www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 9/3 (2016) 12-25

Research article/Araştırma makalesi

Biochemical and physiological responses of metal toxicity in some barley and wheat varieties from Central Anatolia

Elif ÖZTETİK *[1]

[1] Anadolu University, Faculty of Science, Department of Biology, 26470, Eskişehir, Turkey

Abstract

The factors like increased traffic load due to population denstity, environmental pollution, industrial activities and improper practices of agricultural applications could be counted as a source of heavy metal accumulation in air, water and soil. The effects of heavy metals which accumulated in soils continue for a long period of time as a result of food chain between organisms and considered as a serious threat to human health. With this study, toxic effects of heavy metals on germination, roots and shoots lengths, water, protein, glutathione (GSH) contents and glutathione *S*-transferase (GST) activities of barley (*Hordeum vulgare* cv. Ince 04) and wheat (*Triticum aestivum cv*. Yunus) varieties were investigated through different concentrations of single lead chloride ($PbCl_2$) and cadmium chloride ($CdCl_2$) (0, 1.5 and 3.0 mM) and combined $PbCl_2 + CdCl_2$ (1.5 + 1.5 and 3.0 + 3.0 mM) applications. In conclusion, it was shown that biochemical and physiological mechanisms were affected differently with varying concentrations of heavy metals and Ince 04 (barley) were found to be more tolerant to heavy metal stress by comparing to Yunus (wheat). For more tolerant variety (Ince 04), the observed values as follows after 3.0 mM $PbCl_2 + CdCl_2$ treatment; 80% decrease in germination, 75 and 65% decrease of lengths in roots and shoots, 73 and 65 % decrease of water contents in roots and shoots, 134 and 40% increase of GSH contents in roots and shoots, 15 and 50 % increase of protein contents in roots and shoots and 70 and 39 % increase of GST activities in roots and shoots.

Key words: barley, germination, GST, heavy metals (Cd, Pb), wheat

---------- * ----------

Orta Anadolu'ya ait bazı arpa ve buğday varyetelerinin metal toksitesine verdiği biyokimyasal ve fizyolojik tepkiler

Özet

Nüfus yoğunluğu nedeniyle artan trafik yükü, çevresel kirlenme, endüstriyel aktiviteler ve yanlış tarım uygulamaları gibi faktörler, hava, su ve topraktaki ağır metal birikiminin kaynağı olarak sayılabilir. Toprakta biriken ağır metallerin etkileri, organizmalar arasındaki besin zinciri etkisi ile daha uzun süreli olmakta ve bu durum insan sağlığı için ciddi bir tehlike olarak kabul edilmektedir. Bu çalışmada, hem tekli kurşun klorid ($PbCl_2$) ve kadmiyum klorid ($CdCl_2$) (0, 1.5 ve 3.0 mM) ve hem de kombine $PbCl_2 + CdCl_2$ (1.5 + 1.5 ve 3.0 + 3.0 mM) uygulamaları ile ağır metallerin arpa (*Hordeum vulgare* cv. Ince 04) ve buğday (*Triticum aestivum cv*. Yunus) varyetelerindeki çimlenme, kök ve yaprak uzunluğu, su, protein ve glutatyon (GSH) miktarları ile glutatyon *S*-transferaz (GST) aktiviteleri üzerindeki toksik etkileri araştırılmıştır. Sonuç olarak, farklı ağır metal konsantrasyonlarının biyokimyasal ve fizyolojik mekanizmaları farklı şekillerde etkilediği ve Ince 04 (arpa) çeşidinin ağır metal stresine Yunus (buğday) çeşidine nazaran daha toleranslı olduğu bulunmuştur. Daha toleranslı olan çeşit (Ince 04) için, 3.0 mM $PbCl_2 + CdCl_2$ uygulaması sonrasında gözlenen değerler; çimlenmede % 80 oranında azalma, kök ve yaprak uzunluklarında %75 ve 65 oranlarında azalma, su miktarlarında kökler ve yapraklarda %73 ve 65 oranlarında azalma, GSH miktarlarında kökler ve yapraklarda %134 ve 40 oranlarında artma, protein miktarlarında kökler ve yapraklarda %15 ve 50 oranlarında artma ile GST aktivitelerinde kökler ve yapraklarda %70 ve 39 oranlarında artma şeklindedir.

Anahtar kelimeler: arpa, çimlenme, GST, ağır metaller (Cd, Pb), buğday

* *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: ++902223350580 Ext:4730; Fax.: +902223204910; E-mail: eoztetik@anadolu.edu.tr
 BioDiCon. 608-1016

1. Introduction

Archeological evidence indicates that about 10,000 years ago human cultures began the practice of agriculture in several areas of the World (Levetin and McMahon, 2011) and this is considered as beginning of civilization. However, the World is faced with two major problems today. These are insufficient resources of food and environmental pollution. These problems are in close interactions with each others, as increasing human population cause to more environmental pollution and increasing pollutants has an important impact on the health quality of living organisms.

Heavy metals (HMs) are kept under environmental pollutant category due to their toxic effects on plants, animals and human being. HM can be divided into four major groups based on their health importance; (i) Essential metals: Copper (Cu), zinc (Zn), cobalt (Co), chromium (Cr), manganese (Mn) and iron (Fe), (ii) Non-essential metals: Barium (Ba), aluminium (Al), lithium (Li) and zirconium (Zr), (iii) Less toxic metals: Tin (Sn) and arsenic (As) and (iv) Highly toxic metals: Mercury (Hg), cadmium (Cd) and lead (Pb) (Sharma and Agrawal, 2005). There are different sources of HMs in the environment such as natural, agricultural, industrial, domestic effluent, atmospheric sources and other sources, like refuse incineration, landfills and transportation (automobiles, diesel-powered vehicles and aircraft) (Gill, 2014). HMs are persistent, bioaccumulative and they do not readily break down in the environment or not easily metabolized. Therefore, they can accumulate in soils and plants (Sharma and Agrawal, 2005) and have an important impact on the health of animals and humans via food chains. Among non-nutrient and highly toxic metals, Cd and Pb attract more attention than others (Sharma and Agrawal, 2005; Nagajyoti et al., 2010), because of their wide range of use and distribution (Table 1).

Due to increased industrilization, traffic load and improper practices of agricultural applications, Turkey is facing Cd and Pb (although other HMs) contamination problems similar to other countries. At the moment, Central Anatolia is the main grain supplier of Turkey and wheat and barley are the traditional products of this high plateau region. According to market year (MY) 2015 values, total wheat area in Central Anatolia was 3,000,000 ha (with 6.550.000 metric tones [MT] production), which was more than 38% of total wheat area (7.860.000 ha) in Turkey. However, the land area and the production dropped to 2.980.000 ha and 5.100.000 MT according to MY 2016 in Central Anatolia. Similarly, barley planting area is slightly lower compared to the previous year due to a large amount of fallow area in Central Anatolia and production was 5.400.000 MT in MY 2016, which is down from 7.400.00 MT in MY 2015 (USDA Foreign Agricultural Service, 2016). As seen from the given values and as expressed by Yücel and Yücel (2013), wheat and barley plantable areas are reaching to their limits in Turkey and gradually shrinking each year in Central Anatolia.

Table 1. Sources of Cd and Pb contaminations from different industries (Modified from Sharma and Agrawal, 2005; Nagajyoti et al., 2010)

Industry	**Cd**	**Pb**
Mining operations and ore outcrops	✓	✓
Metallurgy and electroplating	✓	✓
Waste	✓	✓
Sewage sludge	✓	✓
Chemical industries	✓	✓
Dyes and pigments	✓	✓
Petroleum refining	✓	✓
Coal burning	✓	
Phosphate fertilizers	✓	
Photography	✓	
Textiles	✓	
Nuclear technology	✓	
Chlor-alkali production	✓	
Leaded gasoline		✓
Print		✓

The exposure of plants to toxic levels of HMs triggers a wide range of physiological and biochemical changes (Dubey, 2011; Villiers et al., 2011). The general visual evidence of HM toxicity is a plant growth reduction (Sharma and Dubey, 2007), which including leaf chlorosis, necrosis, turgor loss, decreased rate of seed germination and a impeded photosynthetic apparatus, often correlated with plant death (Dalcarso et al., 2010). There are also changes in membrane permeability, uptake of other elements, water balance (Sharma and Dubey, 2005) and enzyme activities (Reddy et al., 2005), etc. These effects are related to physiological, ultrastructural and biochemical alterations in plant tissues due to presence of HMs (Gamalero et al., 2009). However, while the potential mobilization of metals in soil depends primarily on metal contents, dissolved organic matter, fertilization, soil pH and other characteristics of soil, like temperature, aeration, moisture and cation exchange capacity are counted as other important factors. HM toxicity to

plants also varied with plant species, its size, the root system, availability of the specific metal, concentration, chemical form, soil composition and pH (Gill, 2014).In cells, excess HMs cause those altered effects through the formation of highly toxic reactive intermediates such as superoxide radicals ($^{\bullet}O_2^-$), hydroxyl radicals ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Halliwell, 1995). These reactive intermediates are collectively known as **R**eactive **O**xygen **S**pecies (ROS) (Weckx and Clijsters, 1997) and induces oxidative stress in the plant cells. With their toxic effects, they creates some abnormal situations in transpiration, stomatal movements, water intake, protein synthesis, membrane stability and hormonal balance in plants generally (Seregin and Ivanov, 2001). Although, to protect themselves from potentially damaging effects of the oxidative stress induced by ROS, plants have some antioxidant (or oxygen radical detoxifying) enzymes (like glutathione *S*-transferases [GST]) and low molecular weight non-enzymatic antioxidants (like glutathione [GSH]) as defense mechanisms (Gill and Tuteja, 2010; Verma and Dubey, 2003).

GSTs (EC.2.5.1.18) have been found in all living species, including plants, animals, bacteria (Mannervik and Danielson, 1988) and archaea (Öztetik and Çakır, 2014). They are a superfamily of multifunctional enzymes that catalyze the conjugation of thiol group of the GSH to diverse electrophilic centres on lipophilic molecules, with the formation of rather less active end products. In plants, the resulting water soluble and less toxic glutathione *S*-conjugates are coupled to internal compartmentation due to the lack of effective excretion pathways (Rea, 1999). In addition to their enzymatic activities, GSTs have been demonstrated that they also participate in antioxidative defences to protect organisms against the adverse effects of ROS reactions (Hajime et al., 2005). Their existence in plants was first shown with the discovery of the relationship between the GST from maize and it's protective activity against injury of herbicide atrazine (Frear and Swanson, 1970). On the other hand, GSH (g-L-glutamyl- L-cysteinyl-glycine) is an important thiol compound against active oxygen species, including toxic metals, for cell protection (May et al. 1998). Biological activity of GSH depends on the unique amino acid cysteine, which is responsible for the binding of HMs (Backor and Loppi, 2009). GSH plays a key role in the cellular antioxidant defence mechanism in many ways: (i) GSH detoxify ROS through ascorbate - glutathione cycle, (ii) GST catalyze the conjugation of GSH with metal ions, (iii) GSH is also utilized by phytochelatin synthase (PCS) in the synthesis of phytochelatins (PCs) (Yadav, 2010).

In this study, toxic effects of HM on germination, roots and shoots lengths, water, protein, GSH contents and GST activities of barley (*Hordeum vulgare* cv. Ince 04) and wheat (*Triticum aestivum cv*. Yunus) varieties were investigated through different concentrations of both single and combined cadmium chloride ($CdCl_2$) and lead chloride ($PbCl_2$) applications.

2. Materials and methods

1.1. Plant materials

In this study, *Hordeum vulgare* L. cv. Ince 04 and *Triticum aestivum* L. cv. Yunus cultivars were used, as they are commonly planted in the area of Central Anatolia. They are used for bread and beer making and as an animal food. Plant seeds were obtained from Transitional Zone Agricultural Research Institute (Eskisehir, Turkey) and both are registered varieties. Some of the specifications of those varieties were given in Table 2. All seed were pure, means they have never been treated with any additive reagents. At the beginning of germination and growth experiments, seeds were washed and immersed in tap water for 2h and then in distilled water for a further 2h.

Table 2. Morphologic, agricultural and quality specifications of Ince 04 and Yunus varieties (Tabulated from the information at Transitional Zone Agricultural Research Institute website)

Specifications	***Hordeum vulgare* L. cv. Ince 04**	***Triticum aestivum* L. cv. Yunus**
Morphologic specifications	Two-rowed ear, white grain, 95-105 cm height	Spelt white ear, red grain, 110-115 cm height
Agricultural specifications	Winter and lodging resistant, Min-Max yield: 200-500 kg/daa	Winter and lodging resistant, Min-Max yield: 600-820 kg/daa
Quality specifications	1000-Grain weight: 33-49 g., Hectolitre weight: 62-73 kg/hl, % of protein: 7.7-13.6	1000-Grain weight: 38-41 g., Hectolitre weight: 78-80 kg/hl, % of protein:12.5-14

1.2. Seed germination and seedling growth

Twenty seeds were placed in each petri dish and germination was tested on wet Whatman (No. 42mm) filter paper. A piece of filter paper was placed on a petri dish and moistened with 3 ml of different concentrations of single $PbCl_2$ and $CdCl_2$ (0, 1.5 and 3.0 mM) and combined $PbCl_2$ + $CdCl_2$ (1.5 + 1.5 and 3.0 + 3.0 mM) solutions. The Pb and Cd solutions were freshly prepared. The controls were arranged by dampening the filter paper with 3 ml of distilled water (dH_2O) in a growth chamber (NUVE ID-501) at 22°C (± 1°C) in the dark for 3 days. Afterwords, seeds exposed to 16 h photoperiod for 7 days. Germinated seeds were counted on 10 days after initiation of treatment. Each treatment was replicated three times. Seeds were considered as germinated when the radicle touched the seed bed (Yücel, 2000). Germination rate is estimated by using the following formula: Germination percentage = Seeds germinated / Total seeds x 100 (Manmathan and Lapitan, 2013).

1.3. Root and shoot lengths measurements

Plant root and shoot were separated and their lengths measured using a measuring scale.

1.4. Water contents

After fresh weights of roots and shoots were measured with an analytical balance, those plant parts were kept in the incubator at 70°C for 48h. At the end of this period, samples were placed into desiccator until they reach to room temperature. Afterwords, dried samples were measured with an analytical balance again. Water contents of the samples were calculated according to the following formula: Water contents (%) = Fresh weight (FW) – Dry weight (DW) / Fresh weight (FW) x 100 (Wojcik and Tukendorf, 1999).

1.5. Plant growth conditions

For each variety, 20 seeds were germinated on Whatman (No. 42 mm) filter papers soaked with dH_2O in a growth chamber at 22°C (± 1°C) in the dark for 3 days and further exposed to 16 h photoperiod for 7 days. After germination, the seedlings were transferred to plastic beakers containing 250 ml of Hoagland solution, including 2mM $Ca(NO_3)_2$, 1mM $NH_4H_3PO_4$, 3mM KNO_3, 0.5mM $CuCl_2$, 50mMKCl, 25mM H_3BO_3, 2mM $ZnCl_2$, 0.5mM $(NH_4)_6MO_7O_{24}$, 1mM $MgSO_4$, 2mM $MnCl_2$ and 20mM Na_2Fe-EDTA (Hoagland, D. R., Arnon 1950). Concentrations of Mg^{2+} were maintained at 1mM by the addition of $MgCl_2$ and pH of the nutrient solution was adjusted to 6.5 ± 0.1 with 0.1M NaOH. The solution was aerated continuously and replaced with fresh solution every week. Beakers were arranged in a randomized block design with Pb and Cd treatment applied in triplets. Ten plants were arranged in a beaker with 8 cm in diameter. After 3 days of growth in the chamber, different single $PbCl_2$ and $CdCl_2$ (0, 1.5 and 3.0 mM) and combined $PbCl_2$ + $CdCl_2$ (1.5 + 1.5 and 3.0 + 3.0 mM) solutions were applied into the nutrient medium. Plants were exposed to a 16 h photoperiod for further 7 days. Seedlings were harvested 10 days after the application of treatments. After roots and shoots were separated, they are pulverized with liquid N_2 for further analysis. Otherwise, pulverized materials stored at -80°C, for later assays.

1.6. GSH detemination

Pulverized roots and shoots were homogenized in a ratio of 1:4 w/v, with 5% (w/v) TCA by using UltraTurrax at 13,500 rpm for 90 s at 4°C. The homogenate was centrifuged at 4°C, 12,000 rpm for 15 min and the pH of the supernatant was adjusted to 4.0 - 5.0 with 1M NaOH. The content of GSH in crude extract was determined using the Ellman (DTNB) procedure (Ellman, 1959). The absorbance of the reaction mixture was read at 412 nm with the help of the standard curve calibrated by using reduced GSH.

1.7. Total protein determination

Protein content of the samples was determined according to the method of Lowry et al. (1951).

1.8. Preparation of cytosolic extract

Pulverized roots and shoots were extracted, in a ratio of 1:3 w/v, with 100 mM pH 7.0 phosphate buffer, including 0.05 mM DTE, 1 mM EDTA and 3.5 % (w/v) PVPP at 4 °C. The mixture was then homogenized for 4 × 30 s. periods by Ultra-Turrax at 13 500 rpm on ice. The crude homogenate was centrifuged at 15,000 rpm for 30 min. at 4 °C. The pellet was discarded and the supernatant fraction was immediately subjected to protein determination and enzyme activity measurements, after passed through a layer of sterile filter paper (Oztetik, 2015).

1.9. Enzyme analytical method

All enzyme activity assays were conducted at 25°C by using a spectrophotometer equipped with thermoregulated cell holder. The GST activities with 1-chloro-2,4 dinitrobenzene (CDNB) as substrate were determined spectrophotometrically by monitoring the formation of the conjugation product at 340 nm according to the method of Habig et al. (1974), as modified previously (Oztetik 2005). The reactions were started with the addition of wheat or barley cytosol and followed for 3 min, which is in the linear period of the reaction. Incubation mixtures without the enzyme source were used as blanks (non-enzymatic reactions). The activity was calculated from the slopes of initial reaction rates using the ε values of CDNB of 9.6 mM^{-1} cm^{-1} (Habig and Jakoby 1981).

2.10. Statistical analyses

The values of GST activities, root and shoot lengths, soluble protein, GSH, water contents and germination rates are presented as a percentage of the corresponding controls. The absolute values for control samples from experiments are indicated in the legends of figures. Statistical comparisons were carried out by using two-sample t test

for germination rates and by 2x2 factorial design for the other parameters using the Minitab (16.2.0) statistics software. Data presented are the averages of at least three independent experiments, each of them in three replications.

3. Results

In this study, the toxic effects of different concentrations of Pb and Cd solutions were detected by single and combined applications on *Hordeum vulgare* L. cv. Ince 04 and *Triticum aestivum* L. cv. Yunus varieties. In the light of obtained data, results were compared with the control samples by paying special attention to some biochemical and physiological parameters tested, like seed germination, roots and shoots lengths, water, protein, GSH contents and GST activities.

1.10. Germination

Without any treatment, the absolute values of seed germinations for controls of Yunus and Ince-04 samples were recorded as 18 and 19 out of 20 seeds, respectively. However, according to the results, the germination of seeds of both plant varieties, which were used in these experiments was found to be affected with Pb and Cd toxicity (Figure 1). Except the stationary increase with 1.5mM Cd treatment for both plants, the seed germination decreased significantly with the increase of Pb and Cd concentrations in a dose dependent manner.

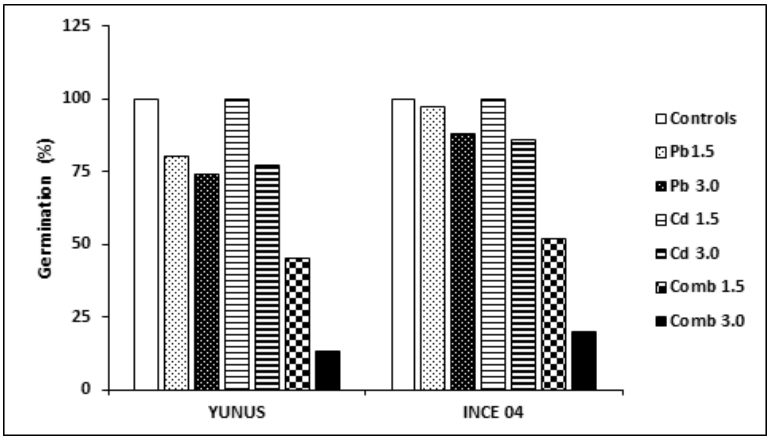

Figure 1. Effects of metal treatments on seed germination. Changes in values were depicted as a percentage of their controls. The absolute values can be seen in text

The reduction in seed germination for Yunus variety was observed more significant than of Ince 04, by comparing to their respective controls. When Pb and Cd were applied together, their inhibitory effects on seed germinaton considerably exceeded the effects of their single applications. As seen from Figure 1, while the application of 1.5 and 3.0 mM Pb+Cd caused 48 and 80% of reduction in seed germination for Ince 04 respectively, these values increased up to 55 and 87% for Yunus variety, by comparing to controls.

1.11. Root and shoot lengths

The absolute values of lengths of shoots and roots for controls of Yunus and Ince-04 samples were observed as 13.33, 13.79, 8.72 and 10.98 cm, respectively. As a result of treatment with different concentrations of metal solutions, root and shoot length of crop seedlings were significantly affected by increasing Pb and Cd concentrations and root growth seem to be more sensitive than shoot growth (Figure 2). The root length of crop seedlings at 3.0mM Pb and Cd were significantly less (62 and 50% for Yunus and 75 and 60% for Ince 04, respectively) than that of the control seedlings. At low concentrations of Pb and Cd (1.5mM), induced growth were observed on barley roots. For roots, Pb and Cd induced reduction in growth was dose dependent and Cd effect was more pronounced than Pb. However, in shoots, while Cd was showing the same dose dependent manner as in the roots, Pb cause a growth induction with its higher concentration (3.0mM) in barley (Ince 04). Both of those shoot and root growth reductions in Yunus variety were found to be higher than of Ince 04, by comparing to their respective controls. Similar to inhibition of seed germination, application of Pb + Cd combinations caused an additive effects on root and shoot lengths reduction.

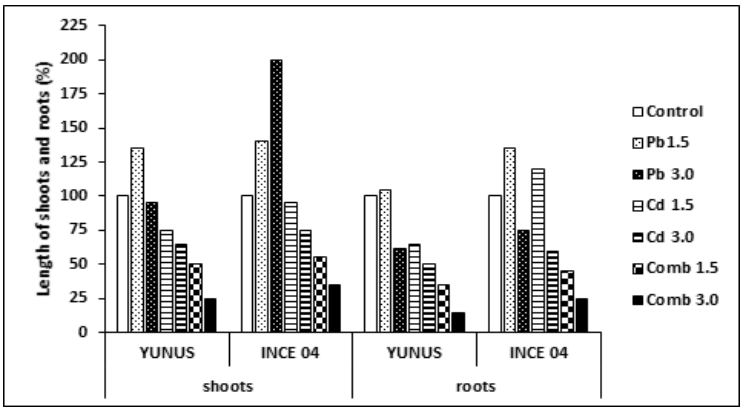

Figure 2. Effects of metal treatments on lengths of shoots and roots. Changes in values were depicted as a percentage of their controls. The absolute values can be seen in text

1.12. Water contents

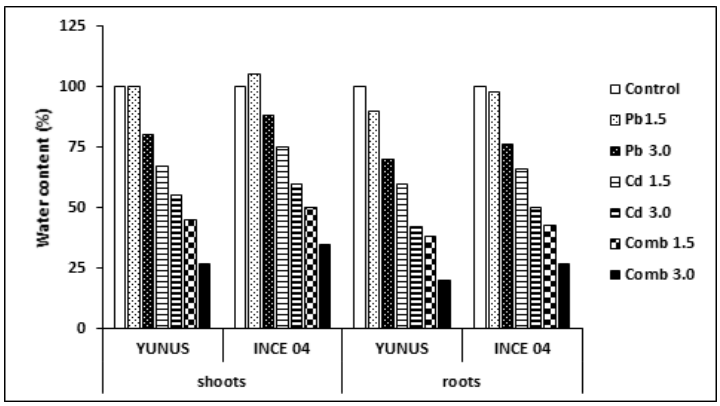

Figure 3. Effects of metal treatments on water contents. Changes in values were depicted as a percentage of their controls. The absolute values can be seen in text

The absolute values of water contents for controls of Yunus and Ince-04 samples were recorded as 89.22 and 96.0 % for shoots, 69.22 and 87.55 % for roots, respectively. However, the water contents of both plant varieties were found to be adversely affected with applied metal toxicity, in which the water contents of shoots and roots were decreased with increasing Pb and Cd concentrations (Figure 3). As for both parts of plants examined in differrent varieties, the effects of metals were in dose dependent manner and the water contents of roots more sensitive to metals than that of the shoots by comparing to their relative controls. On the ther hand, the combined application of Pb + Cd exceeded the effects of their single applications as similar to above findings in seed germination and lengths of shoots and roots. As depicted in Figure 3, while 55 and 73% of decreases were observed in water contents of Yunus shoots (with 1.5 and 3.0mM of Pb + Cd treatment, respectively), the corresponding values for Ince 04 shoots were again found to be less than their respective controls by 50 and 65%. The roots water contents were observed to be more reduced, as 62 and 80% (for Yunus), 57 and 73% (for Ince 04) of decreases were recorded compared to control values.

1.13. GSH contents

The absolute values of GSH contents for controls of Yunus and Ince-04 samples were calculated as 8.00 and 10.00 μg/ml for shoots, 22.55 and 30.00 μg/ml for roots, respectively. After treatment, GSH content of the roots was greatly enhanced with Pb and Cd at all tested concentration. By contrast, the shoots appeared less sensitive to metals exposure (Figure 4). The increase of GSH concentration in the roots of both plants was dose dependent. While 220 and 234% of increases were observed in GSH contents of Ince04 roots (with 1.5 and 3.0mM of Pb + Cd treatment, respectively), the contents of GSH were found to be 160 and 171% for Yunus roots for the same concentrations, compared to the controls.

In the case of plant shoots, even Yunus shoots had lower GSH levels than their own roots, they were still having some GSH levels with 3.0mM Cd and 1.5mM Pb + Cd treatment (115 and 125% of control, respectively). Similar to roots, Ince 04 shoots have shown dose dependent manner with increasing metal concentrations. However, even the application of Pb + Cd combinations caused a slight additive effects in GSH contents in Ince 04 shoots, which is not observed for Yunus shoots with 3.0mM Pb + Cd application, by comparing to other parameters tested.

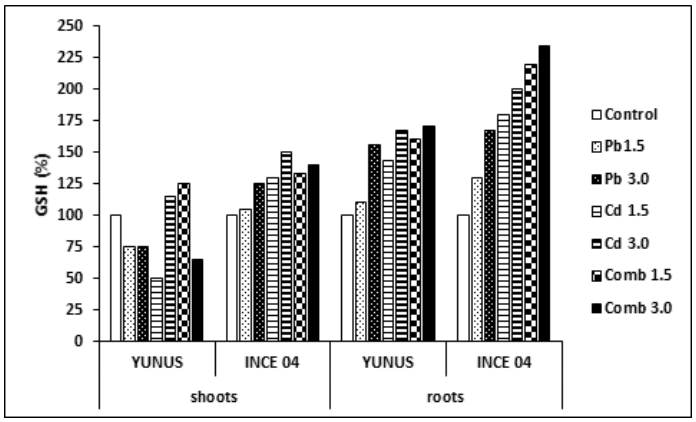

Figure 4. Effects of metal treatments on GSH contents. Changes in values were depicted as a percentage of their controls. The absolute values can be seen in text.

1.14. Total protein contents

The absolute values of protein contents for controls of Yunus and Ince-04 samples were measured as 2.00 and 3.00 mg/ml for shoots, 1.00 and 1.50 mg/ml for roots, respectively. After exposured to metal solutions, the increase in protein content of shoots were observed more significant than roots, by comparing to their respective controls (**Figure 5**). The maximum increase were found in protein contents of Ince 04 shoots with 1.5 and 3.0mM of Pb + Cd treatment as 135 and 150% of controls. The corresponding values for Yunus shoots were 120 and 135% for the same concentrations. For both parts of plants examined in differrent varieties, the effects of Pb and Pb + Cd combinations were in dose dependent manner, but increasing Cd concentrations were not effective for neither shoots or roots in that manner. For shoots, the combined application of Pb + Cd exceeded the effects of their single applications far more than the roots.

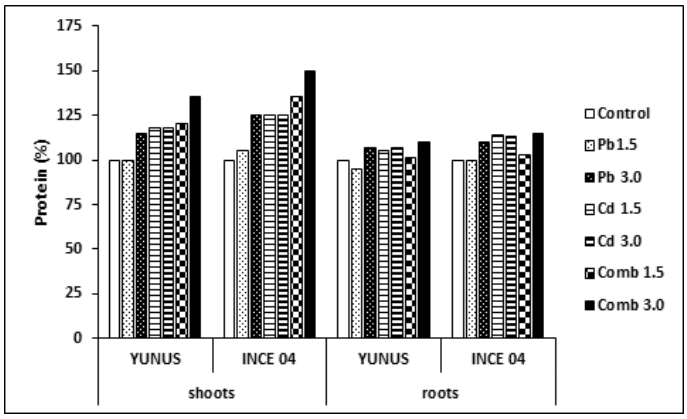

Figure 5. Effects of metal treatments on protein contents. Changes in values were depicted as a percentage of their controls. The absolute values can be seen in text.

3.6. Enzyme activity

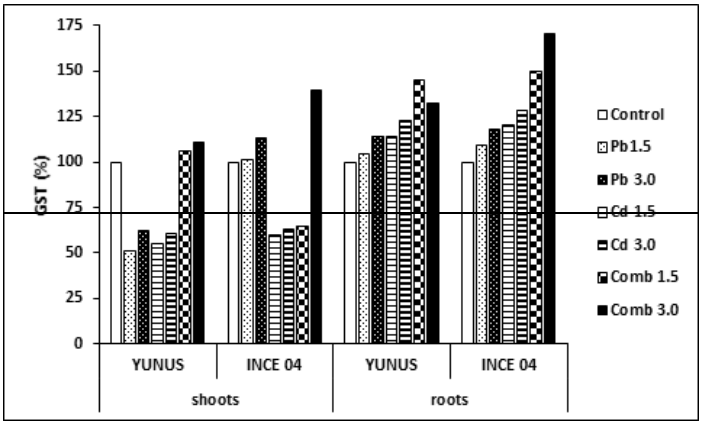

Figure 6. Effects of metal treatments on GST activities. Changes in values were depicted as a percentage of their controls. The absolute values can be seen in text.

Before tratment, the absolute values of GST activities for controls of Yunus and Ince-04 samples were determined as 600.00 and 700.00 nmol/min/mg for shoots, 750.00 and 800.00 nmol/min/mg for roots, respectively. Similar to GSH pattern, all Pb, Cd and Pb + Cd concentrations significantly increased the GST activities of the roots. However, the shoots appeared less sensitive to metals exposure (Figure 6), comparing to their respective controls. The maximum increase in roots were observed with 1.5 and 3.0mM of Pb + Cd treatments (145 and 132 % for Yunus, 150 and 170% for Ince 04) and except 3.0mM Pb + Cd combination for Yunus roots, all other stimulations caused in GST activities were dose dependent for both plants parts. For shoots, only slight GST activity increases were seen with both Pb + Cd applications for Yunus variety by comparing to controls. On the other hand, inhibitory effect of Cd was more pronounced than Pb for Ince 04 shoots and only 3.0mM Pb + Cd application caused 139% increase in this plant, which can be counted as additive effects considering the 35% decrease in activity with 1.5mM Pb + Cd.

3.7. Statistical analysis

For the method of ANOVA, it is presumed (or hypothesized) the data were distributed normally. According to Bartlett's test, variances were found to be homogenous. For the germination of seeds, all parameters, except Cd 1.5 treatment, were found to be significantly different at $\alpha = 0.05$ level for Yunus and Ince-04. (Table 3) The comparison between plants and plants'organs for the measured parameters were performed by 2x2 factorial design test. For all parameters, data were found to be significantly different at $\alpha = 0.05$ level according to plants and organs (Table 4).

Table 3. Two-sample t test results for germination of seeds

		p-value
Germination of seeds	Control	0,249
	Pb 1.5	0,000*
	Pb 3.0	0,000*
	Cd 1.5	0,225
	Cd 3.0	0,001*
	Comb 1.5	0,001*
	Comb 3.0	0,003*

*Significant at $p < 0.05$

Table 4. 2x2 factorial design results for lengths of shoots and roots, water, GSH and protein contents and GST activities

		Lengths of shoots and roots	Water contents	GSH contents	Protein contents	GST activities
		p-value	p-value	p-value	p-value	p-value
Control	A	0,000*	0,000*	0,000*	0,000*	0,000*
	B	0,000*	0,000*	0,000*	0,000*	0,000*
	AXB	0,000*	0,000*	0,000*	0,000*	0,000*
Pb 1.5	A	0,000*	0,000*	0,000*	0,000*	0,000*
	B	0,000*	0,000*	0,000*	0,000*	0,000*
	AXB	0,000*	0,000*	0,000*	0,000*	0,000*
Pb 3.0	A	0,000*	0,000*	0,000*	0,000*	0,000*
	B	0,000*	0,000*	0,000*	0,000*	0,000*
	AXB	0,000*	0,002*	0,000*	0,000*	0,000*
Cd 1.5	A	0,000*	0,000*	0,000*	0,000*	0,000*
	B	0,000*	0,000*	0,000*	0,000*	0,000*
	AXB	0,000*	0,003*	0,000*	0,000*	0,000*
Cd 3.0	A	0,000*	0,000*	0,000*	0,000*	0,000*
	B	0,000*	0,000*	0,000*	0,000*	0,000*
	AXB	0,000*	0,000*	0,000*	0,000*	0,000*
Comb 1.5	A	0,000*	0,000*	0,000*	0,000*	0,000*
	B	0,000*	0,000*	0,000*	0,000*	0,000*
	AXB	0,000*	0,023*	0,000*	0,000*	0,000*
Comb 3.0	A	0,000*	0,000*	0,000*	0,000*	0,000*
	B	0,000*	0,000*	0,000*	0,000*	0,000*
	AXB	0,715	0,731	0,000*	0,000*	0,000*

A plants, *B* plants'organs ; *Significant at $p < 0.05$

4. Conclusions and discussion

1.15. Germination

The probability of germination and subsequent survival of plants strongly influenced by the environment occupied by a seed and variations in germination percentage are interpreted as reflecting adaptation to that specific environmental conditions. As a phase in the reproductive cycle, seed germination has a great importance for species fitness (Navarro and Guitian, 2003). In the literature, the effects of HMs on seed germination were reported for different species of plants though to a different extent. While Mahmood et al (2007) observed that Mg, Na, Pb and Zn did not affect the seed germination in rice, wheat and barley seedlings, a study of Mel'nichuk (1990) showed that Cd at low concentrations promotes seed germination. However, there are also several reports demonstrating that HMs inhibit germination of seeds in different plant species. For example, effects of Pb and Cd in barley and wheat (Titov et al., 1996), Pb in rice (Verma and Dubey, 2003), Cd in barley (Munzuroglu and Zengin, 2006) and Pb in wheat (Lamhamdi etal., 2011) have been reported and all are showing dose dependent manner with increasing Pb and Cd concentrations. Although the seed coating initially provides some protection from metal stress prior to germination, eventually cracks or becomes more permeable upon germination. The current literature suggests that seed germination is affected by metals in two ways; (i) primarily via disturbance of several nutritional mechanisms and (ii) by metal inhibition of water uptake (Kranner and Colville, 2011). Therefore, we can infer that Ince 04 germinated better than Yunus according to our results. Because, firstly the seeds of Yunus were more affected by Pb and Cd and that the seed coat of this genotype degraded quicker than that of Ince 04, resulting in increased metal toxicity induced nutrition disturbances within the seed. Secondly, the metal perniciousness suppressed water uptake in the seed of Yunus was more than in Ince 04.

1.16. Root and shoot lengths

In this study, the lengths of root and shoot were chosen as indices of growth performance, because growth is defined as an irreversible increase in the mass, weight or volume of a living system (Opik and Rolfe, 2005). The effects of HMs on both root and shoot growth were documented in previous literature, with that root growth is particularly sensitive to HMs (Seregin and Ivanov, 2001; Sharma and Agrawal, 2005). Mahmood et al (2007) announced that, among the metals used (Cu, Pb, Zn, Mg and Na), while Mg did not affect the shoot lengths in barley, rice and wheat, it increased the root lengths in all seedlings and Na only increased the root lengths of barley and rice. Similarly, Hagemeyer and Breckle (1996) and Wu et al (2003) have shown that there is some potentially positive impact of Pb and Cd on barley growth at lower concentrations and it is also confirmed with our results for both Pb and Cd in barley roots. According to the authors, the main explanations for this enhancement in growth include increased Fe solubility and availability in calciocolous plants, prevention of P toxicity or promotion of P uptake, reduction in growth rate and prevention of Ca depletion, alternation of growth regulators and protection against Cu/Mn toxicity (Barcelo and Poschenrieder, 2002). For shoots, Mahmood et al (2007) confirmed some contrary results, while Cu, Pb and Zn increasing the shoot length in barley seedlings, the same concentrations of these metals were significantly inhibitory in wheat. As also confirmed by our study, the induction in barley shoots can be attributed to an increase in the synthesis of cell wall polysaccharides resulting from Pb exposure (Obroucheva et al 1998). On the other hand, numerous studies have shown the inhibitory effects of HMs on root and shoot lengths in different plant species. For example, inhibitory effects of 500 mg l^{-1} Cd in shoots and roots of wheat (Öncel et al., 2000), 3mM and 3.5mM Cd in roots and shoots of barley (Munzuroglu and Zengin, 2006) and 2mM Cd in roots of barley (Haluskova et al., 2009) have been recorded. In addition to these, the early results of Titov et al (1996) and Lamhamdi et al., (2011) reported that Pb and Cd caused stunting of roots and shoots of barley and wheat seedlings in relation to increased concentrations used. When Cd and Pb entering the root, only a small fractions were translocated upward to the shoots, because endodermis acts as a barrier to metal uptake. At higher concentrations, this barier is broken and and the metal flux enters the vascular tissues. At this stage, the rate of transport depends on the chemical composition of the cell walls. The accumulation of HMs in the cell walls may reduce the plasticity of the latter and in this way reduce cell elongation. As the root cells are in direct contact with metals, the affected roots may cause a slower movement of metals to the shoots and this is most probably related to greater inhibition of roots by metal toxicity than shoots. Several workers have reported the inhibition of root growth and of cell divisions in root tips, with mitotic abnormalities, damages to microtubules and destabilization of the cellular membranes (Seregin and Kozhevnikova, 2008). Consequently, our present study reveals that, Pb and Cd have a strong and quickly manifested effect on seedlings, which shows up as a decrease in root and shoot growth and inhibitory effects of metals were more pronounced on Yunus than on Ince 04 seedlings.

1.17. Water contents

Water plays an important role in the plant life, because it is the most abundant and at the same time the most limiting resource, that plants need to grow and function for the best physiological efficiency or productivity in terms of dry weight (Gonzalez and Reigosa Roger, 2001). Sometimes decreases in tissue water content may be more important

than decreases in water potential (or pressure potential) (Gonzalez and Gonzalez-Vilar, 2001). It had already been reported that, HMs lower water uptake and its transport in plants (Becerril et al., 1989). The mainly accepted cause is reduction of the number of hairy roots, which decreases the absorption area of roots for water. Moreover, HMs [particularly Cd (Das et al., 1997; Costa and Morel, 1994) and Pb (Parsy et al., 1998)] disturbs water relations by decreasing the number and diameter of vessels and duct tubes as well as decreasing membrane permeability for water. Therefore, root and shoot water uptakes were lowered and growth was interrupted (Wojcik and Tukendorf 1999). Many researchers reported a decline in water content in plants treated with Cd and Pb previously. For example, inhibitory effects of 54µM Cd in barley shoots (Vassilev et al., 1997) and 500ppm Cd in mustard leaves (Singh and Tewari, 2003) have been recorded similar to ours. On the other hand, as water contents of plants can be counted in one of the indices in dry weight (or growth in general terms), the results of Öncel et al., 2000 (Cd in wheat), who reported the increase in dry weight can be considered as non-consistent with the dry weight decrease reported in the above literature. Although, more recent studies of Ozturk et al (2003), Gohar et al (2003) and Shafi et al (2010) were announced similar results to ours. Of those, the latter also indicated that the combined stresses of NaCl and Cd resulted in further reduction of shoot and root dry weight when compared with their individual effects. This was also indicated in our results for combined effects of Pb and Cd. Between the varieties examined, Ince 04 was found to be less affected from metal toxicity when compared to Yunus.

1.18. GSH contents

Antioxidants are considered to play an important role in the detoxification of toxic oxygen species generated in presence of metal ions. As mentioned shortly in Introduction section, to protect from oxidative stress conditions induced by free radicals, plants produce low molecular weight thiols that show high affinity for toxic metals (Chandra et al 2009). The most important and critical low molecular weight biological thiols are GSH and cystein. Of those, GSH is known as a sulfur containing tri-peptide thiol and the chemical reactivity of the thiol group of GSH makes it particularly suitable to serve a broad range of biochemical functions in all organisms. This reactivity along with the relative stability and high water solubility of GSH makes it an ideal biochemical to protect plants against stresses including oxidative stress, HMs and certain exogenous and endogenous organic chemicals (Cobbett and Goldsbrough, 2002; Foyer and Noctor, 2005; Rausch et al., 2007; Mullineaux and Rausch, 2005). The conjugation of GSH with such molecules (including HMs) is governed by GSTs (Edwards and Dixon, 2005) and conjugates are subsequently transported to the vacuole to protects plant cell from their harmful effect (Klein et al., 2006; Yazaki, 2006). On the other hand, GSH is a substrate for phytochelatin (PC) synthesis (Freeman et al., 2004) and PCs form complexes with toxic metal ions in the cytosol and subsequently transported them into the vacuole (Salt and Rauser, 1995). Therefore, protect plants from the deleterious effect of HMs. In the literature, there are several studies showing that the involvement of GSH in tolerance of plants to metal toxicity (Hall, 2002; Wu and Zhang, 2002; Mendoza-Cozatl et al., 2002; Freeman et al., 2004) and in the reduction of ROS generated during stress (Foyer and Noctor, 2005; Shao et al., 2005, 2008) with different plant species. However, as presented by Xiang et al (2001), elevation of GSH does not aways correlate with enhanced tolerance to HMs. In the literature, there are also some studies showing the correlation between these two parameters (Freeman et al., 2004, 2005; Guo et al., 2008). For example, Ozturk et al (2003) have already reported the effect of Cd on GSH contents of root and shoots of two wheat cultivars. Similar to our results, they observed that, there are some slight increases of GSH contents in the shoots, but roots showed a massive increases under elevated metal concentrations. Although, our studies indicated that, while Yunus shoots have observed as having the lowest GSH levels, which might be attributed to the synthesis of PCs, as PCs was accompanied by a decrease in cell GSH pool, the increases in GSH contents of roots by metal applications were more marked in Ince-04 than in the Yunus (60 and 63% differences of root GSH contents between two varieties after 1.5 and 3.0mM Pb+Cd treatments). This indicates that GSH contents are possibly involved in the greater metal tolerance of Ince-04 either by increasing the antioxidative defense mechanism or enhancing metal-binding peptides. After the scrutinization of GSH contents in shoots, we can conclude that, metals taken up by roots and cannot be transported into the shoots or sequestered in vacuoles in both varieties, especially in Ince-04.

1.19. Protein contents

When excess ROS formed within cells, as a common consequence of HM toxicity, it can cause not only peroxidation of lipids, inactivation of enzymes or damage in DNA and RNA, but also provoke oxidation and modification of cellular proteins (Ogawa and Iwabuchi, 2001). An enhanced level of protein oxidation and activation of antioxidant apparatus indicate the presence of oxidative stress in several plant species (Haluskova et al., 2009). In this concept, histidine, arginine, lysine, proline, methionine and cysteine residues are the most common sites of oxidation in proteins and usually only one amino acid residue in a given protein is modified by oxidation. These modifications correspond to site-specific processes, amino acid residues at metal binding sites being specific targets (Nagajyoti et al., 2010). However, inside cells, proteins such as ferritins, metallothioneins (MTs) and PCs and related peptides,

participate in excess metal storage and detoxification. When these systems are overloaded, oxidative stress defense mechanisms are activated (Patra et al., 2004) to overcome the existing metal toxicity. In the literature, there are some controversal results concerning the reduction in protein contents as a result of exess metal ions (Arora et al., 2002; De Dorlodot et al., 2005; Singh and Tewari, 2003). However, the previous results of Ali et al (1998) and Zhu et al (1999) and more recent study of Chandra et al., (2009) have all been shown the increase in total soluble protein contents of the roots and shoots in plants examined. Recently, Lamhamdi et al (2011) have reported the significant increase of soluble protein contents in shoots of wheat seedlings with the increase of Pb concentration, by comparing to roots. This was also the results of our studies, although we observed that the effects of Pb and Pb + Cd combinations were in dose dependent manner for both parts of plants examined and Cd concentrations were not effective in that manner. As substraction, metal toxicity induces an increase of protein content in exposed plant seedlings, likely to the induction of stress proteins under metal exposure (Mesmar and Jaber, 1991). These stress proteins possibly comprise various antioxidant enzymes and other enzymes involved in GSH and PC biosynthesis and also some heat shock proteins (Srivastava et al., 2004).

1.20. Enzyme activities

In plants, HM toxicity is attributed to three mean reasons; (i) stimulation of ROS production, (ii) direct interaction with proteins due to their affinities for thioyl-, histidyl- and carboxyl-groups and (iii) displacement of essential metal ions from specific binding sites (Sharma and Dietz, 2009). In this context, metal ions play an important role in the antioxidant network, as these are essential cofactors of most antioxidant enzymes. When interacting with enzymes, Cd^{2+} and Pb^{2+} usually produce 50% inhibition in enzyme activities at the following molar concentrations: (10^{-6} to 3×10^{-5}) and (10^{-5} to 2×10^{-4}), respectively (Kositsin, 1991). In most cases, the inhibition exerted by Cd and Pb results from the interaction between the metals and enzyme SH-groups. These metals interact with SH-groups that are essential for the enzyme reaction center and the stabilization of the enzyme tertiary structure. In the latter case, metals affect enzyme conformation. For example, Cd binding to sulfhydryl groups of structural proteins and enzymes leads to misfolding and inhibition of activity and/or interference with redox-enzymatic regulation have been reported by Hall (2002) and Dalcorso et al., (2008). Again, Verma and Dubey (2003) has announced a decline in the activity of catalase (CAT) in Pb stressed plants. Such a decrease appears to be due to a decline in enzyme synthesis or a change in the assembly of of enzyme subunits (Sharma and Dubey, 2005). On the other hand, activities of several enzymes (CAT, guaiacol peroxidase [POD], superoxide dismutases [SOD], ascorbate peroxidase [APX], glutathione reductases [GR]) are reported to be enhanced by metal treatments (Singh and Tewari, 2003; Verma and Dubey, 2003; Sharma and Dubey, 2004). Those marked enhancement possibly results from changes in enzyme synthesis, immobilization of enzyme inhibitors or as a result of effector molecules which are synthesized under metal phytotoxicity (Sharma and Dubey, 2005). In the recent studies of Haluskova et al (2009) and Lamhamdi et al (2011), the increase in the GST activities has been shown in barley and wheat seedlings. According to Haluskova et al (2009), Cd induced root growth inhibition is strongly correlated with increased GST activity and an association between increased GST activity and root growth inhibition was also observed during other HM (Pb, Ni, Hg, Co, Cu amd Zn) treatments. These observations are well corraborated with our results, as we have also observed Cd and Pb induced root growth inhibition correlated with increased GST activity in the roots of both plants examined. Moreover, the studies of Lamhamdi et al (2011) showed that Pb treatment caused a progressive increase in GST activity and generally the activity in roots was higher than in the shoots of wheat seedlings, as similar to our findings for both plants examined during experiments. Especially, Ince-04 roots have shown the highest GST activity, by comparing to other plant and plant parts. Apparently, in response to oxidative stress, where basal antioxidant mechanisms are exhausted, the more effective responses are activated, like GSTs and an increase in antioxidant enzyme activities neutralizes free radicals.

Finally, as depicted above with our present study, exposure of plants to high levels of HMs could be counted as a abiotic elicitor of oxidative stress which is causing many alterations from seed germinations to enzyme activities by comparing to normal metabolisms of plants. In conclusion, it was shown that biochemical and physiological mechanisms were affected differently with varying concentrations of HMs and Ince 04 (barley) were found to be more tolerant to HM stress by comparing to Yunus (wheat). It should be kept in mind that, as mostly edible parts of food plants tolerate a relatively high concentrations of HMs and being the major source of HM intake for human through consumption, they are likely to create a greater health risk than those which are more sensitive and show definite symptoms of toxicity. Therefore, first of all, different biomarkers will be used in future experiments aimed to search for molecules able to provide a protection against the deleterious effect of HMs and attempts have been made to generate transgenic plants using several different genes regulating GSH, PC and/or cysteine synthesis pathways. On the other hand, the usage of those tolerant plants through environmentally, technically and economically viable processes like phytoextraction, phytostabilization or rhizofiltration, could have the potential to provide remediation of HMs from soil.

Acknowledgements

The author would like to thank Dr. Şükrü Acıtaş and Dr. İlhan Usta for statistical analysis.

References

Ali, G., Srivastava, P. S., Iqbal, M. (1998). Morphogenic response and proline content in *Bacopa monnieri* cultures grown under copper stress. Plant Science, 138(2), 191-195.

Arora, A., Sairam, R.K., Srivastava, G.C. (2002). Oxidative stress and antioxidative system in plants. Current Science, 82(10), 1227-1238.

Backor, M., Loppi, S. (2009). Interactions of lichens with heavy metals. Biologia Plantarum, 53(2), 214-222.

Barcelo, J., Poschenrieder, C. (2002). Fast root growth responses, root exudates, and internal detoxication as clues to the mechanisms of aluminium toxicity and resistance: a review. Environmental and Experimental Botany, 48(1), 75-92.

Becerril, J.M., Gonzalez-Murua, C., Munoz-Rueda, A., De Felipe, M.R. (1989). Changes induced by cadmium and lead in gas exchange and water relations of clover and lucerne. Plant Physiology and Biochemistry, 27(6), 913-918.

Chandra, R., Bharagava, R.N. Yadav, S., Mohan, D. (2009). Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. Journal of Hazardous Materials, 162(2-3), 1514-1521.

Cobbett, C., Goldsbrough, P. (2002). Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. Annual Review of Plant Biology, 53, 159-182.

Costa, G., Morel, J.L. (1994). Water relations, gas exchange and amino acid content in Cd-treated lettuce. Plant Physiology and Biochemistry, 32(4), 561-570.

Dalcorso, G., Farinati, S., Furini, A. (2010). Regulatory networks of cadmium stress in plants. Plant Signaling and Behavior, 5(6), 1-5.

DalCorso, G., Farinati, S., Maistri, S., Furini, A. (2008). How plants cope with cadmium: staking all on metabolism and gene expression. Journal of Integrative Plant Biology, 50(10), 1268-1280.

Das, P., Samantaray, S., Rout, G.R. (1997). Studies on cadmium toxicity in plants: A review. Environmental Pollution, 98(1), 29-36.

De Dorlodot, S., Lutts, S., Bertin, P. (2005). Effects of ferrous iron toxicity on the growth and mineral composition of an interspecific rice. Journal of Plant Nutrition, 28(1), 1-20.

Dubey, R.S. (2011). Metal toxicity, oxidative stress and antioxidative defense system in plants. In S.D. Gupta (Ed.), *Reactive oxygen species and antioxidants in higher plants* (pp. 177-203). Boca Raton, Fla, USA: CRC Press.

Edwards, R., Dixon, D. (2005). Plant glutathione transferases. Methods in Enzymology, 401, 169-186.

Ellman, G.L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82(1), 70-77.

Frear, D.S., Swanson, H.R. (1970). Biosynthesis of S-(4-ethylamino-6-isopropylamino-2-s-triazine) glutathione: Partial purification and properties of glutathione *S*-transferase from corn. Phytochemistry, 9(10), 2123-2132.

Freeman, J.L., Persans, M.W., Nieman, K., Albrecht, C., Peer,W., Pickering, I.J., Salt, D.E. (2004). Increased glutathione biosynthesis plays a role in nickel tolerance in *Thlaspi* nickel hyperaccumulators. Plant Cell, 16(8), 2176-2191.

Freeman, J.L., Garcia, D., Kim, D., Hopf, A., Salt, D.E. (2005). Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in *Thlaspi* nickel hyperaccumulators. Plant Physiology, 137(3), 1082- 1091.

Foyer, C.H., Noctor, G. (2005). Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. Plant Cell, 17(7), 1866-1875.

Gamalero, E., Lingua, G., Berta, G., Glick B. R. (2009). Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Canadian. Journal of Microbiology, 55(5), 501-514.

Gill, M. (2014). Heavy metal stress in plants: a review. International Journal of Advanced Research, 2(6), 1043-1055.

Gill S.S., Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stres tolerance in crop plants. Plant Physiology and Biochemistry, 48(12), 909-930.

Gohar, Z.N., Ahmad, R., Gul, H. (2003). Growth and development of cotton roots at various soil textures under soil conditions. Pakistan Journal of Botany, 35(5), 949-959.

Gonzalez, L., Reigosa Roger, M.J. (2001). Plant water status. In M.J. Reigosa Roger (Ed.). Handbook of Plant Ecophysiology Techniques (pp.185-191). Dordrecht, The Netherlands: Kluwer Academic Publisher.

Gonzalez, L., Gonzalez-Vilar, M. (2001). Determination of relative water content. In M.J. Reigosa Roger (Ed.), Handbook of Plant Ecophysiology Techniques (pp. 207-212). Dordrecht, The Netherlands: Kluwer Academic Publisher.

Guo, J., Dai, X., Xu, W., Ma, M. (2008). Overexpressing GSH1 and AsPCS1 simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana*. Chemosphere, 72(7), 1020-1026.

Habig, W.H., Pabst, M.J., Jakoby, W.B. (1974). Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249(22), 7130-7139.

Habig, W.H., Jakoby, W.B. (1981). Assays for differentiation of glutathione *S*-transferases. Methods in Enzymology, 77, 398-405.

Hagemeyer, J., Breckle, S.W. (1996). Growth under trace element stress. In Y. Waisel, U. Kafkafi (Ed.), *Plant roots: The hidden half* (pp. 415-433). New York, USA: Marcel Dekker.

Hall, J.L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany, 53(366), 1-11.

Halliwell, B. (1995). How to characterize an antioxidant: An update? Biochemistry Society Symposium, 61, 73-101.

Haluskova, L , Valentovicova, K., Huttova, J., Mistrik, I., Tamas, L. (2009). Effect of abiotic stresses on glutathione peroxidase and glutathione *S*-transferase activity in barley root tips. Plant Physiology and Biochemistry, 47(11-12), 1069-1074.

Hoagland, D. R., Arnon, D. I. (1950). *The water-culture method for growing plants without soil.* California Agricultural Experiment Station, Berkeley.

Klein, M., Burla, B., Martinoia, E. (2006). The multidrug resistance-associated proteins (MRP/ABCC) subfamily of ATP-binding cassette transporters in plants. FEBS Letters, 580(4), 1112-1122.

Kositsin, A.V. (1991). Interaction between metals and enzymes. In N.V. Alekseeva-Popova (Ed.), *Tolerance of plant species grown in the wild to heavy metals* (pp. 15-22). Leningrad: Lenuprizdat.

Kranner, I., L. Colville, L. (2011). Metals and Seeds: Biochemical and molecular implications and their significance for seed germination. Environmental and Experimental Botany, 72(1), 93-105.

Lamhamdi, M. Bakrim, A. Aarab, A., Lafont, R., Sayah, F. (2011). Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedlings growth. Comptes Rendus Biologies, 334(2),118-126.

Levetin, E., McMahon, K. (2011). *Plants and Society*. USA: McGraw-Hill.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randal, R.J. (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193(1), 265-275.

Navarro, L,. Guitian, J. (2003). Seed germination and seedling survival of two threatened endemic species of the northwest Iberian Peninsula. Biological Conservation, 109(3), 313-320.

Mahmood, T., Islam, K.R. Muhammad, S. (2007). Toxic effects of heavy metals on early growth and tolerance of cereal crops. Pakistan Journal of Botany, 39(2), 451-462.

Manmathan, H., Lapitan, N. L. (2013). *Measuring germination percentage in wheat*. Bio-protocol 3(16): e 866. http://www.bio-protocol.org/e866. (Date of access: 29.11.2016).

Mannervik, B., Danielson, U. H. (1988). Glutathione transferases- structure and catalytic activity. CRC Critical Reviews in Biochemistry and Molecular Biology, 23(3), 283-337.

May, M.J., Vernoux, T., Leaver, C., Van Montagu, M., Inze, D. (1998). Glutathione homeostasis in plants: Implications for environmental sensing and plant development. Journal of Experimental Botany, 49(321), 649-667.

Mel'nichuk, Yu.P. (1990). *The effect of cadmium ions on the cell division and plant growth*. Kiev: Naukova Dumka.

Mendoza-Cozatl, D., Devars, S., Loza-Tavera, H., Moreno-Sanchez, R. (2002). Cadmium accumulation in the chloroplast of *Euglena gracilis*. Physiologia Plantarum, 115(2), 276-283.

Mesmar, M.N., K. Jaber, K. (1991).The toxic effect of lead on seed germination, growth, chlorophyll and protein contents of wheat and lens. Acta Biologica Hungarica, 42(4), 331-344.

Mullineaux, P., Rausch, T. (2005). Glutathione, photosynthesis and the redox regulation of stress-responsive gene expression. Photosynthesis Research, 86(3), 459-474.

Munzuroglu, O., Zengin, F.K. (2006). Effect of cadmium on germination, coleoptile and root growth of barley seeds in the presence of gibberellic acid and kinetin. Journal of Environmental Biology, 27(4), 671-677.

Nagajyoti, P. C., Lee, K. D., Sreekanth, T.V.M. (2010). Heavy metals, occurrence and toxicity for plants: A review. Environmental Chemistry Letters, 8(3), 199-216.

Obroucheva, N.V. Bystrova, E.I. Ivanov, V.B. Anupova, O.V. Seregin, I.V. (1998). Root growth responses to lead in young maize seedling. Plant and Soil, 200(1), 55-61.

Ogawa, K., Iwabuchi, M. (2001). A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. Plant and Cell Physiology, 42(3), 286-291.

Ono, H., Ozaki, K., Yoshikawa, H. (2005). Identification of cytochrome P450 and glutathione-S-transferase genes preferentially expressed in chemosensory organs of the swallowtail butterfly, *Papilio xuthus* L. Insect Biochemistry and Molecular Biology, 35(8), 837-846.

Opik, H., Rolfe, S. A. (2005). *The Physiology of flowering plants*. Cambridge: Cambridge University Press.

Öncel, I., Keleş, Y. Ustun, A.S. (2000). Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedling. Environmental Pollution, 107(3), 315-320.

Oztetik, E. (2005). *Purification of glutathione S-transferases and genetic characterization of zeta isozyme from Pinus brutia, Ten.* (PhD Dissertation). Ankara, Turkey: Middle East Technical University.

Oztetik, E. (2015). Biomarkers of ecotoxicological oxidative stress in an urban environment: using evergreen plant in industrial areas. Ecotoxicology, 24(4), 903-914.

Oztetik, E., Cakir, A. (2014). New food for an old mouth: New enzyme for an ancient archaea. Enzyme and Microbial Technology, 55, 58-64.

Ozturk, L., Eker, S., Ozkutlu, F. (2003). Effect of cadmium on growth and concentrations of cadmium, ascorbic acid and sulphydryl groups in durum wheat cultivars. Turkish Journal of Agriculture and Forestry, 27(3), 161-168.

Parys, E., Romanowska, E., Siedlecka, M., Poskuta, J.W. (1998). The effect of lead on photosynthesis and respiration in detached leaves and in mesophyll protoplasts of *Pisum sativum*. Acta Physiologiae Plantarum, 20(3), 313-322.

Patra, M., Bhowmik, N.,Bandopadhyay, B., Sharma, A. (2004). Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environmental and Experimental Botany, 52(3), 199-223.

Rausch, T., Gromes, R., Liedschulte, V., Muller, I., Bogs, J., Galovic, V., Wachter, A. (2007). Novel insight into the regulation of GSH biosynthesis in higher plants. Plant Biology, 9(5), 565-572.

Rea, P. A. (1999). MRP subfamily ABC transporters from plants and yeast. Journal of Experimental Botany, 50 (Special issue), 895-913.

Reddy, A.M., Kumar, S.G., Jyotsnakumari, G., Thimmanayak, S., Sudhakar, C. (2005). Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). Chemospher, 60(1), 97-104.

Salt, D.E., Rauser, W.E. (1995). MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiology, 107(4), 1293-1301.

Seregin, I.V., V. B. Ivanov, V.B. (2001). Physiological Aspects of Cadmium and Lead Toxic Effects on Higher Plants. Russian Journal of Plant Physiology, 48(4), 523-544.

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

Shafi, M., Guoping, Z., Bakht, J., Khan, M.A., Islam, E.U., Dawood, M. K. (2010). Effect of cadmium and salinity stresses on root morphology of wheat. Pakistan Journal of Botany, 42(4), 2747-2754.

Shao, H.B., Liang, Z.S., Shao, M.A., Sun, Q., Hu, Z.M. (2005). Investigation on dynamic changes of photosynthetic characteristics of 10 wheat (*Triticum aestivum* L.) genotypes during two vegetative-growth stages at water deficits. Colloids and Surfaces B: Biointerfaces, 43(3-4), 221-227.

Shao, H.B., Chu, L.Y., Lu, Z.H., Kang, C.M. (2008). Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. International Journal of Biological Science, 4(1), 8-14.

Sharma, R.K., Agrawal, M. (2005). Biological effects of heavy metals: An overview. Journal of Environmental Biology, 26(2), 301-313.

Sharma, S.S., Dietz, K.J. (2009). The relationship between metal toxicity and cellular redox imbalance. Trends in Plant Science, 14(1), 43-50.

Sharma, P., Dubey R.S. (2004). Ascorbate peroxidase from rice seedlings: Properties of enzyne isoforms, effects of stresses and protective roles of osmolytes. Plant Science, 167(3), 541-550.

Sharma, P., Dubey, R.S. (2005). Lead toxicity in plants. Brazilian Journal of Plant Physiology, 17(1), 35-52.

Sharma, P., Dubey, R.S. (2007). Involvement of oxidative stress and role of antioxidative defense systemin growing rice seedlings exposed to toxic concentrations of aluminum. Plant Cell Reports, 26(11), 2027-2038.

Singh, P.K., Tewari, R.K. (2003). Cadmium toxicity induced chnages in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. Journal of Environmental Biology, 24(1), 107-112.

Srivastava, S., Tripathi, R.D., Dwivedi, U.N. (2004). Synthesis of phytochelatins and modulation of antioxidants in response to cadmium stress in Cuscuta reflexa – an angiospermic parasite. Journal of Plant Physiology, 161(6), 665-674.

Titov, A.F. Talanova, V.V., Boeva, N.P. (1996). Growth responses of barley and wheat seedlings to lead and cadmium. Biologia Plantarum, 38(3), 431-436.

Transitional Zone Agricultural Research Institute website. (2016). Registered Varieties. http://arastirma.tarim.gov.tr/gktaem/Menu/12/Tescilli-Cesitlerimiz (Date of access: 07.12.2016).

USDA Foreign Agricultural Service. (2016). Turkey Grain and Feed Annual Report, 2016. E. Leonardi. (Ed). (GAIN Report No. TR6015). http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Ankara_Turkey_3-29-2016.pdf (Date of access: 10.12.2016).

Vassilev, A., Yordanov, I., Tsonev, T. (1997). Effects of Cd^{2+} on the physiological state and photosynthetic activity of young barley plants. Photosynthetica, 34(2), 293-302.

Verma, S., Dubey, R.S. (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Science, 164(4), 645-655.

Villiers, F. Ducruix, C., Hugouvieux, V. (2011). Investigating the plant response to cadmium exposure by proteomic and metabolomic approaches. Proteomics. 11(9), 1650-1663.

Weckx, J.E.J., Clijsters, H.M.M. (1997). Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiology and Biochemistry, 35(5), 405-410.

Wojcik, M., Tukendorf, A. (1999). Cd-tolerance of maize, rye and wheat seedlings. Acta Physiologiae Plantarum, 21(2), 99-107.

Wu, F., Zhang, G., Dominy, P. (2003). Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. Environmental and Experimental Botany, 50(1), 67-78.

Wu, F.B., G.P. Zhang. (2002). Alleviation of cadmium-toxicity by application of zinc and ascorbic acid in barley. Journal of Plant Nutrition, 25(12), 2745-2761.

Xiang, C., Werner, B.L., Christensen, E.M., Oliver, D.J. (2001). The biological functions of glutathione revisited in Arabidopsis transgenic plants with altered glutathione levels. Plant Physiology, 126(2), 564-574.

Yadav, S.K. (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South African Journal of Botany, 76(2), 167-179.

Yazaki, K. (2006). ABC transporters involved in the transport of plant secondary metabolites. FEBS Letters, 580(4), 1183-1191.

Yücel, E. (2000). Ebe Karaçamın biyolojik ve ekolojik özellikleri. Eskisehir; Birlik Ofset Matbaacılık.

Yücel, M., Yücel, E. (2013). On the ecotoxicological effects of heavy metal pollution of industrial origin determination of wheat varieties. Biological Diversity and Conservation, 6(3), 6-11.

Zhu, Y.L. Pilon-Smits, E.A.H. Tarun, A.S., Weber, S.U., Jouanin, L. Terry, N. (1999). Cadmium tolerance and accumulation in Indian mustard is enhanced by overexpressing g-Glutamylcysteine synthetase. Plant Physiology, 121(4), 1169-1177.

(Received for publication 04 October 2016; The date of publication 15 December 2016)