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## Wheat Biological Responses to Stress Caused by Cadmium, Nickel and Lead

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**Abstract**

Several stressors like different types of heavy metals are found in the soil and can affect the growth and genomic integrity of wheat grains (*Triticum aestivum* L.). The aim of this study was to compare the effect of exogenous Cd (30, 60, 120 mg/kg), Ni (50, 100 and 150 mg/kg) or Pb (100, 200 and 300 mg/kg) on wheat agronomic characteristics through the assessment of oxidative stress indices at protein and gene expression levels, photosynthetic pigments and genetic aberrations using RAPD analysis that were studied during two winter seasons (2015/2016 and 2016/2017). The results showed that all stressors significantly decreased the vegetative growth parameters, altered the activities of antioxidants enzymes in seedlings (after 30 days) and grains (after 5 months) and differently affected their expression levels in seedlings leaves and roots. Pb treated plants showed the poorest agronomic characteristics as it exhibited the worst affected wheat height, number of tillers, fresh and dry weight, flag leaf area as well as yield. Pb treatment caused poorest plant performance, it showed the highest proline content, least protein and chlorophyll contents, thus affects the overall plants growth followed by Cd and Ni, respectively. Furthermore, high Pb and Cd doses revealed highest degree of polymorphism and lowest degree of genome stability. Altogether, heavy metals accumulated mainly in wheat straw and induced genotoxic effect which consequently altered normal plant metabolism and pigment content which resulted in a significant reduction in wheat yield and quality. Moreover, Pb induced more genotoxic and phytotoxic effects than Cd and Ni.

**Keywords:** Cadmium; Nickel; Lead; Antioxidants; vegetative growth parameters; RAPD

## 1. Introduction

Pollution concerns the deterioration of chemical, physical and biological properties of the surrounding environment air, water and soil (Ahmad et al., 2015b). Heavy metal pollution is one of the most global environmental problems worldwide facing humanity. Unlike organic pollutants that can be easily degraded to harmless small molecules, heavy metals belong to a group of immutable, non-biodegradable, inorganic chemical constituents and their total concentration in soils persists for a long time after their introduction (Wuana & Okieimen, 2011; Malar et al., 2014). Despite heavy metals are natural constituents of soils in trace amount, they become toxic to soil as they decrease soil fertilization which consequently cause plant cellular damage when their quantity exceeds the normal limit (Al Naggar et al., 2014; Placek et al., 2016; Jalmi et al., 2018). Moreover, this adverse effect has been extended to human populations via food chain (Xie et al., 2016).

Among heavy metal ions, cadmium (Cd), nickel (Ni) and lead (Pb) contaminants are an emerging problem all over the world due to anthropogenic, technogenic and geogenic activities (Wuana & Okieimen, 2011; Rizwan et al., 2016). In agricultural soils, naturally Cd concentration ranged from 0.01 to 0.7 mg kg<sup>-1</sup> soil (Szolnoki et al., 2013), while the mean content of Pb is 50 mg kg<sup>-1</sup> soil (Nagajyoti et al., 2010) and Ni concentration, with respect to the World Health Organization (WHO), is 15-30 mg kg<sup>-1</sup>soil (Kabata-Pendias & Pendias, 2001).

Nickel (Ni) is an essential micronutrient for plant growth and development; it has high biological activity as an integral component of various biomolecules (including metalloenzymes). However, it becomes toxic at high concentration (Sirhindi et al., 2016). In contrast, Cd and Pb are non-essential elements and at very low concentration they can cause morphological, physiological, and biochemical dysfunctions in plants (Shu et al., 2012; Meng et al., 2017).

Wheat (*Triticum aestivum* L.) is the most domesticated-cultivated-food crop and it is considered the most important human food grain source. As other plants, wheat is sensitive to various biotic and abiotic factors like, heavy metals (Parlak, 2016; Ali et al., 2018). Heavy metals have many direct and indirect damaging effects on plants growth (Jalmi et al., 2018). Heavy metals induced-phytotoxicity is reported to initiate secondary oxidative stress by importing the formation of harmful reactive oxygen species (ROS) or modification of the antioxidant defense system as well as replacement of enzyme cofactors and transcription factors, cellular redox and ionic transport imbalance, DNA damage and protein oxidation, thus inhibiting growth and grain yield (Sardar et al., 2013; Shahid et al., 2014; Theophanides & Anastassopoulou, 2017; Jalmi et al., 2018).

The present study was conducted to evaluate and compare the effect of different concentrations of Cd, Ni or Pb (the concentration range for each metal was chosen between the maximum allowable concentrations in soil and toxic soil concentration for plants) on wheat agronomic characteristics through studying the biochemical assessments of oxidative stress indices, detection of the genotoxic effect using RAPD assay and the evidence on vegetative growth parameters. RAPD technique, is a reliable and sensitive PCR based technique, has the potential to identify the DNA polymorphisms by detecting DNA damage (e.g. DNA adducts, DNA breakage) and mutations in plants (Jhang & Shasany, 2012; Gjorgieva et al., 2013). Differences in RAPD profiles between untreated and treated samples would be acted as a marker and elucidate the potential genotoxic effects of environmental hazardous (Atienzar & Jha, 2006; Azimi et al., 2013).

Soil samples were collected from one of the famous farms in Egypt which is Al-Gabal Al-Asfar farm (25 km north east of Cairo). Al-Gabal Al-Asfar farm was irrigated with sewage water of Cairo city for more than eighty years and there are many field crops cultivated in it such as wheat, tomato, potato, onion, garlic, maize, eggplant, bean, cauliflower, clover,

cabbage and strawberry beside pecan nuts and date palm. Total contents of Cd, Ni, Pb, iron, zinc and cobalt were found to increase as time goes on in soils irrigated with sewage at El-Gabal El-Asfer (Omran et al., 1988; Abdel-Sabour et al., 1996; Saber et al., 2016). Therefore, El-Gabal El-Asfer farm was chosen for the present study.

## **2. Materials and methods**

### **2.1. Materials**

The soil sample was collected from the surface layer (0-30 cm) of Al-Gabal Al-Asfar farm. Physiochemical characteristics of soil were illustrated in **Supp 1**. Wheat grains were obtained from the wheat Research- Crop Research Institute, Ministry of agriculture. Metals concentrations were selected according to the guideline values for metals concentrations in Egyptian (Al Naggar et al., 2014; Shokr et al., 2016) and global soils (Sipos & Póka, 2002; Toth et al., 2016) where the maximum allowable Cd, Ni and Pb concentrations in soil (0.3, 50 and 60 ppm) and toxic concentration in soil for plants (3-8, 100-150 and 100-400 ppm), respectively.

### **2.2. Growth conditions and treatments**

To study the effect of either Cd, Ni or Pb polluted soils on wheat agronomic characteristics, two pot experiments were conducted under natural conditions at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate (31°5' 35.93" latitude N and 30°56' 54.33" longitude E) during winter seasons of (2015/2016) and (2016/ 2017). Control soil was a pot (30 cm width and 35 cm height) filled with 9 kg of soil without any treatment, while treatments were prepared by using aqueous solution of either Cd (30, 60 and 120 ppm) using Cadmium sulfate octahydrate ( $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ ) or Ni (50, 100 and 150 ppm) using Nickel chloride ( $\text{NiCl}_2$ , anhydrous) or Pb solution (100, 200 and 300 ppm) using Lead acetate trihydrate ( $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ ) and applied to respective pots with four replicates. Seeds of wheat

were then uniformly sown in pots. After harvesting of wheat plants, another seed of wheat was grown in the same pot without fresh treatment of heavy metals. All plants were regularly irrigated and basal fertilizers (NPK) were applied in liquid forms for all pots. Nitrogen (N) as urea ( $91.575 \text{ mg kg}^{-1}$  dry soil) was splatted in three doses, the first active dose (20%) directly at the time of sowing of wheat and the remaining dose (80%) was applied in two equal splits at tillering and flower initiation stages, phosphorus (P) as calcium superphosphate ( $18.315 \text{ mg kg}^{-1}$  dry soil) and potassium (K) as potassium sulphate ( $29.304 \text{ mg kg}^{-1}$  dry soil) applied at the time of sowing of wheat.

### 2.3. Plant sampling

Plant sampling and analysis are summarized in **Figure 1**. First plant samples were collected **one month** after sowing (on December 14<sup>th</sup>, 2015). Roots were carefully separated from the soils, washed with tap water and the whole plants (seedlings) were rinsed in distilled water. Plants were separated into roots, stems and leaves. Each part was separately homogenized in 9 volumes phosphate buffer (0.1 M, pH 7.4), centrifuged at 3000 rpm for 10 minutes and the supernatant was collected for biochemical investigation. Glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities as well as proline content were estimated in whole seedling. Total protein content and the expression level of superoxide dismutase (SOD), GPx and catalase (CAT) were estimated in seedling roots and leaves. DNA stability was also estimated in seedling roots.

At flowering stage (**after two months**) in the first and second seasons (on January 19<sup>th</sup>, 2016 and 2017), the plant pigment (chlorophyll a, chlorophyll b and total chlorophyll) and flag leaf area ( $\text{cm}^2$ ) were determined using the method of Quarrie and Jones (1979), where:

$$\text{Leaf area} = \text{Maximum length} \times \text{Maximum width} \times 0.75.$$

Where 0.75 is a coefficient factor for wheat

At full maturity of wheat (**harvesting after five months**) in the first and second seasons (on 21, April, 2016 and 2017), the plants were picked out and straw and spike (grains) were separated. The mean of two seasons was recorded for some agronomic traits (fresh and dry weight of plant, plant height, weight and number of spikes and weight of 100 grains as well as number of tillers per plant). Finally, Cd, Ni or Pb contents were estimated in straw, grains and post-harvest soil samples.

#### **2.4. Biochemical and molecular analysis**

**The activity of GPx** was measured following the method of Paglia and Valentine (1967). GPx catalyzes the oxidation of GSH by cumene hydroperoxide. GPx activity was determined by subtracting the excess GSH after enzymatic reaction from the total GSH in absence of the enzyme. GSH reacts with DTNB to form yellow colored chromophore which was measured at 412 nm spectrophotometrically.

**The GST activity** was determined according to the method of Habig et al. (1974). Ten microliters of p-nitrobenzyl chloride (1 mM) was added to 1.365 mL phosphate buffer (0.1 M, pH 6.5) and mixed well. 25 µl of either plant supernatant (test) or phosphate buffer, pH 7.4 (blank) were added and incubated at 37 °C for 5 min, then 100 µl GSH (5 mM) was added to start the reaction and incubated for 20 min at room temperature. The absorbance of samples was read against blank at 310 nm using UV spectrophotometer.

**Proline content** was estimated by using the method given by Bates et al. (1973). Ninhydrin can form a red product with proline at acidic pH. The plant material (100 mg) was added to 2 mL aqueous sulfosalicylic acid (3%), grounded and centrifuged for 5 min at room temperature. The reaction mixture was prepared (100 µL of 3% sulfosalicylic acid, 200 µL glacial acetic acid and 200 µL acidic ninhydrin) and 100 µL of the plant extract supernatant was added, mixed well, incubated at 96 °C for 60 min and the reaction was terminated on ice. The samples were extracted with 1 mL toluene then vortexed for 20 s, and left for 5 min to



allow the separation of the organic and water phases. The chromophore containing toluene was removed and the absorbance was measured at 520 nm using toluene as a reference. The proline concentration can be determined using a standard curve of L-Proline (20-100 µg/ml) and calculated on fresh weight basis and expressed as micromole per gram FW (µmol/g FW). **The total protein level** was measured using the method of Gornall et al. (1949) that depends on the formation of colored complex when protein reacts with cupric ions in alkaline medium and the absorbance of samples were recorded at 546 nm.

#### **Reverse transcriptase Polymerase chain reaction**

The total RNA was isolated from different plant parts (leaves and roots) using Easy-RED™ solution. One microgram of isolated RNA was reverse transcribed into complementary DNA (cDNA) using reverse transcriptase (Maxime RT PreMix kit, iNtRON Biotechnology, Korea). The resulting cDNA was used as templates for subsequent PCR amplification using specific primers for SOD, GPx, CAT and G3PDH as internal control using PCR thermal cycler (ESCO, Swift MiniPro Thermal Cycler, USA). Primer sequences and PCR conditions were summarized in **Supp 2**. Semi-quantitative PCR analysis was performed using 2X PCR Master mix (i-Taq™) with standard buffer (iNtRON Biotechnology, Korea). The PCR product was resolved on 1.5% Agarose electrophoresis and stained with ethidium bromide. DNA bands were revealed by UV light and the band intensity was analyzed using UVitec software. The expression levels were determined by calculating the relative density of each SOD, GPx and CAT band to GAPDH.

#### **DNA extraction and RAPD procedures**

Total genomic DNA was extracted from root tips of seedlings (100 mg) by chloroform-phenol method. Chloroform-phenol mixture was used to precipitate proteins while DNA is precipitated by ethanol and dissolved in Tris-EDTA buffer. The PCR amplification was carried out with ten primers (10- base pair each, Biolegio, Korea, **Supp 3**) and genomic DNA

as a template. PCR was performed in a reaction mixture of 25 µl containing approximately 1 µl primer, 1 µl template DNA, 12.5 µl master mix and 10.5 µl nuclease free water. After PCR cycle, 10 µl of PCR product was resolved on 1% Agarose electrophoresis at 80 mA for 20 minutes. DNA bands were revealed by UV light. DNA polymorphism represented in RAPD profile included the change in number of bands, presence or absence of bands compared with the control profile. Polymorphism was calculated according to Gjorgieva et al. (2013) as following:

$$P (\%) = [(a + d)/c] \times 100$$

Where, a is the number of new bands detected in samples, d is the number of disappeared bands and c is the total number of bands in control. In addition, genomic template stability (GTS, %) was calculated as following:

$$GTS (\%) = (1 - p / n) \times 100$$

Where, p is the total number of RAPD polymorphic bands detected in each metal treated sample and n the number of total bands in the control (Gjorgieva et al., 2013; Mohamed et al., 2017).

**Chlorophyll a, b and total chlorophyll contents** were determined as described by Moran (1982). One cm<sup>2</sup> of fresh flag leaf of plant was taken and placed into tubes containing 5 mL of N,N-dimethylformamide. The tubes were kept at 25 °C in darkness for 24 h and shaken 15 s every 12 h. Samples were filtered and immediately measured for light absorption at 664 (Abs<sub>664</sub>) and 647 (Abs<sub>647</sub>) nm using spectrophotometer. Chlorophyll contents (mg/cm<sup>2</sup>) were calculated based on the equations:

$$\text{Chlorophyll a} = (12.64 \times \text{Abs}_{664}) - (2.99 \times \text{Abs}_{647})$$

$$\text{Chlorophyll b} = (23.26 \times \text{Abs}_{647}) - (5.6 \times \text{Abs}_{664})$$

$$\text{Total Chlorophyll} = (7.04 \times \text{Abs}_{664}) + (20.27 \times \text{Abs}_{647})$$

**Heavy Metals content**

Heavy metals (Cd, Ni or Pb) contents in wheat plants and post-harvesting soils were measured as suggested by Chapman and Pratt (1961) and Lindsay and Norvell (1978), respectively. For the mature-grown, straw and grains from each concentration were separated, dried in oven at 72 °C for 48 hours and powdered. 0.5 g dry powder (straw or grains) was added to 8 ml concentrated H<sub>2</sub>SO<sub>4</sub> and left to next day. The mixture was heated and the temperature was raised gradually to 370 °C. When sample color changed to pale brown, sample cold, 0.5-1 ml of 70 % perchloric acid was added and the mixture was further boiled until the release of dense white fumes. After cooling, 20 ml distilled water was added and heated until a clear solution was obtained. At room temperature, the mixture was filtered and transferred quantitatively to a 50 ml volumetric flask by adding double-distilled water. While, the soil sample (20g) were mixed with 40 ml diethylenetriaminepentaacetic acid (DTPA) and secured upright on shaker for 2 hours. Then the suspensions were filtered. The obtained samples' solutions were analyzed through atomic absorption spectrophotometer, PERKIN ELMER 3300.

## **2.5. Statistical analysis**

Data were analyzed using IBM SPSS software package version 20.0. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data was described as mean  $\pm$  standard deviation (mean  $\pm$  SD). Data were analyzed by using one-way ANOVA to compare between more than two groups and Post Hoc test (Tukey) for pairwise comparisons. Significance of the obtained results was judged at the 5% level. The marked changes observed in RAPD profiles (disappearance and/or appearance of bands compared with control) were evaluated (Gjorgieva et al., 2013).

## **3.Results**

### **3.1Activities and relative gene expression of antioxidant enzymes**

239 **After 30 days**, most Cd, Ni or Pb contaminated soils significantly elevated **GPx and GST**  
 240 **activities** in seedlings compared to control (**Table 1**). A non-significant elevation in GPx  
 241 activity was observed at Cd 60, 120 ppm and Pb at 300 ppm and in GST activity at Cd 120  
 242 ppm and Ni 100 ppm. While, lowest Ni concentration (50 ppm) showed a significant  
 243 suppression in GST activity compared to control.

244 In addition, the expression level of GPx was significantly up-regulated in seedlings leaves at  
 245 different treatments except Pb at 200 ppm compared to control. While seedlings roots were  
 246 diversely affected by metals contamination where Cd (30 and 60 ppm) showed non-  
 247 significant increase and Ni (150 ppm) showed a significant up-regulation and other metals  
 248 showed a significant reduction in GPx expression level compared to control (**Supp 4 and**  
 249 **Table 2**). Cd, Ni or Pb stressed soils were significantly up-regulated the expression level of  
 250 CAT in seedlings leaves and roots except Cd significantly down-regulated CAT expression  
 251 level in seedlings roots compared to control. This down regulation was peaked at lower Cd  
 252 concentration and declined at higher Cd concentration. The expression level of SOD was  
 253 significantly down-regulated in both seedlings leaves and roots compared to control.  
 254 Moreover, 200 and 300 ppm Pb treatment resulted in maximum down-regulation of SOD  
 255 expression about 52 and 58 %, respectively.

256 After 5 months, GST activity in grains showed a non- significant change compared to control  
 257 except Cd 30 ppm showed a significant elevation. In addition, Cd 60 ppm, Ni 50 ppm and Pb  
 258 100, 200 and 300 ppm showed a non- significant elevation in grain GPx activity.

259 Notably, in seedlings and grains the activities of GPx and GST were maximum at lower Cd  
 260 concentration followed by a decline at higher Cd concentrations compared to control (**Table**  
 261 **1**). GPx and GST activities of seedlings increased about 61.9 and 76.9 %, while this increase  
 262 was around 16.6 and 14.6 % for grains at 30 ppm Pb compared to the control. GPx activity  
 263 was lower in grains than that in seedlings and vice versa in GST.

### 3.2 Proline and total protein contents in seedling parts

Proline content was significantly increased in either Cd, Ni or Pb- stressed wheat plants (**Table 3**). It was found to increase in a dose dependent manner, showing highest elevation at Pd 300 ppm (about 363%).

Total protein content was significantly elevated in roots and leaves after both Cd and Ni treatments than corresponding untreated control parts, except Cd at 120 ppm showed a non-significant decrease by only 3% in roots (**Table 3**). On the other hand, in Pb stressed plants the total protein content was significantly decreased in roots while it was significantly elevated in leaves except 100 ppm showed a non-significant change.

### 3.3 Chlorophyll content and flag leaf area

Cd, Ni and Pb treatments adversely affected the plant pigment system. A significant decline in chlorophyll a, chlorophyll b, total chlorophyll and flag leaf area was observed in metal-stressed plants and the magnitude of decline was in a dose dependent manner (**Table 3**). The concentration of chlorophyll a was found to be higher than chlorophyll b in plant leaves. Pd treatment especially at 300 ppm showed the highest decrease in chlorophyll a, chlorophyll b and total chlorophyll by about 36 % and flag leaf area was decreased by about 46%.

### 3.4 Vegetative growth parameters

After 5 months, Cd, Ni and Pb ions had negatively impacted the plant growth parameters. This inhibition was in a dose dependent manner and the highest metals concentration produced the shortest plant and the least number of tillers/plant compared to control (**Table 4**). A significant reduction in wheat height by 21.67, 16.11, 21.11 % and the number of tillers/plant by about 29 % was observed in Cd (120 ppm), Ni (150 ppm) and Pb (300 ppm) stressed soils, respectively. In addition, fresh and dry weight of wheat were clearly affected by Cd, Ni and Pb treatments, reaching lowest values at 300 ppm Pb, 150 ppm Ni and 120 ppm Cd, respectively. Moreover, spike weight, number of spikes/pot and weight of 100

grains were remarkably reduced by applying different concentrations of Cd, Ni and Pb (Table 4). Interestingly, the highest Pb concentration (300 ppm) showed the maximum inhibitory effect on plant growth parameters. On the contrary, Ni 50 ppm resulted in no statistically change in wheat height, dry weight, spike weight and number of spikes/ pot compared to control.

### 3.5 DNA polymorphism profile

RAPD profiles generated following the metal exposure were altered and different from those of control DNA as indicated in Figure 2 and Table 5. Generally, in this study, heavy metals treatment leads to normal fragment pattern disappearance as the total number of disappeared fragments was higher than the number of appeared bands. A variation in band intensities in comparison with control profiles was also observed. The maximum change in RAPD profiles was obtained at 120 ppm Cd and 300 ppm Pb concentrations (P= 100 % and GTS= 0 %), as compared with control profile. The percentages of polymorphism are in the range of 73.1-100% and the values of GTS ranged from 26.9% to zero %. Samples treated with Cd, showed an increase in the percentages of polymorphism and a decrease in GTS with increasing the concentration, although the pattern of Ni and Pb induced genotoxicity did not follow the concentration gradient.

### 3.6 Heavy metals content in wheat straw and grains as well as in post-harvest soils (after 5 months)

In the present study, heavy metals (Cd, Ni and Pb) accumulation in different wheat parts (straw and grains) was directly proportional with metals concentrations in soil (Table 6). Metals were strongly accumulated in straw more than in grains. The highest Cd concentration (120 ppm) showed the maximum accumulation in straw and grains by about 14 and 16 fold, respectively than control ( $P < 0.05$ ). As well as, Ni (150 ppm) and Pb (300 ppm) treated soils

313 were found to increase Ni and Pb accumulation in straw and grains by lower levels than Cd  
314 compared to control.

315 The chemical analysis of Cd, Ni and Pb treated-soils indicated that the use of different  
316 quantities of heavy metals significantly increased its availability to 27.2, 4.08, 1.44 fold in  
317 first season and 12.5, 7.1, 1.56 fold in second season, respectively, compared to control  
318 (**Table 6**).

#### 4. Discussion

Detecting and evaluating biological changes that result from chemical contamination are essential steps to determine the significance of such contamination (Stegemann et al., 2018). High concentration of heavy metals becomes toxic to biota and humans and significantly reduces plant production. The present study revealed a significant decrease in vegetative growth parameters by Cd, Ni or Pb toxicity. Moreover, Pb treated plants showed the poorest growth as indicated by the highest reduction in wheat height, number of tillers, fresh and dry weight, flag leaf area as well as yield components. Pb intoxication also resulted in the highest proline content, least protein and chlorophyll contents, thus affected the overall plants growth followed by Cd and Ni, respectively. Furthermore, high Pb and Cd doses revealed highest degree of polymorphism and lowest degree of genome stability.

Heavy metals induce irreparable physiological and biochemical dysfunctions in plants by inducing the rapid generation of ROS and may cause oxidative stress and cell death (Shu et al., 2012; Shahid et al., 2014; Jalmi et al., 2018). In 2015, studies reported that the non-enzymatic and enzymatic antioxidants like SOD, peroxidase (POD), ascorbate peroxidase (APX) and CAT showed up or down regulation to cope and repair the damage and this confer the stress tolerance of plants (Ahmad et al., 2015a; Sofo et al., 2015).

Shahabivand et al. (2016) reported that Cd toxicity significantly altered the activities and the transcription levels of CAT (decreased), APX and GST (increased) in leaf samples. Also Aydin et al. (2016), illustrated that the gene expression pattern of CAT under different Cd concentrations revealed a positive correlation, and there is no correlation found between the mRNA level and CAT enzyme activity in tomato leaves. Shu et al. (2012) stated that Pb treatment had a stimulating effect on SOD activity at low concentration and an inhibitory effect



at higher concentration in cuttings while in seedlings SOD activity was increased by increasing Pb concentration. In addition, 2 mM Ni solution, increased the expression levels of Fe-SOD, CAT, POD, and APX in soybean seedlings than the control levels (Sirhindi et al., 2016).

In the present study, the activities of GPx and GST in wheat seedlings (after 30 days) and grains (after 5 months) were increased by Cd, Ni or Pb- stress in an irregular manner not concentration dependent. Interestingly, the highest Cd (120 ppm), Pb (300 ppm) and Ni (150 ppm) concentrations showed the lowest GPx activities in seedlings while it altered in grains. Li et al. (2012) reported that GPx may be considered as an intrinsic defense against Pb-induced oxidative damage in perennial ryegrass and he also stated that the antioxidant enzymes (including; GPx, SOD, CAT and POD) responded to Pb exposure at an early stage. Genes encoding SOD and GPx were upregulated within a few hours after Pb treatment and after 24 or 48 h, their expression levels were returned to the levels of control or below and after 7 d the SOD activity was below that in untreated control (Li et al., 2012). Liu et al. (2009) also reported that the SOD gene expression was substantially increased within 3 h of Pb treatment and then gradually declined to a stable level in *A. thaliana* seedlings. In agreement with these findings, the activities of GPx and GST after 30 days (in wheat seedlings) more than that after 5 months (in grains). Therefore, the antioxidant enzymes might be responsible for the early stage defense to heavy metals- induced oxidative stress and may play an important role in eliminating the metals toxicity.

Furthermore, the expression level of GPx in leaves was significantly up-regulated (except in Pb at 200 ppm) after heavy metals stress, while in roots Cd (30 and 60 ppm) showed non-significant changes, Ni (150 ppm) showed a significantly up-regulation and other metals showed a significant reduction in GPx expression level. Cd, Ni or Pb stressed soils were significantly up-regulated the expression level of CAT in seedlings leaves and roots (except Cd treatments in

roots). A protective mechanism was suggested by the increased expression of specific genes encoding antioxidant enzymes (Baek & Skinner, 2003). On the other hand, the expression level of SOD was significantly down-regulated in both seedlings leaves and roots. This indicates the consumption of antioxidant enzymes to protect the membrane stability and to scavenge oxidative damage ( $O_2^-$  and  $H_2O_2$  production) induced by heavy metals (Shahabivand et al., 2016; Sirhindi et al., 2016). The altered enzymatic levels indicate the tolerance of wheat plants against heavy metals stress.

In this study, total protein content was progressively increased in leaves stressed by Cd, Ni or Pb and in roots stressed by Cd (30, 60 ppm) and Ni, while it was decreased in roots stressed by Cd (120 ppm) and Pb. The increase in total protein may be due to the induction of stress proteins that mainly involved in detoxification, antioxidant processes and the activation of genes for synthesis of specific proteins associated with stress such as some heat shock proteins that maintain the membrane proteins and the plant cell structures (Shu et al., 2012; Alayat et al., 2014). On the other hand, the inhibitory action of Cd (120 ppm) and Pb on roots protein content may be due to metals toxicity that reduces the protein synthesis and elevates protease activity, causing proteins degradation. Nevertheless, heavy metal stress generates ROS that in turn damages proteins, also the binding of metals to protein-SH groups can cause deleterious effect in the normal protein structure (Sirhindi et al., 2016; Natarajan et al., 2018). This decrease agrees with the decrease in GPx expression level in roots concerning Cd (120 ppm) and Pb (100, 200 & 300 ppm) toxicity.

Proline is a pivotal and multifunctional amino acid, plays an essential role not just in plant developmental processes (including; chlorophyll reconstruction, Krebs cycle activation and an energy source) but also in reducing environmental stress (Trovato et al., 2008). Proline

accumulation under heavy metal stress has been reported to be a potential indicator of stress tolerance (Parlak, 2016; Sirhindi et al., 2016). This accumulation may be due to the reduction of its degradation as it acts as an osmoprotectant, macromolecules stabilizer, metal chelator and inhibitor of lipid peroxidation as well as free radical scavenger (Ahmad et al., 2015a; Ahmad et al., 2015b). In consistent with these findings, proline content shows a positive association with increasing the three metals concentrations in the soil and Pb proved to be the strongest inducer for proline accumulation.

Toxic Metal ions directly or indirectly influence DNA synthesis and the replication process (Morales et al., 2016; Theophanides & Anastassopoulou, 2017). Metal ions can interact with DNA through hydrogen bonding of the metal-coordinating water molecules resulting in the disruption of base pair hydrogen bonding and cause changes in the structure of the DNA double helix (Anastassopoulou, 2003; Theophanides & Anastassopoulou, 2017). ROS generated by metal-catalyzed reactions can modify DNA bases; three base substitutions, G/ C, G/ T and C/ T, can occur as a result of oxidative damage by metal (Al-Qurainy, 2009).

The genotoxic effect of Cd, Ni or Pb contamination was investigated in the present study through the analysis of ten different RAPD primers in wheat seedlings roots. The results showed obviously different RAPD amplification products in metals treated samples compared to control sample. According to our results, low metals concentrations may lead to the lower level of genotoxic effects of wheat seedlings than higher concentrations as indicated by higher values of GTS at Cd 30 ppm, Ni 50 ppm and Pb 100, 200 ppm. In addition, the number of missing bands was found more than that of newly appeared bands. These results are in agreement with the study of Gjorgieva et al. (2013) however, their results showed that Ni treatment at concentrations of 150 and 350 ppm showed the highest number of lost bands in bean seedlings than Cd and Pb at

the same concentrations, but in the present study Cd 60 ppm and Pb 100, 300 showed the highest number of missing bands (15 band/ 10 primers). The disappearance of normal DNA bands may be resulted from DNA damage (e.g. single- and double-strand breaks, bulky adducts, modified or oxidized bases) and point mutation that may be induced by heavy metals treatments (Atienzar & Jha, 2006). New RAPD amplification products (extra bands) may be attributed to a change in some oligonucleotide priming sites due to mutations (new annealing events), homologous recombination and/or large deletions (bringing to pre-existing annealing site closer) (Atienzar et al., 1999). The highest number of extra bands (12 band/ 10 primers) was observed in Cd 120 ppm and Ni 100 ppm treated samples suggests that this metal probably cause mutations on genomic level in high doses. Cd and Pb at highest concentrations were detected for the highest level of DNA damage (GTS 0% and P 100%), and for Ni, 100 ppm showed highest genotoxicity (GTS 3.8% and P 96.2%) than other concentrations. There is correlation between results obtain for Cd 300 ppm in the present study (highest GPx and SOD expression level, highest number of missing bands in RAPD profile and lowest GTS; comparing to ether metals treated samples) with a study from 2013 (Gjorgieva et al., 2013) and 2010 (Wan-Ibrahim et al., 2010) where author's deduced the correlation between genotoxicity and high antioxidant level. In (2017) Meng et al. provided the molecular mechanisms that investigated Cd toxicity on DNA and protein metabolism at protein levels. Cd toxicity was found to inhibit DNA glycosylase, ligation enzymes expressions and gap-filling, therefore, decreasing DNA repair capacity. At the protein level, Cd induced the heat shock protein response, initiation of protein refolding as well as degradation by ubiquitin proteasome pathway. In addition, excess Cd also induced antioxidant responses (Meng et al., 2017).

In present study, chlorophyll a, b and total chlorophyll contents were drastically reduced in wheat flag leaf with increasing the concentrations of externally supplied metals and Pb had the least contents followed by Ni and Cd, respectively. This reduction may be occurred due to (i) the disturbance in the uptake of  $Mg^{2+}$  and essential elements (such as Mn and Fe) (Parlak, 2016), (ii) the inhibition of chlorophyll biosynthesis due to the interaction of heavy metals with –SH group of enzymes involved in that process (Shu et al., 2012) and (iii) the increase in chlorophyllase (the first enzyme of the chlorophyll catabolic pathway) activity, which resulted in chlorophyll degradation (Liu et al., 2008). The reduction in chlorophyll content and consequent inhibition of photosynthesis are indicating symptoms of metals toxicity and generally reduce plants growth (Ci et al., 2010; Deswal & Laura, 2018; Marzilli et al., 2018). In this study, Cd, Ni or Pb at different concentrations showed a significant reduction in wheat height, number of tillers, fresh and dry weight, flag leaf area as well as yield components (weight and number of spikes and weight of 100 grains) and the highest reduction was observed in Pb, these results are consistent with who reported that metal stress created disorders in metabolic system of plants and quickly inhibited the cell division and cell elongation process leading to reduction of leaf blade area, leaf density and plant stem growth with accompanying decrease in numbers of flowers and fruits as well as shoot and root growth.

In the present study Cd, Ni and Pb were accumulated more in straw (leaves+ stem) than grains of wheat. Note also, the content of metal in straw and grains was increased in a dose dependent manner corresponding to metal concentration in the soil and Cd showed the highest accumulation in straw and grains. These results corroborate with the findings of Dang et al. (1990); Jafarnejadi et al. (2011) and Ali et al. (2018) who reported that metal uptake by grains was directly related to the applied heavy metal in the soil and accumulation of metal in plants

differed greatly among organs or tissues and was found to follow soil > root > shoot > grain. Moreover, the DTPA extractable elements (Cd, Ni or Pb) from soil were increased with increasing levels of applied elements. In addition, the metals levels were found to be higher than the maximum allowable limits for Cd (0.3 ppm), Ni (50 ppm) and Pb (60 ppm) in soils (Al Naggar et al., 2014; Shokr et al., 2016; Toth et al., 2016). Sardar et al. (2013) and Placek et al. (2016) reported that removal of heavy metals through leaching or uptake by the plants is much lower than the rate of their entry into the soil, leading to gradual accumulation of elements in the soil. The studies of Dang et al. (1990) and Deswal and Laura (2018) deduced that the phytotoxicities and metals accumulation in soil were found to be in the order:  $\text{Cd}^{2+} > \text{Ni}^{2+} > \text{Pb}^{2+}$  in different crop species by using the same concentrations of heavy metals. In the present study, similar trend was observed for metals accumulation in soil, however, the phytotoxicities was found to be in the order:  $\text{Pb}^{2+} > \text{Cd}^{2+} > \text{Ni}^{2+}$  where metals concentrations were selected according to guideline values for metals in soils.

## Conclusions

The studied heavy metals (i.e. Cd, Ni and Pb) have a negative impact on plant growth and development. They are readily taken up and accumulated in plant parts causing proportionate changes in the biochemical responses (concerning the contents of protein, proline, chlorophyll, antioxidant system and DNA stability), reduction in vegetative growth parameters (concentration dependent) and finally led to a significant reduction in wheat yield and quality. The changes occurred in RAPD profiles, can be successfully used as a sensitive marker for detecting metal induced DNA damage and showed the potential as a reliable assay for genotoxicity. Finally, we concluded the importance of increasing the public awareness of the threat of heavy metals

pollution and also keep the soil, water and fertilizers away from heavy metals contamination, especially cadmium, nickel and lead.

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## References:

- Abdel-Sabour, M. F., Ismail, A. S., & Abou Naga, H. (1996). Environmental impact of Cairo sewage effluent on El-Gabal El-Asfer farm. *Egyptian Journal of Soil Science*, 36, 329-342.
- Ahmad, P., Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., John, R., Egamberdieva, D., & Gucel, S. (2015a). Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Front Plant Sci*, 6, 868. doi:10.3389/fpls.2015.00868
- Ahmad, P., Sarwat, M., Bhat, N. A., Wani, M. R., Kazi, A. G., & Tran, L. P. (2015b). Alleviation of cadmium toxicity in *Brassica juncea* L.(Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. *PloS one*, 10(1), e0114571.
- Al-Qurainy, F. (2009). Toxicity of heavy metals and their molecular detection on *Phaseolus vulgaris* (L.). *Australian Journal of Basic Applied Sciences*, 3(3), 3025-3035.
- Al Naggar, Y., Naiem, E., Mona, M., Giesy, J. P., & Seif, A. (2014). Metals in agricultural soils and plants in Egypt. *Toxicological and Environmental Chemistry*, 96(5), 730-742.
- Alayat, A., Souiki, L., Grara, N., Djebbar, M. R., Boumedris, Z. E., Benosmane, S., . . . Berrebbah, H. (2014). Effects of cadmium on water content, soluble protein, proline changes and some antioxidant enzymes in wheat (*Triticum durum* desf.) leaves. *Annual Research and Review in Biology*, 4(24), 3835-3847.
- Ali, Z., Mujeeb-Kazi, A., Quraishi, U. M., & Malik, R. N. (2018). Deciphering adverse effects of heavy metals on diverse wheat germplasm on irrigation with urban wastewater of mixed municipal-industrial origin. *Environ Sci Pollut Res Int*, 25(19), 18462-18475. doi:10.1007/s11356-018-1996-0
- Anastassopoulou, J. (2003). Metal–DNA interactions. *Journal of Molecular Structure*, 651-653, 19-26. doi:https://doi.org/10.1016/S0022-2860(02)00625-7
- Atienzar, F. A., Conradi, M., Evenden, A. J., Jha, A. N., & Depledge, M. H. (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: Comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a]pyrene. *Environ Toxicol Chem*, 18(10), 2275-2282. doi:10.1002/etc.5620181023

- Atienzar, F. A., & Jha, A. N. (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutat Res*, 613(2-3), 76-102. doi:10.1016/j.mrrev.2006.06.001
- Aydin, S. S., Büyük, I., Gündüzer, E. G., Büyük, B. P., Kandemir, İ., Duman, D. C., & Aras, S. (2016). Effects of Lead (Pb) and Cadmium (Cd) Elements on Lipid Peroxidation, Catalase Enzyme Activity and Catalase Gene Expression Profile in Tomato Plants. *Journal of Agricultural Sciences*, 22(4), 539-547.
- Azimi, A., Shahriari, F., Fotovat, A., Qale, R. K., & Agje, K. H. (2013). Investigation of DNA changes in wheat (*Triticum aestivum* L.) induced by cadmium using random amplified polymorphic DNA (RAPD) analysis. *African Journal of Biotechnology*, 12(16), 1921-1929. doi:10.5897/AJB12.2836
- Baek, K.-H., & Skinner, D. Z. (2003). Alteration of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat lines. *Plant Science*, 165(6), 1221-1227. doi:https://doi.org/10.1016/S0168-9452(03)00329-7
- Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39(1), 205-207. doi:10.1007/BF00018060
- Chapman, H. D., & Pratt, P. F. (1961). Methods of analysis for soils, plants, and waters. Riverside: University of California, Division of Agricultural Sciences.
- Ci, D., Jiang, D., Wollenweber, B., Dai, T., Jing, Q., & Cao, W. (2010). Cadmium stress in wheat seedlings: growth, cadmium accumulation and photosynthesis. *Acta Physiologiae Plantarum*, 32(2), 365-373. doi:10.1007/s11738-009-0414-0
- Dang, Y. P., Chhabra, R., & Verma, K. S. (1990). Effect of Cd, Ni, Pb and Zn on growth and chemical composition of onion and fenugreek. *Communications in Soil Science and Plant Analysis*, 21(9-10), 717-735. doi:10.1080/00103629009368265
- Deswal, M., & Laura, J. S. (2018). Effect of heavy metals cadmium, nickel and lead on the seed germination and early seedling growth of *Pisum sativum*. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 4(2), 368. doi:10.26479/2018.0402.29
- Gjorgieva, D., Kadifkova Panovska, T., Ruskovska, T., Bačeva, K., & Stafilov, T. (2013). Mineral nutrient imbalance, total antioxidants level and DNA damage in common bean (*Phaseolus vulgaris* L.) exposed to heavy metals. *Physiology and Molecular Biology of Plants : An International Journal of Functional Plant Biology*, 19(4), 499-507. doi:10.1007/s12298-013-0196-0
- Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J Biol Chem*, 177(2), 751-766.
- Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem*, 249(22), 7130-7139.
- Jafarnejadi, A. R., Homaei, M., Sayyad, G., & Bybordi, M. (2011). Large Scale Spatial Variability of Accumulated Cadmium in the Wheat Farm Grains. *Soil and Sediment Contamination: An International Journal*, 20(1), 98-113. doi:10.1080/15320383.2011.528472
- Jalmi, S. K., Bhagat, P. K., Verma, D., Noryang, S., Tayyeba, S., Singh, K., . . . Sinha, A. K. (2018). Traversing the Links between Heavy Metal Stress and Plant Signaling. *Front Plant Sci*, 9, 12. doi:10.3389/fpls.2018.00012



- Jhang, T., & Shasany, A. K. (2012). Random amplified marker technique for plants rich in polyphenols. *Methods Mol Biol*, 862, 61-74. doi:10.1007/978-1-61779-609-8\_6
- Kabata-Pendias, A., & Pendias, H. (2001). *Trace elements in soils and plants* (3rd ed.). Boca Raton, FL, USA: CRC Press.
- Li, H., Luo, H., Li, D., Hu, T., & Fu, J. (2012). Antioxidant enzyme activity and gene expression in response to lead stress in perennial ryegrass. *Journal of the American Society for Horticultural Science*, 137(2), 80-85.
- Lindsay, W. L., & Norvell, W. A. (1978). Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. *Soil Science Society of America Journal*, 42(3), 421-428. doi:10.2136/sssaj1978.03615995004200030009x
- Liu, D., Li, T. Q., Jin, X. F., Yang, X. E., Islam, E., & Mahmood, Q. (2008). Lead induced changes in the growth and antioxidant metabolism of the lead accumulating and non-accumulating ecotypes of *Sedum alfredii*. *J Integr Plant Biol*, 50(2), 129-140. doi:10.1111/j.1744-7909.2007.00608.x
- Malar, S., Vikram, S. S., Favas, P. J. C., & Perumal, V. (2014). Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Botanical Studies*, 55(1), 54. doi:10.1186/s40529-014-0054-6
- Marzilli, M., Di Santo, P., Palumbo, G., Maiuro, L., Paura, B., Tognetti, R., & Coccozza, C. (2018). Cd and Cu accumulation, translocation and tolerance in *Populus alba* clone (Villafranca) in autotrophic in vitro screening. *Environ Sci Pollut Res Int*, 25(10), 10058-10068. doi:10.1007/s11356-018-1299-5
- Meng, J., Wang, W., Li, L., Yin, Q., & Zhang, G. (2017). Cadmium effects on DNA and protein metabolism in oyster (*Crassostrea gigas*) revealed by proteomic analyses. *Scientific Reports*, 7(1), 11716. doi:10.1038/s41598-017-11894-7
- Mohamed, R. A., Aboulila, A. A., El-Kholya, S. Z., & Hamza, A. (2017). Evaluation of genetic polymorphism, genomic template stability, condition factor and hemato-biochemical parameters in response to slow reduction in water level during Nile tilapia (*Oreochromis niloticus*) harvest. *The Thai Journal of Veterinary Medicine*, 47(4), 435-448.
- Morales, M. E., Derbes, R. S., Ade, C. M., Ortego, J. C., Stark, J., Deininger, P. L., & Roy-Engel, A. M. (2016). Heavy Metal Exposure Influences Double Strand Break DNA Repair Outcomes. *PloS one*, 11(3), e0151367-e0151367. doi:10.1371/journal.pone.0151367
- Moran, R. (1982). Formulae for determination of chlorophyllous pigments extracted with n,n-dimethylformamide. *Plant Physiology*, 69(6), 1376-1381.
- 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. doi:DOI 10.1007/s10311-010-0297-8
- Natarajan, A., Vijayarengan, P., & Vijayaragavan, M. (2018). Influence of cadmium on growth and biochemical contents of tomato plants. *Journal of Plant Stress Physiology*, 4, 04-06.
- Omran, M. S., Waly, T. M., Abd Elnaim, E. M., & El Nashar, B. M. B. (1988). Effect of sewage irrigation on yield, tree components and heavy metals accumulation in navel orange trees. *Biological Wastes*, 23(1), 17-24. doi:https://doi.org/10.1016/0269-7483(88)90041-9
- Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*, 70(1), 158-169.

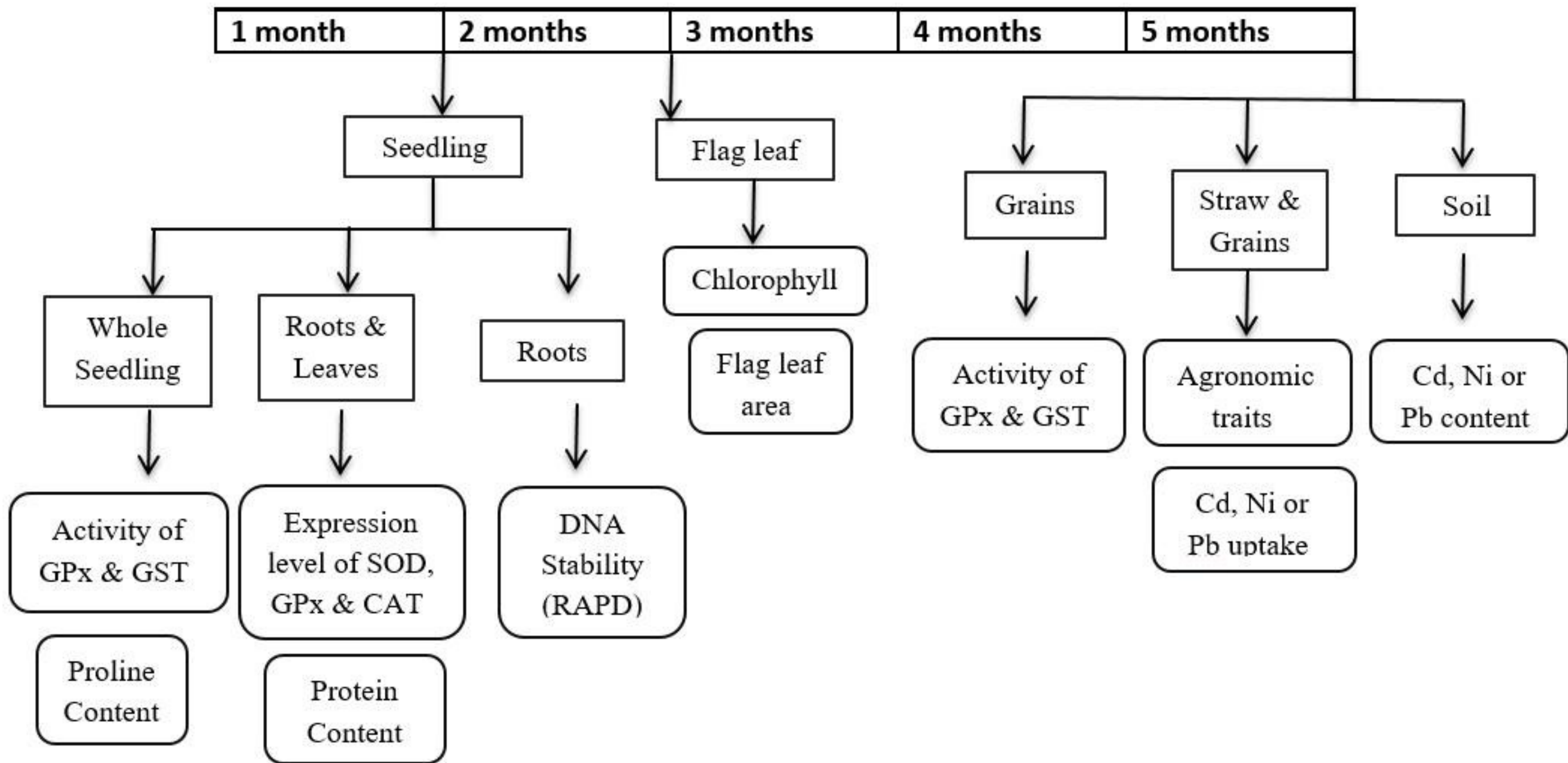
- Parlak, K. U. (2016). Effect of nickel on growth and biochemical characteristics of wheat (*Triticum aestivum* L.) seedlings. *NJAS-Wageningen Journal of Life Sciences*, 76, 1-5.
- Placek, A., Grobelak, A., & Kacprzak, M. (2016). Improving the phytoremediation of heavy metals contaminated soil by use of sewage sludge. *International Journal of Phytoremediation*, 18(6), 605-618. doi:10.1080/15226514.2015.1086308
- Rizwan, M., Ali, S., Adrees, M., Rizvi, H., Zia-Ur-Rehman, M., Hannan, F., . . . Ok, Y. S. (2016). Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. *Environ Sci Pollut Res Int*, 23(18), 17859-17879. doi:10.1007/s11356-016-6436-4
- Saber, M., Hoballah, E., Abouziena, H., Haggag, W. M., El-Ashry, S., & Zaghloul, A. (2016). Management of Sewage Farming in Arid Region: Egyptian Experience. *International Scientific Researches Journal*, 72(3), 28-56. doi:10.21506/j.ponte.2016.3.7
- Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M. B., . . . Tauqeer, H. M. (2013). Heavy metals contamination and what are the impacts on living organisms. *Greener Journal of Environmental Management and Public Safety*, 2(4), 172-179.
- Shahabivand, S., Maivan, H. Z., Mahmoudi, E., Soltani, B. M., Sharifi, M., & Aliloo, A. A. (2016). Antioxidant activity and gene expression associated with cadmium toxicity in wheat affected by mycorrhizal fungus. *Zemdirbyste-Agriculture*, 103(1), 53-60.
- Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M., & Pinelli, E. (2014). Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. *Rev Environ Contam Toxicol*, 232, 1-44. doi:10.1007/978-3-319-06746-9\_1
- Shokr, M. S., El Baroudy, A. A., Fullen, M. A., El-beshbeshy, T. R., Ramadan, A. R., Abd El Halim, A., . . . Jorge, M. C. O. (2016). Spatial distribution of heavy metals in the middle Nile delta of Egypt. *International Soil and Water Conservation Research*, 4(4), 293-303. doi:https://doi.org/10.1016/j.iswcr.2016.10.003
- Shu, X., Yin, L., Zhang, Q., & Wang, W. (2012). Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. *Environ Sci Pollut Res Int*, 19(3), 893-902. doi:10.1007/s11356-011-0625-y
- Sipos, P., & Póka, T. (2002). Threshold limit values for heavy metals in soils in the function of spatial and temporal variation of geochemical factors. Paper presented at the Proceedings of XVII. Congress of Carpathian-Balkan Geological Association Bratislava, September 1st-4th.
- Sirhindi, G., Mir, M. A., Abd-Allah, E. F., Ahmad, P., & Gucel, S. (2016). Jasmonic Acid Modulates the Physio-Biochemical Attributes, Antioxidant Enzyme Activity, and Gene Expression in Glycine max under Nickel Toxicity. *Frontiers in Plant Science*, 7, 591. doi:10.3389/fpls.2016.00591
- Sofo, A., Scopa, A., Nuzzaci, M., & Vitti, A. (2015). Ascorbate Peroxidase and Catalase Activities and Their Genetic Regulation in Plants Subjected to Drought and Salinity Stresses. *Int J Mol Sci*, 16(6), 13561-13578. doi:10.3390/ijms160613561
- Stegemann, T., Kruse, L. H., Brutt, M., & Ober, D. (2018). Specific Distribution of Pyrrolizidine Alkaloids in Floral Parts of Comfrey (*Symphytum officinale*) and its Implications for Flower Ecology. *J Chem Ecol*. doi:10.1007/s10886-018-0990-9
- Szolnoki, Z., Farsang, A., & Puskás, I. (2013). Cumulative impacts of human activities on urban garden soils: Origin and accumulation of metals. *Environmental Pollution*, 177, 106-115. doi:https://doi.org/10.1016/j.envpol.2013.02.007

- Theophanides, T., & Anastassopoulou, J. (2017). The effects of metal ion contaminants on the double stranded DNA helix and diseases. *Journal of Environmental Science and Health, Part A*, 52(10), 1030-1040. doi:10.1080/10934529.2017.1328950
- Toth, G., Hermann, T., Da Silva, M. R., & Montanarella, L. (2016). Heavy metals in agricultural soils of the European Union with implications for food safety. *Environ Int*, 88, 299-309. doi:10.1016/j.envint.2015.12.017
- Trovato, M., Mattioli, R., & Costantino, p. (2008). Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei*, 19(4), 325-346.
- Wan-Ibrahim, W., Sidik, K., & Kuppusamy, U. (2010). A high antioxidant level in edible plants is associated with genotoxic properties. *Food Chemistry*, 122(4), 1139-1144.
- Wuana, R. A., & Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology*, 2011, 20. doi:10.5402/2011/402647
- Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L., & Fu, J. (2016). Effect of Heavy Metals Pollution on Soil Microbial Diversity and Bermudagrass Genetic Variation. *Frontiers in Plant Science*, 7, 755-755. doi:10.3389/fpls.2016.00755

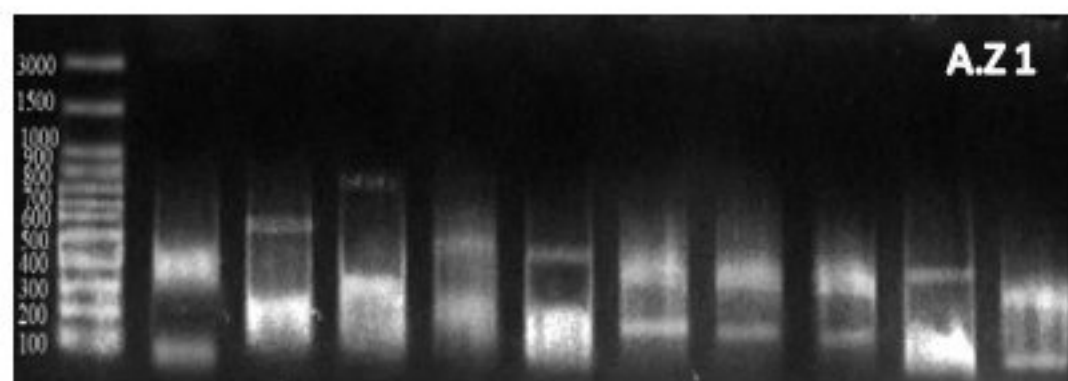
681 **Figure Legend**

682 **Figure 1. Experimental protocol (Plant sampling and analysis).**

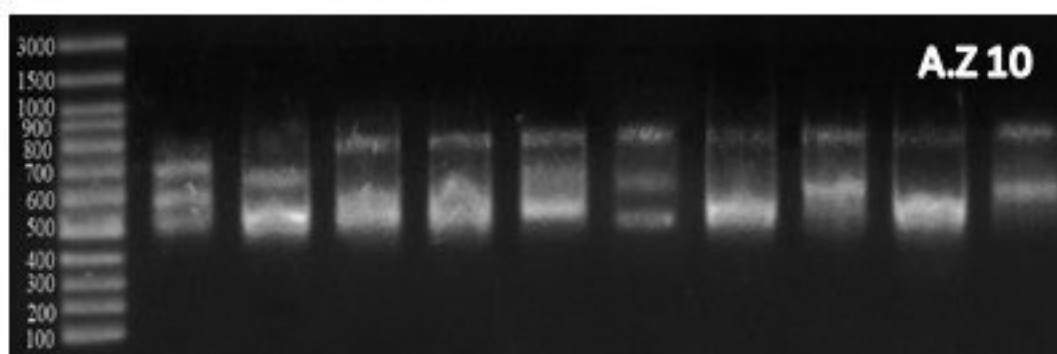
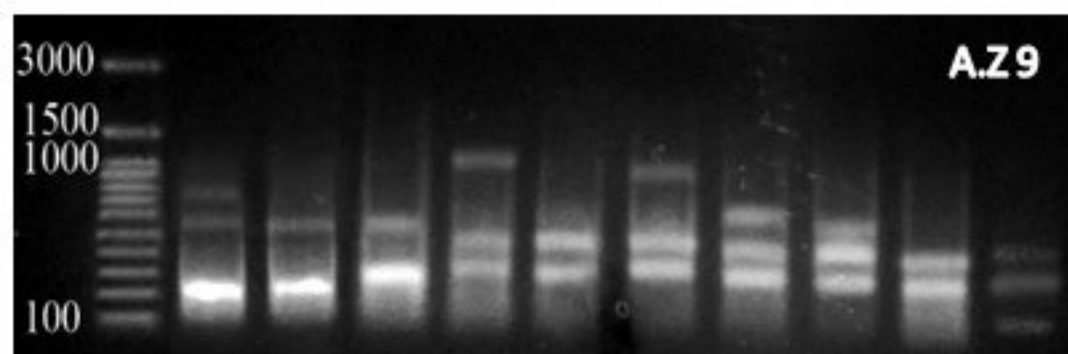
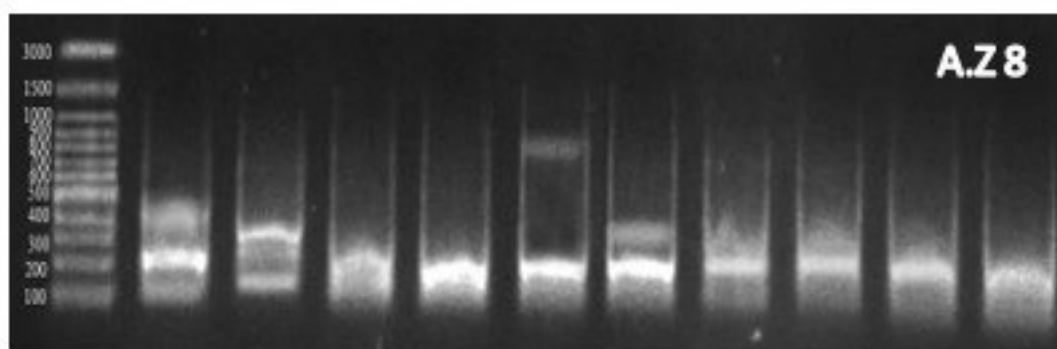
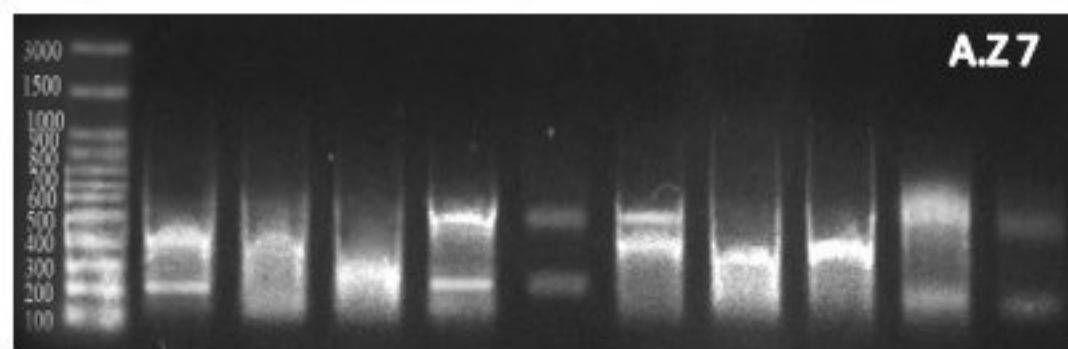
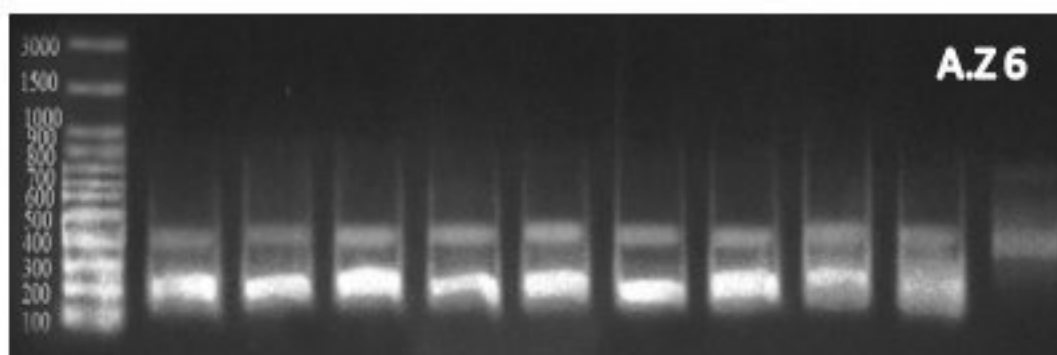
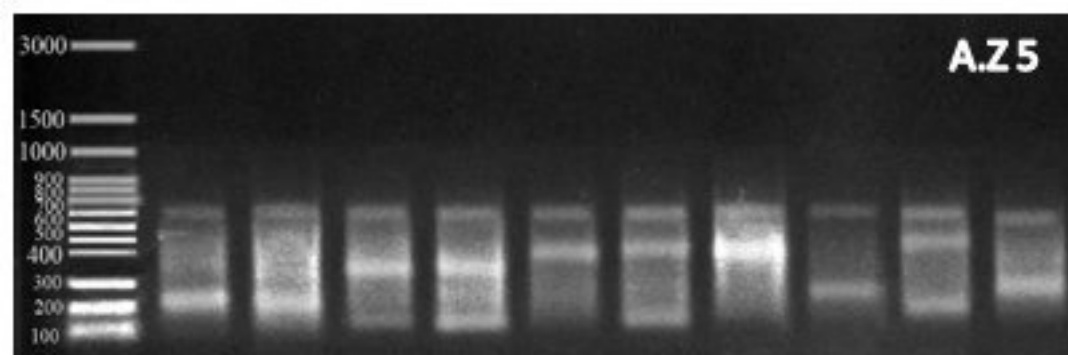
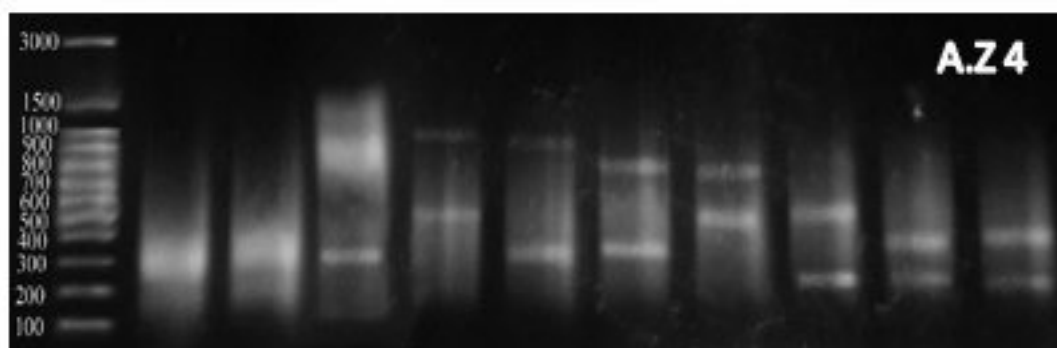
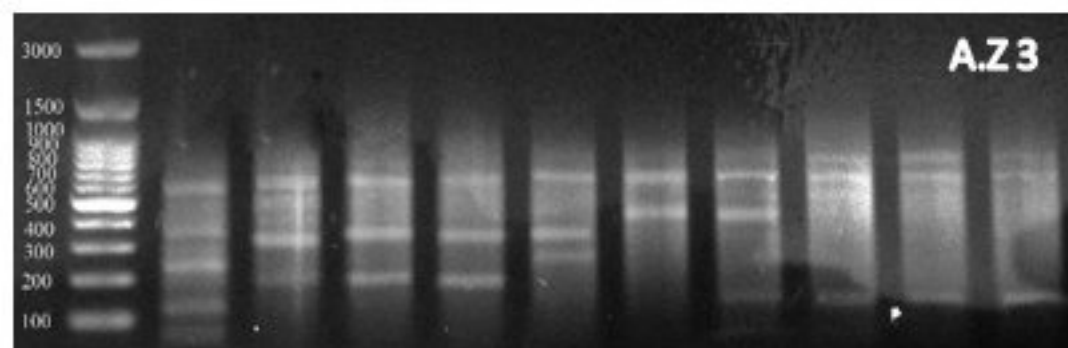
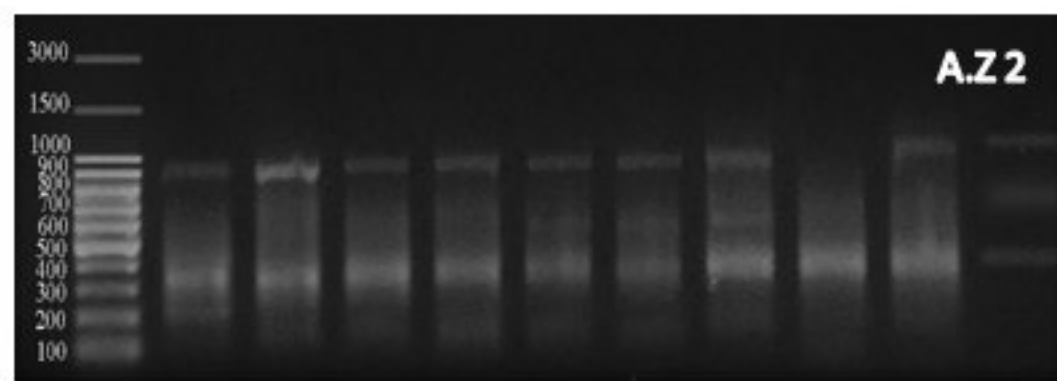
683 **Figure 2. RAPD profiles of genomic DNA from root-tips of wheat seedlings exposed to**  
684 **varying Cd, Ni and Pb concentrations.** Lanes M: DNA ladder; 1: control; 2: Cd 30  
685 ppm; 3: Cd 60 ppm; 4: Cd 120 ppm; 5: Ni 50 ppm; 6: Ni 100 ppm; 7: Ni 150 ppm; 8: Pb  
686 100 ppm; 9: Pb 200 ppm and 10: Pb 300 ppm.



M 1 2 3 4 5 6 7 8 9 10



M 1 2 3 4 5 6 7 8 9 10





Soil samples from Al-Gabal Al-Asfar farm were collected and treated with different concentrations of Cd, Ni and Pb

Wheat grains were sown in

### Biochemical Parameters after 2,3 & 5 months

Activity and  
Expression level of  
antioxidants

Proline and protein  
Contents

DNA Stability (RAPD)

Agronomic  
characteristics

Cd, Ni or Pb content  
in Straw, Grains and  
soil

### Results

Altered antioxidants  
enzymes.

Pb showed the highest  
proline, least protein and  
chlorophyll contents

High Pb and Cd doses  
revealed highest  
polymorphism and lowest  
genome stability

Heavy metals accumulated  
mainly in wheat straw and  
induced genotoxic effect.

Pb toxicity showed poorest agronomic characteristics and induce more genotoxic and phytotoxic effects followed by Cd and Ni.