scientists can use durum wheat as the indicator plant to discover the zinc deficiency in specific region. They reported the deficiency of zinc in the wheat occurs in soil where the plants are unavailable absorbed zinc from soils or where available zinc in the soils is low or decreases because of some specific reasons.

In the wheat varieties, the symptoms of zinc deficiency will firstly appear on the middle aged leaves of plants. Depending on the wheat varieties, the symptoms of zinc deficiency can appear in the middle aged leaves or old leaves. In the leaves, the color will change from green in the healthy plants to gray- green in the zinc- deficient plants. The leaves also have some necrotic spots that are gradually developing.

Takkar (1989) in India, Graham (1992) in Australia, Cakmak et al. (1996) reported the influence of zinc deficiency to the wheat production. Cakmak et al. (1997) and Cakmak et al. (1998) researched the responses of different plant varieties including rye, bread wheat, durum wheat and triticale on the situation of zinc deficiency and how this situation affected to the morphological and physiological characteristics of corresponding plants. Rye and triticale are considered as the most tolerant zinc– deficient cereal varieties while oat and durum wheat are more sensitive to zinc deficient soils. To correct the deficiency in plants, the suggestion is applying the ZnO or ZnSO₄. $7H_2O$ in soils. In practice, the application of ZnSO₄ is more common than ZnO. Other method is foliar application of zinc. To increase the grain yield, the foliar application is less effective than the soil application. In contrast, to increase the grain zinc concentration, the soil application is less effective than foliar application.

So, depending on the objective of experiment, people can choose the appropriate method to analyze. Increasing the zinc concentration in grain is a popular issue in the modern world. The reason is the higher zinc concentration in grain can help improve the zinc deficiency problem in human health. Breeding of new cereal varieties that contains the high zinc concentration in grain is also another approach. Using the molecular variation, scientists can improve the concentration of micronutrients in plants (Welch and Graham, 2004; White et al., 2005).

In rural regions of developing countries, using the supplementations to increase the concentration of micronutrients such as zinc is not the most appropriate solution. This method is not effective when applying in large scale and also expensive for farmers. In contrast, traditional plant breeding or biotechnology to enrich the concentration of zinc in cereal seeds is considered as effective and sustainable methods (Bouis et al., 2000; Frossard et al., 2000). The emmer wheat (*Triticum dicoccum*) and *Triticum dicoccoides* have the higher concentration of micronutrients such as zinc rather than other *Triticum* species. These kinds of varieties can be used for plant

breeding to improve the concentration of micronutrients in grains. Hao (2007) and Pearson (2008) discovered the late planting and proper irrigation or nitrogen fertilizers can also increase the concentration of zinc and iron in wheat and rice varieties.

3.2.5 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 (Nriagu, 1996; Watanabe, 1997).

In the past, lead is 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 have 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 plants 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. Therefore, the cereal products are of particular concern to scientists because of their potential role in transporting the heavy metals to the human body. According to the

European Union legislation, the permissible concentration of lead for wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) is 0.235 mg kg⁻¹ dry weight.

Lead is a physiological and neurological toxin. Lead is primarily accumulated in the skeleton of human body. It can remain the bones from 10 to 30 year (Rabinowitz et al., 1980). 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. James (1985) reported the concentrations of lead that were absorbed by children normally higher than by adults.

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). According to Sarkar and Jane (1986), when the concentration of lead is high, it can reduce the development of root hair and significantly affect 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.

4 MATERIALS AND METHODS

4.1 Plant materials and conditions of cultivation

The study was carried out in 2011- 2013 at the Czech University of Life Science in Prague, at the Department of Chemistry.

Plant material: 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 6 below. Total 15 samples of *Triticum* species were investigated (Table 1) and the used procedures and methods for all analyses were identical for all of them.

Number	Number	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	<i>T. monococcum</i> 2101 ²
9	2361	01C0204040	242007	T. monococcum 2102 ²
10	2362	01C0204044	242019	<i>T. monococcum</i> 2103 ²
11	2363	01C0200948	412048	Rudico ³
12	2364	01C0203989	412013	Kahler Emmer ³
13	2365	01C0201282	412048	<i>T. dicoccon</i> (Tapioszele) ³
14	2366	01C0200117	412013	Krajova-Horny Tisovnik (Malov) ³
15	2367	01C0204501	412013	<i>T. dicoccon</i> No 8909 ³

Table 1. Characteristics of analysed wheat sample (¹ spring wheat, ² einkorn wheat, ³ emmer wheat)

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
- A 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)
- H₂O₂ 30%, TraceSelect (Fluka)
- 1.5% HNO₃
- 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) is 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 ^oC. After passing through the catalytic furnace at 650 ^oC, mercury was trapped in gold amalgamator. It was released at high temperature and the atomic absorption was measured.

4.3.2 Determination of cadmium, zinc and lead

The concentrations of lead (Pb), cadmium (Cd), and zinc (Zn) in *Triticum* 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 H₂O₂ 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% HNO₃. The dissolving was accelerated by sonication. Then digests were transferred to probes and adjusted with 1.5% HNO₃ to 12 ml.

Measurement of cadmium and lead concentrations in the digests: Cadmium and lead concentrations in grain of *Triticum* 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 2.

Element	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) ₂ HPO ₄	(NH ₄) ₂ HPO 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	

Table 2. The parameters of measurement of Cd and Pb in *Triticum* species using Varian AA

 280Z spectrometer

Measurement of zinc concentration in the digests: The concentration of zinc in *Triticum* species was measured using AAS with air-acetylene flame technique. We used a spectrometer Varian SpectrAA 110. The chosen wavelength was 213.9 nm with deuterium background correction. Standard solution ASTASOL (Analytika, CR) of zinc was used in the preparation of a calibration curve for the measurement.

All samples of *Triticum* 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 \pm 3SD of blanks) which were 0.07 ng ml⁻¹ for cadmium, and 0.21 ng ml⁻¹ for lead and 6 ng ml⁻¹ for zinc.

4.3.3 Replicates and statistical analysis

All experiments were conducted in triplicate. For all measurements averages and standard errors were calculated in Microsoft Excel 2007. Statistical evaluation was performed with ANOVA, using the statistical package SPSS 9.0 with Tukey's HSD (Honestly Significant Difference) test ($\alpha = 0.05$).

5 RESULTS

5.1 Determination of mercury (Hg), cadmium (Cd), lead (Pb) and zinc (Zn) in the analyzed grain wheat species (mg kg⁻¹dry 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 3 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.

Wheat variety	Hg	Cd	Pb	Zn
SW Kadrilj ¹	0.0024 ± 0.0004 ^d	0.0358 ± 0.0028 bc	0.2779 ± 0.0266 ^b	36.9816 ± 1.3360^{ab}
Granny ¹	0.0009 ± 0.0002 ^{ab}	0.0338 ± 0.0014 bc	0.0620 ± 0.0130^{a}	35.1871 ± 2.7328 ^a
Jara ¹	0.0087 ± 0.0012 ^e	0.0133 ± 0.0005 ^a	0.0781 ± 0.0015 ^a	48.9195 ± 0.6013 ^{cd}
Kaerntner Frueher ¹	0.0015 ± 0.0003 abcd	0.0262 ± 0.0048 ^{ab}	0.2950 ± 0.1749 ^b	46.4025 ± 2.2734 bc
Postoloprstska presivka 6 ¹	0.0012 ± 0.0001 abcd	0.0357 ± 0.0018 bc	0.1058 ± 0.0305 ^a	37.4371 ± 1.3727 ^{ab}
Escana ²	0.0006 ± 0.0001 ^a	0.0543 ± 0.0012^{d}	0.0483 ± 0.0142 ^a	$40.2356 \pm 10.9861^{abc}$
Schwedisches Einkorn ²	0.0007 ± 0.0002 ^a	0.0570 ± 0.0019^{d}	0.0416 ± 0.0194^{a}	35.3430 ± 0.2390^{a}
T. monococcum 2101 2	0.0008 ± 0.0001 ^{ab}	0.0580 ± 0.0009 ^d	0.0455 ± 0.0024 ^a	36.6893 ± 2.0559^{abc}
<i>T. monococcum</i> 2102 ²	0.0015 ± 0.0001 ^{abcd}	0.0505 ± 0.0084 ^{cd}	0.1308 ± 0.0979 ^{ab}	$57.0025 \pm 1.1328^{\text{de}}$
T. monococcum 2103 ²	0.0022 ± 0.0001 ^{cd}	0.0542 ± 0.0024 ^d	0.0677 ± 0.0261 ^a	$35.1871 \pm 2.7328^{\text{g}}$
Rudico ³	0.0012 ± 0.0001 ^{abc}	0.0232 ± 0.0015^{ab}	0.0745 ± 0.0052 ^a	$67.4047 \pm 1.9899^{\mathrm{fg}}$
Kahler Emmer ³	0.0018 ± 0.0003 bcd	0.0334 ± 0.0015 bc	0.0319 ± 0.0052 ^a	$66.6986 \pm 0.5582^{\text{ efg}}$
<i>T. dicoccon</i> (Tapioszele) 3	$0.0014 \pm 0.0002^{\text{ abcd}}$	0.0273 ± 0.0048 ^{ab}	0.0659 ± 0.0399 ^a	67.1244 ± 2.8065 ^{fg}
Krajova-Horny Tisovnik (Malov) ³	0.0009 ± 0.0001 ^{ab}	0.0186 ± 0.0052^{ab}	0.0397 ± 0.0225 ^a	$61.4951 \pm 1.2800^{\text{ ef}}$
<i>T. dicoccon</i> No 8909 ³	0.0013 ± 0.0001 abcd	0.0337 ± 0.0065 bc	0.1268 ± 0.0435 ^{ab}	47.8992 ± 1.9912 ^{cd}

Table 3. Concentration of mercury (Hg), cadmium (Cd), lead (Pb) and zinc (Zn) in the analyzed grain wheat species (mg kg⁻¹ dry matter)

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 Concentration 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 3 and Figure 1. The concentration of mercury in the grain wheat was present in very small quantity. The concentration ranged between 0.0006 ± 0.0001 mg kg⁻¹ dry matter and 0.087 ± 0.0012 mg kg⁻¹ dry matter. When considering on different varieties, the highest value was characteristic for the Jara variety (0.0087 ± 0.0012 mg kg⁻¹ dry matter). On the other hand, the lowest values were measured in Escana (0.0006 ± 0.0001 mg kg⁻¹ dry matter) and Schwedisches Einkorn (0.0007 ± 0.0002 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 Concentration 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 3, Figure 2). The most distinctive varieties were Jara, Escana, Schwedisches Einkorn, *T. monococcum* 2101 with average absorbed cadmium amounts 0.0133 ± 0.0005 , 0.0543 ± 0.0012 , 0.0570 ± 0.0019 , 0.0580 ± 0.0009 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 (0.0133 ± 0.0005 mg kg⁻¹ dry matter) and the highest was found in *T. monococcum* 2101 (0.0580 ± 0.0009 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 Concentration 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 3, Figure 3). High lead contents were typical for Kaerntner Frueher, SW Kadrilj and *T. dicoccon* No 8909 varieties (0.2950 ± 0.1749 , 0.2779 ± 0.0266 and 0.1268 ± 0.0435 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)

5.1.4 Concentration 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 35.1871 ± 2.7328 and 67.4047 ± 1.9899 mg kg⁻¹ dry matter. The most distinctive species and varieties were Granny (35.1871 ± 2.7328 mg kg⁻¹ dry matter), Schwedisches Einkorn (35.3430 ± 0.2390 mg kg⁻¹ dry matter) and *T. monococcum* 2103 (35.1871 ± 2.7328 mg kg⁻¹ dry matter).



Figure 4. 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 (47.8992 \pm 1.9912 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 5. 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 6). 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 6. 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 Influence of different growth stages (boot growth, stage 11, stage 10.2 and leafstage 10.2) on the accumulation of mercury (Hg) (mg kg⁻¹dry matter)



Figure 7. Wheat growth development according to Feekes

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

Figure 7 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 4. Influence of different growth stages (boot growth, stage 11, stage 10.2 and leaf stage 10.2) on the accumulation of mercury (mg kg⁻¹dry matter)

Stage of growth	Hg (mg kg ⁻¹ dry matter)
Boot growth stage	0.0028 ^a
Stage 11	0.0031 ^a
Stage 10.2	0.0152 ^b
Leaf-stage 10.2	0.0214 ^c
The content of mercury in 4 growth stage of investigated wheat was described in the Table 4. The maximum mercury content was of 0.0214 mg kg⁻¹dry matter for the leaf–stage 10.2 and of 0.0152 mg kg⁻¹dry matter in the stage 10.2, respectively, while in the boot growth stage, it was lowest (0.0028 mg kg⁻¹dry 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 Determination of mercury (Hg) in the analyzed grain wheat species in different wheat growth stages (mg kg⁻¹dry 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⁻¹dry matter) and expressed to one standard deviation. All varieties showed statistically differences (p < 0.05) (Table 5).

The higher mercury content was found in Schwedisches Einkorn in boot growth stage and leaf-stage 10.2, with the concentration 0.0210 ± 0.0016 and 0.0280 ± 0.0068 mg kg⁻¹dry matter, respectively, in comparison with the stage 11 when the lowest mercury level was recorded (0.0017 ± 0.0001 mg kg⁻¹dry 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 0.0017 ± 0.0001 mg kg⁻¹dry matter to 0.0042 ± 0.0007 mg kg⁻¹dry 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 0.0048 mg kg⁻¹dry matter. In contrast, *T. monococcum* 2101 had lower concentration of mercury, only 0.0016 \pm 0.0001 mg kg⁻¹ dry matter.

Table 5. The concentration 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⁻¹dry matter)

Wheat variety	Boot growth stage	Stage 11	Stage 10.2	Leaf-stage 10.2
SW Kadrilj ¹	0.0189 ± 0.0029^{b}	0.0019 ± 0.0002 ^a	0.0023 ± 0.0004^{ab}	0.0163 ± 0.0003 ^a
Granny ¹	0.0136 ± 0.0003^{a}	0.0026 ± 0.0001 ^{abc}	0.0048 ± 0.0001 ^c	0.0160 ± 0.0002 ^a
Jara ¹	0.0198 ± 0.0013 ^b	0.0023 ± 0.0001^{ab}	0.0048 ± 0.0003 ^c	0.0246 ± 0.0002 ^{bc}
Kaerntner Frueher ¹	0.0144 ± 0.0002 ^a	$0.0029 \pm 0.0012^{\text{ abcd}}$	0.0026 ± 0.0001 ^{ab}	0.0210 ± 0.0007 ^{ab}
Schwedisches Einkorn ²	0.0210 ± 0.0016 ^b	0.0017 ± 0.0001 ^a	0.0033 ± 0.0011 ^a	0.0280 ± 0.0068 ^c
T. monococcum 2101^{-2}	0.0136 ± 0.0003 ^a	0.0023 ± 0.0001^{ab}	0.0016 ± 0.0001 ^b	0.0265 ± 0.0003 ^{bc}
T. monococcum 2102 ²	0.0111 ± 0.0004 ac	0.0037 ± 0.0001 ^{cd}	0.0030 ± 0.0002 ^a	0.0159 ± 0.0005 ^a
<i>T. monococcum</i> 2103 ²	0.0101 ± 0.0001 ^c	0.0042 ± 0.0007 ^d	0.0026 ± 0.0008^{ab}	0.0217 ± 0.0007^{abc}
Krajova-Horny Tisovnik (Malov) ³	0.0145 ± 0.0005 ^a	0.0035 ± 0.0001 bcd	0.0024 ± 0.0001 ^{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

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 master 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). 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⁻¹dry 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 (Nan et al., 2002; 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⁻¹dry matter; 0.088 mg kg⁻¹dry matter and 29.2 mg kg⁻¹dry 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). Grahm (1992) reported the influence of zinc deficiency to the wheat production. Cakmak et al. (1997) and Cakmak et al. (1998) researched the responses of different plant varieties including rye, bread wheat, durum wheat and triticale on the situation of zinc deficiency. 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 \pm 0.03 mg kg⁻¹dry matter and 80.6 \pm 1.16 mg kg⁻¹dry matter, respectively), whereas the zinc concentration was smaller (28.26 \pm 3.18 mg kg⁻¹dry 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) and Karavoltsos et al. (2008), as well as Harcz et al. (2007).

Similarly, Hermandez-Martinez et al. (2012) in Spain found the higher amount of lead and cadmium in comparison with our work. The contents of lead and cadmium (median, Q1 and Q3) were (26.07; 21.36; 51.63 mg kg⁻¹dry matter) and (18.52; 16.56; 28.50 mg kg⁻¹dry matter), respectively. 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⁻¹dry matter for lead and 234.24 ± 79.88 mg kg⁻¹dry 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. In the experiment provided by Lucena (1993), the mercury availability in soil for plants is low. The amount of absorbing mercury is different among the plant varieties.

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⁻¹dry 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. Our results were appropriate to the data published by Fergusson et al. (1990) with the concentration of cadmium ranging from 0.2 to 1 mg kg⁻¹dry matter, and the concentration of lead lower than 0.75 mg kg⁻¹dry matter.

Several researches on the effects of lead on plants have been reported (Sharma, 2005; Seregin, 2008). According to Sarkar and Jane (1986), 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 (1990 and 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. In the environment, mercury is widely distributed pollutant. Some researches of Hitchcock (1957), Waldron (1975) and Goren (1976) reported the absorbing mercury from the atmosphere by plants.

Lucena (1993) reported the mercury availability in soil for plants is low. 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⁻¹dry matter). The content of zinc ranged from 12.06 to 80.33 mg kg⁻¹dry matter, while the amounts of lead and cadmium were 0.017 - 1.158 mg kg⁻¹dry matter; 0.006- 0.179 mg kg⁻¹dry 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. According to Hodgson (1963), 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.

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 $(0.0133 \pm 0.0005 \text{ mg kg}^{-1} \text{ dry matter})$ and the highest value stands for *T. monococcum* 2101 (0.0580 \pm 0.0009 mg kg⁻¹ dry matter). Wheat variety of high mercury content was represented mainly by spring wheat (Jara variety 0.0087 ± 0.0012 mg kg⁻¹ dry matter). Otherwise, the concentration of zinc was higher than other investigated heavy metals, ranging from $35.1871 \pm 2.7328 \text{ mg kg}^{-1}$ dry matter to $67.4047 \pm 1.9899 \text{ mg kg}^{-1}$ 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 (0.0152 mg kg⁻¹dry matter and 0.0214 mg kg⁻¹dry 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.

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9. APPENDIX

 Table 6. Characteristics of analysed wheat varieties

Wheat species / type	Variety	ECN ¹	BCHAR ²	Origin	Spike - awnedness (26) ³	Caryopsis - colour (40))	Glume- colour (35)	Glume - indumentum (36)	Spike density (25)	Plant - height (3)	Powdery mildew (58)	Country of origin
	Rudico	01C0200948	412048	CZE	7- awned	5 - brown	4 - red	1 - absent	9 - compact	6 – 96-110 cm	9	Czech Republic; legally protected cultivar, Crop Research Institute Prague (2006), ECN 01C0200948
Emmer wheat [<i>Triticum</i> dicoccum Schueb]	Kahler Emmer	01C0203989	412013	DEU	6- short awned	5 - brown	1 - white, straw- yellow	1 - absent	8 - very dense	6 – 96-110 cm	9	Germany; advanced /improved cultivar, ECN 01C0203989
(Schrank)] syn. Triticum turgidum, sp. dicoccon	<i>T.dicoccon</i> (Tapioszele)	01C0201282	412048	HUN	5 – long scurs	5 - brown	4 - brown	1 - absent	7 - dense	6 - 96- 110 cm	9	Not registered ⁴
Schrank	Krajova-Horny Tisovnik (Malov)	01C0200117	412013	CSK	4 - scurs	4 – light brown	2- white, with a gray edge	1 - absent	5 – medium dense	5 - medium 81-95 cm	9	Not registered ⁴
-	T.dicoccum No.8909	01C0204501	412013	DNK	5 – long scurs	5 - brown	1 - white, straw- yellow	1 - absent	7 - dense	7- 115 cm	9	Not registered ⁴

	Triticum monococcum L. var. flavescens KOERN. Escana	01C0201503	242002	ESP	6- short awned	4 – light brown	1 - white, straw- yellow	1 - absent	9 - compact	5 - medium 81-95 cm	8	Spain; traditional cultivar/landrace, seed sample from Gene bank of the Crop Research Institute Prague, ECN 01C0201503
Einkorn wheat (Triticum	Triticum monococcum L. var. vulgare Schwedisches Einkorn	01C0204053	242019	SWE	6- short awned	4 – light brown	1 - white, straw- yellow	1 - absent	9 - compact	6 - 96- 110 cm	9	Sweden; traditional cultivar/landrace, ECN 01C0204053
monococcum L.)	T.monococcum	01C0204039	242007	ALB	5 – long scurs	4 – light brown	1 - white, straw- yellow	1 - absent	5 – medium dense	6 - 96- 110 cm	9	Not registered ⁴
-	T.monococcum	01C0204040	242007	ARM	5 – long scurs	4 – light brown	1 - white, straw- yellow	1 - absent	7 - dense	6 - 96- 110 cm	9	Not registered ⁴
-	T.monococcum	01C0204044	242019	ALB	5 – long scurs	5 - brown	4 - brown	1 - absent	5 – medium dense	6 - 96- 110 cm	9	Not registered ⁴
Spring bread	Granny	01C0204799	635001	CZE	4 - semi- awned	5- brown	1 - white, straw- yellow	1 - absent	3 - lax	5 - medium 81-95 cm	7	Czech Republic; registered cultivar, Selgen, Ltd., Plant Breeding Station Úhřetice (2004), ECN 01C0204799
wneat (1 rificum - aestivum L.)	SW Kadrilj	01C0204877	635000	SWE	2 - awnless	2- yellow	1 - white, straw- yellow	1 - absent	5 - intermediate	5 - medium 81-95 cm	8	Sweden; registered cultivar (in CR 2006), Svalöf Weibull AB, ECN 01C0204877

Kärntner Früher	01C0203840	635104	AUT	1 - awnless	5- brown	4 - red	1 - absent	3 - lax	6 - 96- 110 cm	6	Austria; registered cultivar, Kärntner Saatbaugenossenschaft Reg. G.m.b.H (1960), ECN 01C0203840
Jara	01C0200100	635090	CSK	1 - awnless	5- brown	1 - white, straw- yellow	1 - absent	5 - intermediate	6- 110 cm	8,7	CSK Úhřetice Rdkm. Remo/Úhřetice400 (1975)
Postoloprtská přesívka 6	01C0200043	635090	CSK	1 - awnless	6- amber brown	1 - white, straw- yellow	1 - absent	5- medium dense	7- 115 cm	8,5	CSK Rdkm. S-LV Postoloprty (1922-1941)

Notes: the classifications were done according to Bareš et al. (1985);

¹ identification number of gene bank;

² taxonomical code (botanical characteristics);

³ number of descriptor,

The 1-9 scale in described part express state of descriptor of morphological character within the limits 1 to 9

(9 - The highest level, 0 - variable character); in the case of powdery mildew means 9 - very high resistant, 1 - very sensitive; ⁴Registration of Plant Genetic Resources in the Czech Republic.

Wheat variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C1	0.0020	0.0008	0.0074	0.0018	0.0012	0.0006	0.0008	0.0008	0.0014	0.0021	0.0012	0.0021	0.0017	0.0009	0.0013
C2	0.0024	0.0012	0.0099	0.0014	0.0014	0.0005	0.0006	0.0008	0.0016	0.0022	0.0012	0.0015	0.0014	0.0010	0.0015
C3	0.0027	0.0008	0.0088	0.0013	0.0012	0.0008	0.0005	0.0006	0.0014	0.0023	0.0012	0.0018	0.0012	0.0008	0.0013
Mean	0.0025	0.0009	0.0087	0.0015	0.0012	0.0006	0.0007	0.0008	0.0015	0.0022	0.0012	0.0018	0.0014	0.0009	0.0013
Standard deviation	0.0004	0.0002	0.0012	0.0003	0.0001	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001	0.0003	0.0002	0.0001	0.0001

Table 7. The concentration of mercury (Hg) in the analyzed grain wheat species in (mg kg⁻¹dry matter)

1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 5 = Postoloprstska presivka 6, 6 = Escana, 7 = Schwedisches Einkorn,

8 = *T. monococcum* 2101, 9 = *T. monococcum* 2102, 10 = *T. monococcum* 2103, 11 = Rudico, 12 = Kahler Emmer,

13 = T. dicoccon (Tapioszele), 14 = Krajova-Horny Tisovnik (Malov), 15 = T. dicoccon No 8909

Wheat variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C1	0.0327	0.0321	0.0135	0.0049	0.0348	0.0551	0.0589	0.0577	0.0551	0.0561	0.0215	0.0318	0.0269	0.0242	0.0297
C2	0.0365	0.0345	0.0127	0.0360	0.0378	0.0550	0.0550	0.0574	0.0556	0.0549	0.0237	0.0348	0.0322	0.0140	0.0301
C3	0.0383	0.0347	0.0137	0.0376	0.0344	0.0529	0.0570	0.0591	0.0409	0.0515	0.0243	0.0336	0.0226	0.0175	0.0412
Mean	0.0358	0.0338	0.0133	0.0262	0.0357	0.0543	0.0570	0.0580	0.0505	0.0542	0.0232	0.0334	0.0273	0.0186	0.0337
Standard deviation	0.0028	0.0014	0.0005	0.0048	0.0018	0.0012	0.0019	0.0009	0.0084	0.0024	0.0015	0.0015	0.0048	0.0052	0.0065

Table 8. The concentration of cadmium (Cd) in the analyzed grain wheat species in (mg kg⁻¹dry matter)

1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 5 = Postoloprstska presivka 6, 6 = Escana, 7 = Schwedisches Einkorn,

8 = *T. monococcum* 2101, 9 = *T. monococcum* 2102, 10 = *T. monococcum* 2103, 11 = Rudico, 12 = Kahler Emmer,

13 = T. dicoccon (Tapioszele), 14 = Krajova-Horny Tisovnik (Malov), 15 = T. dicoccon No 8909

Reference material: mean 0.0205, standard deviation 0.0050 (mg kg⁻¹ dry matter)

Wheat variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C1	0.2472	0.0595	0.0790	0.2116	0.0743	0.0463	0.0244	0.0438	0.0794	0.0544	0.0758	0.0372	0.0224	0.0640	0.1614
C2	0.2923	0.0504	0.0764	0.1775	0.1351	0.0634	0.0379	0.0445	0.2437	0.0977	0.0687	0.0268	0.1009	0.0195	0.0779
C3	0.2942	0.0761	0.0790	0.4961	0.1081	0.0353	0.0627	0.0483	0.0692	0.0510	0.0789	0.0317	0.0745	0.0357	0.1411
Mean	0.2779	0.0620	0.0781	0.2950	0.1058	0.0483	0.0416	0.0455	0.1308	0.0677	0.0745	0.0319	0.0659	0.0397	0.1268
Standard deviation	0.0266	0.0130	0.0015	0.1749	0.0305	0.0142	0.0194	0.0024	0.0979	0.0261	0.0052	0.0052	0.0399	0.0225	0.0435

Table 9. The concentration of lead (Pb) in the analyzed grain wheat species in (mg kg⁻¹dry matter)

- 1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 5 = Postoloprstska presivka 6, 6 = Escana, 7 = Schwedisches Einkorn,
- 8 = *T. monococcum* 2101, 9 = *T. monococcum* 2102, 10 = *T. monococcum* 2103, 11 = Rudico, 12 = Kahler Emmer,
- 13 = T. dicoccon (Tapioszele), 14 = Krajova-Horny Tisovnik (Malov), 15 = T. dicoccon No 8909

Reference material: mean 0.0793, standard deviation 0.0624 (mg kg⁻¹ dry matter)

Wheat variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C1	38.3609	38.1475	48.4177	48.0108	39.0194	28.0725	35.0993	38.8166	58.2923	72.7114	66.9932	67.2148	68.4049	60.2772	50.1877
C2	35.6936	32.7609	49.5859	47.3951	36.7272	49.4377	35.3528	38.2138	56.5457	70.4199	65.6528	66.7747	63.9059	61.3787	46.5619
C3	36.8904	34.6529	48.7548	43.8016	36.5646	43.1968	35.5771	42.0376	56.1694	74.7557	69.5683	66.1063	69.0622	62.8293	46.9479
Mean	36.9816	35.1871	48.9195	46.4025	37.4371	40.2356	35.3430	36.6893	57.0025	72.6290	67.4047	66.6986	67.1244	61.4951	47.8992
Standard deviation	1.3360	2.7328	0.6013	2.2734	1.3727	10.9861	0.2390	2.0559	1.1328	2.1690	1.9899	0.5582	2.8065	1.2800	1.9912

Table 10. The concentration of zinc (Zn) in the analyzed grain wheat species in (mg kg⁻¹dry matter)

- 1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 5 = Postoloprstska presivka 6, 6 = Escana, 7 = Schwedisches Einkorn,
- 8 = *T. monococcum* 2101, 9 = *T. monococcum* 2102, 10 = *T. monococcum* 2103, 11 = Rudico, 12 = Kahler Emmer,
- 13 = T. dicoccon (Tapioszele), 14 = Krajova-Horny Tisovnik (Malov), 15 = T. dicoccon No 8909

Reference material: mean 12.0227, standard deviation 6.7555 (mg kg⁻¹ dry matter)

Table 11. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of mercury (Hg)

[Tukeyův HSD test; proměnná H	g mg/kg (int	fofvæ	ariety)		
	Homogenní skupiny, alfa = .050)00 (Neúplné	é wh	ledáv	ání)		
	Chyba: meziskup. PČ = .00000	, sv = 30.00	0				
	variety	Hg mg/kg	1	2	3	4	5
Č. buňky		Průměr					
7	Schwedisches Einkom	0.000633	** **				
6	Escana	0.000633	** **				
8	T. monococcum 2101	0.000733	** **	****			
14	Krajova-Homy Tisovnik (Malov)	0.000900	** **	****			
2	Granny	0.000933	** **	****			
11	Rudico	0.001200	** **	****	** **		
5	Postoloprtská přesívka	0.001267	** **	****	** **	****	
15	T. dicoccum No. 8909	0.001367	** **	****	** **	****	
13	T. dicoccon (Tapioszele)	0.001433	** **	****	** **	****	
9	T. monococcum 2102	0.001467	** **	****	** **	****	
4	Kaertner Frueher	0.001500	** **	****	** **	****	
12	Kahler Emmer	0.001800		****	** **	****	
10	T. monococcum 2103	0.002200			** **	****	
1	SW Kadrilj	0.002367				****	
3	Jara	0.008700					** **

Table 12. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of lead (Pb)

	Tukeyův HSD test; proměnná P	b mg/kg (in	f of va	ariety)
	Homogenní skupiny, alfa = .050	00 (Neúplné	é vyhl	edáv	ání)
	Chyba: meziskup. PC = .00315	, sv = 30.00	0		
_	variety	Pb mg/kg	1	2	
Č. buňky		Průměr			
12	Kahler Emmer	0.031900	** **		
14	Krajova-Homy Tisovnik (Malov)	0.039733	** **		
7	Schwedisches Einkom	0.041667	** **		
8	T. monococcum 2101	0.045533	** **		
6	Escana	0.048333	** **		
2	Granny	0.062000	** **		
13	T. dicoccon (Tapioszele)	0.065933	** **		
10	T. monococcum 2103	0.067700	** **		
11	Rudico	0.074467	** **		
3	Jara	0.078133	** **		
5	Postoloprtská přesívka	0.105833	** **		
15	T. dicoccum No. 8909	0.126800	** **	** **	
9	T. monococcum 2102	0.130767	** **	** **	
1	SW Kadrilj	0.277900		** **	
4	Kaertner Frueher	0.295067		** **	

Table 13. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of cadmium (Cd)

	Tukeyův HSD test; proměnná c Homogenní skupiny, alfa = .050 Chyba: meziskup. PČ = .00004	d mg/kg (int)00 (Neúpln , sv = 30.00	fofva évyh)0	ariety Iedáv) <i>i</i> ání)	
Č buňky	variety	cd mg/kg	1	2	3	4
C. DUIRY	lara	F 10111C1	** **			
3	Jaia Viziouz Horzy Tisouzik (Malarià	0.013500	** **	****		
14	Krajova-Horny Tisovnik (Malov)	0.016067				
11	Rudico	0.023167	****	****		
4	Kaertner Frueher	0.026167	** **	****		
13	T. dicoccon (Tapioszele)	0.027233	** **	****		
12	Kahler Emmer	0.033400		****	****	
15	T. dic oc cum No. 8909	0.033667		****	****	
2	Granny	0.033767		****	****	
5	Postoloprtská přesívka	0.035667		****	****	
1	SW Kadrilj	0.035833		****	****	
9	T. monococcum 2102	0.050533			****	** **
10	T. monococcum 2103	0.054167				** **
6	Escana	0.054333				** **
7	Schwedisches Einkom	0.056967				** **
8	T. monococcum 2101	0.058067				** **

Table 14. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of zinc (Zn)

Tukovův LICD teot: proměnné 7	n na allea lint	f of tr	rictu	1				
Tukeyuv HSD test, promenna Z	n mg/kg (in		mety)				
Homogenni skupiny, alta = .050	00 (Neúpln	ê wh	ledáv	/ání)				
Chyba: meziskup. PC = 11.026	, sv = 30.00	00						
variety	Zn mg/kg	1	2	3	4	5	6	7
	Průměr							
Granny	35.18710	****						
Schwedisches Einkorn	35.34307	****						
SW Kadrilj	36.98163	****	****					
Postolopitská přesívka	37.43707	****	****					
T. monococcum 2101	39.68933	****	****	****				
Escana	40.23567	****	****	****				
Kaertner Frueher	46.40250		****	****				
T. dicoccum No. 8909	47.89917			****	****			
Jara	48.91947			****	****			
T. monococcum 2102	57.00247				****	****		
Krajova-Horny Tisovnik (Malov)	61.49507					****	****	
Kahler Emmer	66.69860					****	****	**:
T. dicoccon (Tapioszele)	67.12433						****	**:
Rudico	67.40477						****	**:
T. monococcum 2103	72.62900							**:
	Tukeyův HSD test; proměnná Z Homogenní skupiny, alfa = .050 Chyba: meziskup. PČ = 11.026 variety Granny Schwedisches Einkorn SW Kadrilj Postoloprtská přesívka T. monococcum 2101 Escana Kaertner Frueher T. dicoccum No. 8909 Jara T. monococcum 2102 Krajova-Horny Tisovnik (Malov) Kahler Emmer T. dicoccon (Tapioszele) Rudico T. monococcum 2103	Tukeyův HSD test; proměnná Zn mg/kg (inf Homogenní skupiny, alfa = .05000 (Neúplac Chyba: meziskup. PČ = 11.026, sv = 30.00 variety Zn mg/kg Průměr Granny 35.18710 Schwedisches Einkorn 35.34307 Schwedisches Einkorn 35.34307 Sv Kadrilj 36.98163 Postolopitská přesívka 37.43707 T. monococcum 2101 39.68933 Escana 40.23567 Kaertner Frueher 46.40250 T. dicoccum No. 8909 47.89917 Jara 48.91947 T. monococcum 2102 57.00247 Krajova-Horny Tisovnik (Malov) 61.49507 Kahler Emmer 66.69860 T. dicoccon (Tapioszele) 67.12433 Rudico 67.40477 T. monococcum 2103 72.62900	Tukeyův HSD test; proměnná Zn mg/kg (inf of va Homogenní skupiny, alfa = .05000 (Neúplné vh Chyba: meziskup. PČ = 11.026, sv = 30.00 variety Zn mg/kg Průměr 1 Schwedisches Einkorn 35.18710 Schwedisches Einkorn 35.98163 SVK Kadrilj 36.98163 Postoloprtská přesívka 37.43707 T. monococcum 2101 39.68933 Kaertner Frueher 46.40250 T. dicoccum No. 8909 47.89917 Jara 48.91947 T. monococcum 2102 57.00247 Krajova-Horny Tisovnik (Malov) 61.49507 Kahler Emmer 66.69860 T. dicoccon (Tapioszele) 67.12433 Rudico 67.40477	Tukeyův HSD test; proměnná Zn mg/kg (inf of variety Homogenní skupiny, alfa = .05000 (Neúplné vyhledáv Chyba: meziskup. PČ = 11.026, sv = 30.000 variety Zn mg/kg Průměr 1 2 Schwedisches Einkorn 35.18710 **** Schwedisches Einkorn 35.34307 **** SV Kadrilj 36.98163 **** **** Postoloprtská přesívka 37.43707 **** **** Kaertner Frueher 46.40250 **** **** Kaertner Frueher 46.40250 **** **** T. dicoccum No. 8909 47.89917 Jara 48.91947 Krajova-Horny Tisovnik (Malov) 61.49507 Kahler Emmer 66.69860 T. dicoccon (Tapioszele) 67.12433 T. monococcum 2103 72.62900	Tukeyův HSD test; proměnná Zn mg/kg (inf of variety) Homogenní skupiny, alfa = .05000 (Neúplné vyhleděvání) Chyba: meziskup. PČ = 11.026, sv = 30.000 variety Zn mg/kg Průměr 1 2 3 Granny 35.18710 **** 5 Schwedisches Einkorn 35.34307 **** 5 Postoloprtská přesívka 37.43707 **** **** Postoloprtská přesívka 37.43707 **** **** T. monococcum 2101 39.68933 **** **** Kaertner Frueher 46.40250 **** **** T. dicoccum No. 8909 47.89917 **** T. monococcum 2102 57.00247 Kaelt Emmer 66.69860 Kahler Emmer 66.69860 T. dicoccon (Tapioszele) 67.12433 T. monococcum 2103 72.62900	Tukeyův HSD test; proměnná Zn mg/kg (inf of variety) Homogenní skupiny, alfa = .05000 (Neúplné vyhledávání) Chyba: meziskup. PČ = 11.026, sv = 30.000 variety Zn mg/kg Průměr 1 2 3 4 Oranny 35.18710 **** Schwedisches Einkorn 35.34307 **** Schwedisches Einkorn 35.34307 **** Postoloprtská přesívka 37.43707 **** T. monococcum 2101 39.68933 **** **** T. monococcum 2101 39.68933 **** **** Kaertner Frueher 46.40250 **** **** **** T. dicoccum No. 8909 47.89917 **** **** **** Krajova-Horny Tisovnik (Malov) 61.49507 ***** Krajova-Horny Tisovnik (Malov) 61.49507 Kahler Emmer 66.69860	Tukeyův HSD test; proměnná Zn mg/kg (inf of variety) Homogenní skupiny, alfa = .05000 (Neúplné vyhledávání) Chyba: meziskup. PČ = 11.026, sv = 30.000 variety Zn mg/kg Průměr 1 2 3 4 5 Schwedisches Einkorn 35.18710 ***** - - - Schwedisches Einkorn 35.34307 **** - - - Schwedisches Einkorn 36.98163 **** * - - Postoloprtská přesívka 37.43707 **** * - - Postoloprtská přesívka 37.43707 **** **** - - - Kaertner Frueher 46.40250 ***** **** - - - T. dicoccum No. 8909 47.89917 - - ***** **** - T. monococcum 2102 57.00247 - - ***** ***** ***** Krajova-Horny Tisovnik (Malov) 61.49507 - - ***** ***** T. dicoccon (Tapioszele) 67.12433 - - - ***** <t< td=""><td>Tukeyův HSD test; proměnná Zn mg/kg (inf of variety) Homogenní skupiny, alfa = .05000 (Neúplné vyhledavání) Chyba: meziskup. PČ = 11.026, sv = 30.000 variety Zn mg/kg Průměr 1 2 3 4 5 6 Variety Zn mg/kg Průměr 1 2 3 4 5 6 Granny 35.18710 **** - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -</td></t<>	Tukeyův HSD test; proměnná Zn mg/kg (inf of variety) Homogenní skupiny, alfa = .05000 (Neúplné vyhledavání) Chyba: meziskup. PČ = 11.026, sv = 30.000 variety Zn mg/kg Průměr 1 2 3 4 5 6 Variety Zn mg/kg Průměr 1 2 3 4 5 6 Granny 35.18710 **** - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Wheat variety	1	2	3	4	7	8	9	10	14
C1	0.0181	0.0133	0.0204	0.0146	0.0199	0.0136	0.0115	0.0099	0.0148
C2	0.0221	0.0137	0.0184	0.0145	0.0202	0.0133	0.0107	0.0101	0.0148
C3	0.0164	0.0138	0.0207	0.0143	0.0229	0.0139	0.0110	0.0102	0.0139
Mean	0.0189	0.0136	0.0198	0.0144	0.0210	0.0136	0.0111	0.0101	0.0145
Standard deviation	0.0029	0.0003	0.0013	0.0002	0.0016	0.0003	0.0004	0.0001	0.0005

Table 15. The concentration of mercury (Hg) in the analyzed wheat species (in the boot growth stage according to Feekes scale) ((mg kg⁻¹dry matter)

1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 7 = Schwedisches Einkorn,

8 = *T.* monococcum 2101, 9 = *T.* monococcum 2102, 10 = *T.* monococcum 2103,

14 = Krajova-Horny Tisovnik (Malov)

Table 16. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of mercury (Hg) in the boot growth stage

	Tukeyův HSD test; proměnná boot growth stage (inf of stage_separately Homogenní skupiny, alfa = .05000 Chyba: meziskup. PČ = .00000, sv = 18.000										
	variety	boot growth stage	1	2	3						
Č. buňky		Průměr									
8	T. monococcum 2103	0.010067			****						
7	T. monococcum 2102	0.011067	****		****						
2	Granny	0.013600	****								
6	T. monococcum 2101	0.013600	****								
4	Kaertner Frueher	0.014467	****								
9	Krajova-Horny Tisovnik (Malov)	0.014500	****								
'1	SW Kadrilj	0.018867		****							
3	Jara	0.019833		****							
5	Schwedisches Einkorn	0.021000		****							

Wheat variety	1	2	3	4	7	8	9	10	14
C1	0.0020	0.0027	0.0023	0.0023	0.0016	0.0024	0.0037	0.0048	0.0034
C2	0.0021	0.0027	0.0023	0.0021	0.0017	0.0023	0.0038	0.0035	0.0035
C3	0.0017	0.0027	0.0022	0.0043	0.0017	0.0022	0.0037	0.0043	0.0036
Mean	0.0019	0.0026	0.0023	0.0029	0.0017	0.0023	0.0037	0.0042	0.0035
Standard deviation	0.0002	0.0001	0.0001	0.0012	0.0001	0.0001	0.0001	0.0007	0.0001

Table 17. The concentration of mercury (Hg) in the analyzed wheat species (in the stage 11 according to Feekes scale) (mg kg⁻¹dry matter)

1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 7 = Schwedisches Einkorn,

8 = *T.* monococcum 2101, 9 = *T.* monococcum 2102, 10 = *T.* monococcum 2103,

14 = Krajova-Horny Tisovnik (Malov)

Table 18. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of mercury (Hg) in the stage 11

	Tukeyův HSD test; proměnná stage 11 (inf of stage_separately) Homogenní skupiny, alfa = .05000 Chyba: meziskup, PČ = .00000, sv = 18.000									
Č. buňky	variety	stage 11 Průměr	1	2	3	4				
5	Schwedisches Einkorn	0.001667	****							
1	SW Kadrilj	0.001933	****							
3	Jara	0.002267	****	****						
6	T. monococcum 2101	0.002300	****	****						
2	Granny	0.002700	****	****	****					
4	Kaertner Frueher	0.002900	****	****	****	****				
9	Krajova-Horny Tisovnik (Malov)	0.003500		****	****	****				
7	T. monococcum 2102	0.003733			****	****				
8	T. monococcum 2103	0.004200				****				
Wheat variety	1	2	3	4	7	8	9	10	14	
--------------------	--------	--------	--------	--------	--------	--------	--------	--------	--------	
C1	0.0024	0.0047	0.0047	0.0024	0.0026	0.0017	0.0029	0.0033	0.0025	
C2	0.0027	0.0048	0.0051	0.0026	0.0028	0.0016	0.0031	0.0018	0.0024	
C3	0.0019	0.0048	0.0047	0.0026	0.0046	0.0015	0.0031	0.0026	0.0024	
Mean	0.0023	0.0048	0.0048	0.0026	0.0033	0.0016	0.0030	0.0026	0.0024	
Standard deviation	0.0004	0.0001	0.0003	0.0001	0.0011	0.0001	0.0002	0.0008	0.0001	

Table 19. The concentration of mercury (Hg) in the analyzed wheat species (in the stage 10.2 according to Feekes scale) (mg kg⁻¹dry matter)

C1, C2, C3= replicates

1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 7 = Schwedisches Einkorn,

8 = *T.* monococcum 2101, 9 = *T.* monococcum 2102, 10 = *T.* monococcum 2103,

14 = Krajova-Horny Tisovnik (Malov)

Table 20. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of mercury (Hg) in the stage 10.2

	Tukeyův HSD test; proměnná stage 10.2 (inf of stage_separately) Homogenní skupiny, alfa = .05000 Chyba: meziskup. PČ = .00000, sv = 18.000								
	variety stage 10.2 1 2 3								
C. buňky		Průměr							
6	T. monococcum 2101	0.001600		****					
1	SW Kadrilj	0.002333	****	****					
9	Krajova-Horny Tisovnik (Malov)	0.002433	****	****					
4	Kaertner Frueher	0.002533	****	****					
8	T. monococcum 2103	0.002567	****	****					
7	T. monococcum 2102	0.003033	****						
5	Schwedisches Einkorn	0.003333	****						
2	Granny	0.004767			****				
3	Jara	0.004833			****				

Wheat variety	1	2	3	4	7	8	9	10	14
C1	0.0161	0.0158	0.0246	0.0204	0.0264	0.0268	0.0162	0.0210	0.0230
C2	0.0162	0.0159	0.0245	0.0202	0.0222	0.0264	0.0162	0.0222	0.0228
C3	0.0166	0.0162	0.0249	0.0218	0.0354	0.0263	0.0153	0.0220	0.0228
Mean	0.0163	0.0160	0.0246	0.0210	0.0280	0.0265	0.0159	0.0217	0.0229
Standard deviation	0.0003	0.0002	0.0002	0.0007	0.0068	0.0003	0.0005	0.0007	0.0001

Table 21. The concentration of mercury (Hg) in the analyzed wheat species (in the leaf-stage10.2 according to Feekes scale) (mg kg⁻¹dry matter)

C1, C2, C3= replicates

1= SW Kadrilj, 2 = Granny, 3 = Jara, 4 = Kaerntner Frueher, 7 = Schwedisches Einkorn,

8 = *T.* monococcum 2101, 9 = *T.* monococcum 2102, 10 = *T.* monococcum 2103,

14 = Krajova-Horny Tisovnik (Malov)

Table 22. One way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of mercury (Hg) in the leaf stage 10.2

	Tukeyův HSD test; proměnná leaf stage 10.2 (inf of stage_sepa Homogenní skupiny, alfa = .05000 Chyba: meziskup. PČ = .00001, sv = 18.000							
	variety	1	2	3				
Č. buňky	Průměr							
7	T. monococcum 2102	0.015900	****					
2	Granny	0.015967	****					
1	SW Kadrilj	0.016300	****					
4	Kaertner Frueher	0.020800	****	****				
8	T. monococcum 2103	0.021733	****	****	****			
9	Krajova-Horny Tisovnik (Malov)	0.022867		****	****			
3	Jara	0.024667		****	****			
6	T. monococcum 2101	0.026500		****	****			
5	Schwedisches Einkom	0.028000			****			

Table 23. Two way factorial analysis of variance (ANOVA), Tukey HSD test, $\alpha = 0.05$ of mercury (Hg) in different growth stages (boot growth, stage 11, stage 10.2 and leaf-stage 10.2)

	Tukey ův HSD test; proměnná Hg mg/kg (inf of stag Homogenní skupiny, alfa = .05000 Chyba: meziskup. PČ = .00001, sv = 96.000stage og growthHg mg/kg123PrůměrPrůměr123								
Č. buňky									
2	stage 11	0.002800	****						
3	stage 10.2	0.003048	****						
1	boot growth stage	0.015222		****					
4	leaf stage 10.2	0.021415			****				