

Full Length Research Paper

Heavy metal accumulations of *Allium cepa* L. as a bioindicator for air pollution in Ereğli, Turkey

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Ereğli, a province with iron-steel industrial activities, is situated on the coast of Black Sea in Northwestern Anatolia. Heavy metal pollution caused by industrial activities is a threat to environmental quality and human health. *Allium cepa* L. is a bioindicator due to its sensitivity to environmental pollution. Fe, Hg, Zn, Hg, Cr showed greater accumulation in its leaves in four different sampling points. A significant decrease at Hg and Pb concentrations was not seen in its leaves. This study showed that the leafy plants contaminated with toxic heavy metals during industrial activities and Ereğli's air quality has been poor.

Key words: Heavy metal, bioindicator, industrial pollution, Western Anatolia.

INTRODUCTION

Sulfur, nitrogen oxides, heavy metals and pollutants such as hydrocarbons with various industrial activities can turn into a new product into the atmosphere with complex chemical and physical reactions. Several studies suggested that metals can alter the composition, rigidity and fluidity of membranes, inhibiting water and nutrient fluxes, cellular division, and thus the normal development of the roots and leaf growth (Geremias et al., 2010). Pollutants such as heavy metals Pb, V, Fe, Co, Cu, Br, and Pb can be absorbed by plant leaves (Rodríguez et al., 2011). Thus, these metals negatively affect the plants. They may be transferred to animals and humans via the food chain (Tamoutsidis et al., 2002; Yildiz et al., 2008).

Heavy metals especially such as Cd, Hg, Pb, Zn, Cu, Al, Ni, Co, Cr, Fe, Cr and Pb, cause a great risk to human health and induce cancer formation and developmental disorders for children. Toxic and carcinogenic metals interact with DNA and nuclear proteins and cause oxidative destruction of biological macromolecules. DNA in plant is greatly enhanced by iron. ATP is known to bind

Fe and cause oxidation of lipids and DNA damage (Valko et al., 2005). Toxic effects of metals involve hepatotoxicity, neurotoxicity and nephrotoxicity and respiratory distress. Hg may be passing to blood easily through entering the respiratory tract and mucous membranes, and reaches all tissues and organs, is absorbed through the respiratory tract and pulmonary edema, bronchitis, can cause significant respiratory tract damage, also affects the nervous system (Adams et al., 2010).

Iron-steel industry in Turkey after the textile and clothing industry is the second largest industry. Ereğli, Turkey's western Black Sea coast is a province of Zonguldak. Iron-steel factory in Ereğli is the main source of air pollution in the long term. Due to industrial activity, the heavy metals have been concentrated in plants. Province inhabitants breathe air with harmful heavy metals. Individuals consuming foods grown in that region are faced with the adverse effects of air pollution. People who are sensitive to heavy metals are at greatest risk in terms of respiratory distress such as asthma and allergic pulmonary disorders. (Uyar et al., 2008; Akca et al., 2007). Cadmium is a serious soil and a significant environmental pollutant. Potential toxic effects even at low concentrations is remarkable. Leaves of plants easily can accumulate cadmium through roots from the soil.

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Atmospheric phenomena, sewage water, industrial wastes and groundwater constitutes the main source of cadmium. However, excessive amounts of heavy metals are toxic to plants. *Allium cepa* (onion) consumed as leafy vegetables was shown to be a useful short-term test systems in biomonitoring of environmental pollution. Compared to other plants, these plants are more sensitive to environmental stress (Blagojević et al., 2009).

The purpose of this research was to measure heavy metal concentrations in leaves of *A. cepa* and in soils at the places polluted with them. Bioaccumulation capability in leaves was compared with concentration of heavy metals in soils. Leaf development was tested as an economic bioassay with *A. cepa* to assess the toxicity of industrial pollutants containing copper, iron and so on.

MATERIALS AND METHODS

Plant samples

The sampling points in four different distances from iron-steel factories were determined. Onion samples were grown in pots and collected from four sampling points. Sampling points were located at different distances and directions from the factory. The first sampling point is 300 m. away from the factory. The second sampling point is 3 km away from, third and fourth sampling point are at distances of 5 to 7 km away from the factory. All plant samples taken from four sampling points were cleaned and washed with 1 M HCl and then washed in deionized water and air dried. Leaves were cut 4 cm in length and dried in an oven at 70°C for 12 h. Dry weight was measured. Correlations between dry weight of leaves and the four different distances from iron-steel factory was assessed by Pearson correlation in SPSS 14 computer program.

Analysis

To determine Cu, Cd, Hg, Zn concentrations in plant tissue, Perkin Elmer Analyst 400 flame atomic absorption deuterium lamp was used. Samples of dried plants were powdered at 70°C for 3 h in the oven for chemical analysis. Powder materials were stored in polyethylene tubes at room temperature. 2 g of powdered plant samples were washed with 5 ml of 65% HNO₃ and 2 ml of 30% H₂O₂ for analysis. Examples in HP-500 CEM MARS 5 (Mathews, NC, USA) microwave oven were turned into ashes at 200°C in 45 min. Later, they were cooled at room temperature. Extracts were passed through Whatman 42 filter paper. Filtrate with 30 ml deionized water was collected in polyethylene tubes and stored at 4°C. To determine the concentration of heavy metals, 30 ml of solution were analysed by Perkin Elmer Analyst 400 model hollow cathode lamp (HCl) and flame atomic absorption spectrometer with deuterium lamp. Data were expressed as mean ±SE.

Reagents

Distilled-deionized water were used for all analytical applications. Polyethylene tubes and glassware were leached with 4% HCl, and washed two-three times with deionized water. A standard solution of Cd, Cu, Hg, and Zn'nin was also prepared with 1% HNO₃. Dilutions of 1000 ppm stock solution stored in polyethylene tubes were analyzed.

Elementer analysis

Soil samples were oven-dried (105°C) to determine Pb, Al, Ni, Co,

Fe, Hg, Cr, Zn and Cu concentrations in soil at four different distances, disaggregated in a porcelain mortar with a pestle, and sieved through a 2 mm nylon mesh sieve. For elemental analysis the <2 mm fraction was ground. To measure Pb, Al, Ni, Co, Cr and Fe concentrations absorbed in four *A. cepa* samples, leaves were dried, and placed on stamps separately. Soil samples of each polluted areas were also placed on stamps. These samples were coated with gold at Poleron 500 sputter coater and analysed by a scanning electron microscope (Jeol 5600).

Multiple linear correlations between metal bioaccumulation in leaves tissues and length of the leaves were performed by Pearson test ($\alpha = 0.5$, 2-tailed).

RESULTS

In the Pearson correlation analysis, for the four sampling points in the various distances from iron-steel factory and for the dry weight of the onions leaves taken from the four sampling points, a negative correlation was found. This value indicated inversely proportional relationship between dry weight of the samples with the sampling point distances. Cu, Cd, Hg, Zn concentrations in tissues of onion by Perkin Elmer Analyst 400 flame atomic absorption spectrometer with deuterium lamp, Cr, Co, Ni, Pb, Al, Fe concentrations by the elemental analysis were measured. The statistical analysis was performed by one-way ANOVA analysis, taking $P \leq 0.05$ as significant.

Heavy metal concentrations in onion tissues at different distances from Eregli Iron-Steel factory were measured by a flame atomic absorption spectrometer Perkin Elmer Analyst 400 model equipped with back-ground correction deuterium lamp and elementary analysis. A significant differences among heavy metal concentrations in four different samples were found (Table 1).

Fe, Co and Cr concentrations decreased with increasing distance values (Figure 1). It was found that the concentrations of heavy metals such as Hg and Pb did not decrease with the distance increasing from the factory. Motor vehicles, soil, coal consumption in the four sampling points affected high levels of Pb and Hg. Correlation coefficient of lead in soil and in leaves of onion is very high correlation. In the case of cadmium, correlations were calculated for the soil and leaves. Cadmium accumulates at an average 0.077 µg/g concentration in the leaves. In the first sampling point, the highest was measured in 0.095 µg/g concentration, the lowest in 0.065 µg/g. Correlation coefficient of lead in soil and leaves of onion concent points to very high correlation. (Table 2).

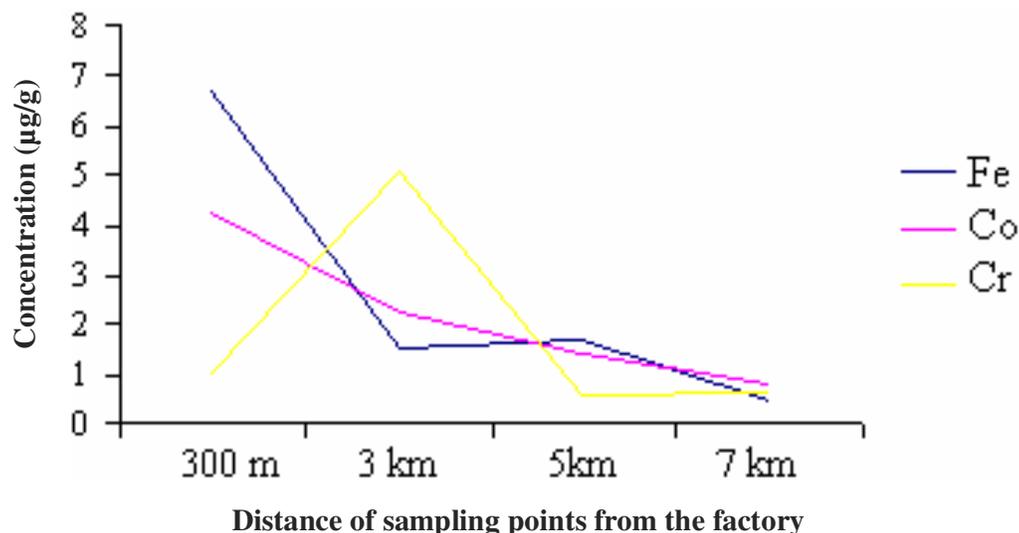
It was determined to accumulate from 5.140 to 9.774 µg/g concentrations in onion tissue and from 69.54 to 171.00 µg/g concentrations in soil. Iron was found from 0.047 to 0.670 µg/g concentrations in the onion samples (Figure 2a, b, c, and d) and from 5.71 to 10.72 µg/g concentrations in soil (Figure 3a, b, c, and d). Concentration raising to 0.670 µg/g in the first sampling points was the highest mean value.

The zinc concentration accumulated in onion from of four sampling points in various distances to Iron-steel

Table 1. The concentration of heavy metals accumulated in *Allium cepa* from the sampling points at different distances from iron-steel factory.

Heavy metals concentrations analysed by Perkin Elmer Analyst 400 model hollow cathode lamp (HCl) and flame atomic absorption spectrometer with deuterium lamp				
Sampling points	Cd ($\mu\text{g/g}$)	Hg ($\mu\text{g/g}$)	Zn ($\mu\text{g/g}$)	Cu ($\mu\text{g/g}$)
300 m distance	0.073 \pm 0.10	6.235 \pm 7.25	0.929 \pm 1.43	0.332 \pm 1.75
3 km distance	0.065 \pm 0.08	7.141 \pm 9.26	0.399 \pm 1.56	0.354 \pm 1.53
5 km distance	0.077 \pm 0.13	5.140 \pm 6.28	0.381 \pm 1.55	0.262 \pm 0.41
7 km distance	0.095 \pm 0.23	9.774 \pm 10.36	0.670 \pm 1.87	0.414 \pm 0.64

Heavy metals concentrations measured by elementary analysis						
Sampling points	Cr ($\mu\text{g/g}$)	Co ($\mu\text{g/g}$)	Ni ($\mu\text{g/g}$)	Pb ($\mu\text{g/g}$)	Fe ($\mu\text{g/g}$)	Al ($\mu\text{g/g}$)
300 m	0.105 \pm 0.09	0.428 \pm 0.11	0.179 \pm 0.24	0.581 \pm 0.67	0.670 \pm 0.71	0.100 \pm 0.24
3 km	0.511 \pm 0.23	0.225 \pm 0.15	0.199 \pm 0.28	0.188 \pm 0.21	0.149 \pm 0.32	0.06 \pm 0.12
5 km	0.058 \pm 0.14	0.140 \pm 0.08	0.074 \pm 0.83	0.129 \pm 0.24	0.170 \pm 0.22	0.002 \pm 0.05
7 km	0.064 \pm 0.88	0.081 \pm 0.18	0.226 \pm 0.35	0.282 \pm 0.38	0.047 \pm 0.15	0.103 \pm 0.20

**Figure 1.** Fe, Co and Cr concentrations ($\mu\text{g/g}$) in sampling points.

factories varies as depending on distance. Zinc concentrations were measured at among 0.381 and 0.929 $\mu\text{g/g}$ values (Table 1) and at among 49.44 - 4.31 $\mu\text{g/g}$ values in soil (Figure 3a, b, c, and d).

Wester region of Ereğli is the most polluted area, with air pollution from iron-steel factory. This region is characterised with high concentrations of metals, including copper and iron.

The average concentration of chromium was measured as 0.158 $\mu\text{g/g}$ in leaves and as 0.82 $\mu\text{g/g}$ in soil. The average Al concentration of onion was determined as 0.0507 $\mu\text{g/g}$ (Table 2).

Multiple linear correlations revealed a significant inverse relationship ($P < 0.1$) between Zn, Cr, Co, Pb, Fe and Al concentrations and length of the leaves (Table 3),

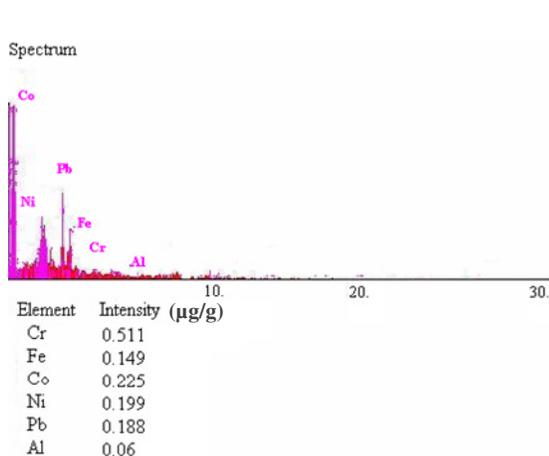
suggesting an effect of these metals in the development of such tissues.

DISCUSSION AND CONCLUSIONS

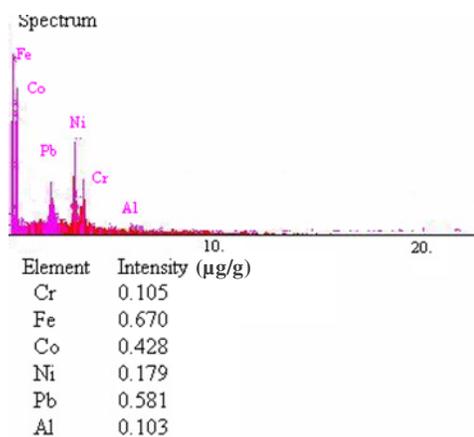
Heavy metals and air pollutants such as nitrogen and sulfur dioxide show directly phytotoxic effect by passing through the surface of plant leaves, and indirectly accumulate in tissues through plant roots by the soil water. Toxic effects such as decrease in the number of chloroplasts in leaf discoloration of the outer epidermal layer, formations of spots on the leaf surface and the destruction of the physiological and biochemical mechanisms create. Geremias et al. (2010) revealed that *A. cepa*

Table 2. Heavy metal concentrations accumulated in *Allium cepa* collected at different distances from iron-steel factory ($P < 0.05$).

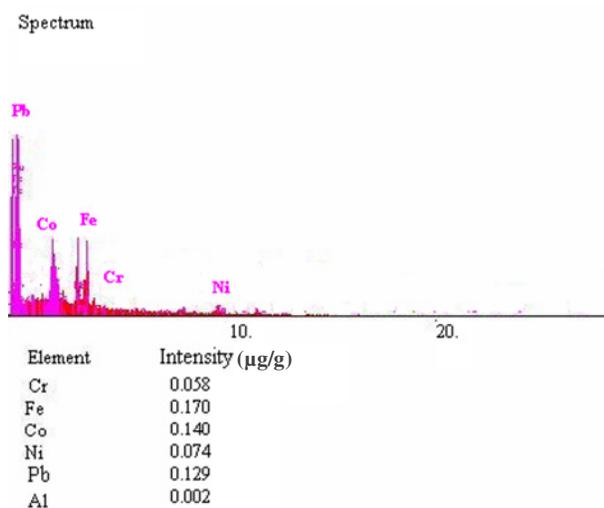
Heavy metals	Mean concentrations ($\mu\text{g/g}$)	The lowest ($\mu\text{g/g}$)	The highest ($\mu\text{g/g}$)
Cd	0.077 ± 0.12	0.065	0.095
Hg	7.072 ± 1.97	5.140	9.774
Cr	0.158 ± 0.23	0.058	0.511
Co	0.219 ± 0.15	0.081	0.428
Ni	0.169 ± 0.06	0.074	0.226
Pb	0.295 ± 0.20	0.129	0.581
Zn	0.594 ± 0.25	0.381	0.929
Cu	0.340 ± 0.62	0.262	0.414
Fe	0.2590 ± 0.279	0.047	0.670
Al	0.0507 ± 0.058	0.0	0.103



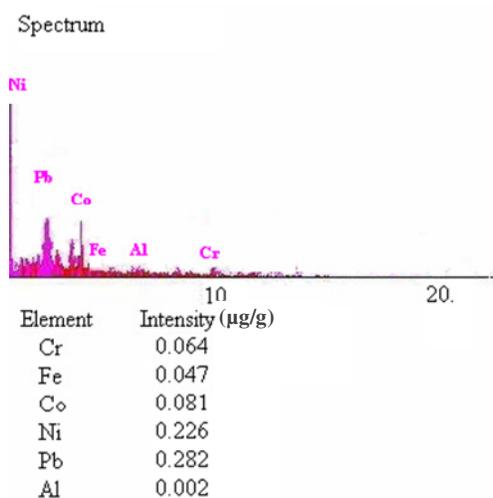
(a)



(b)

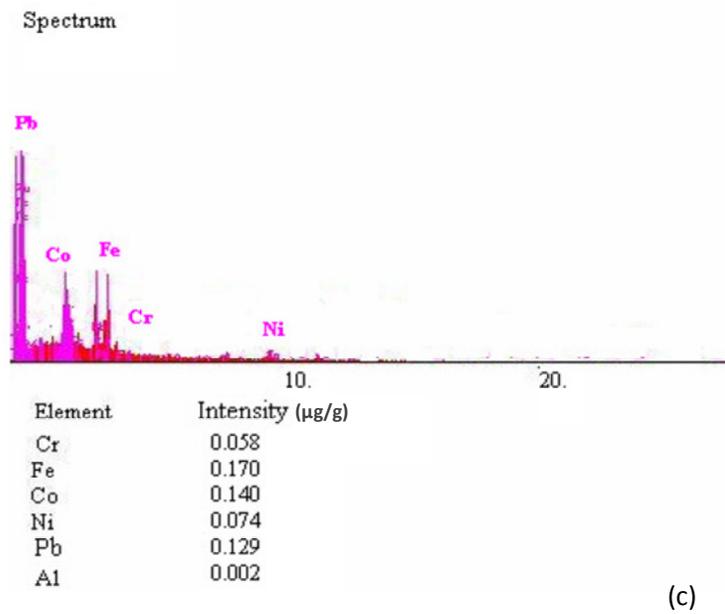
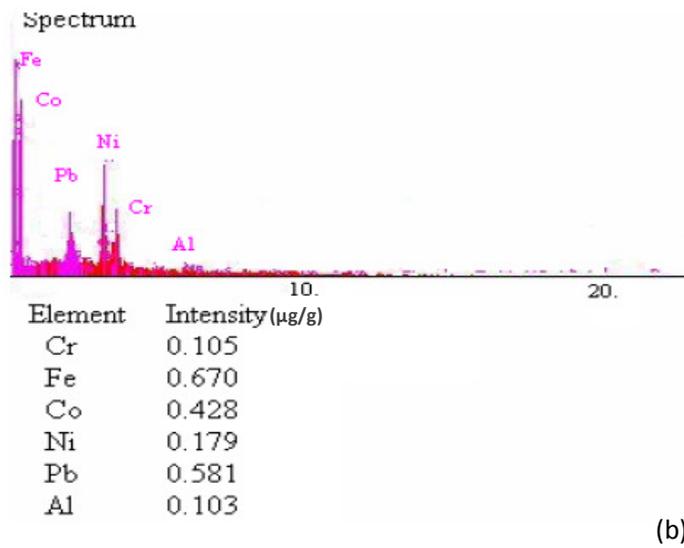
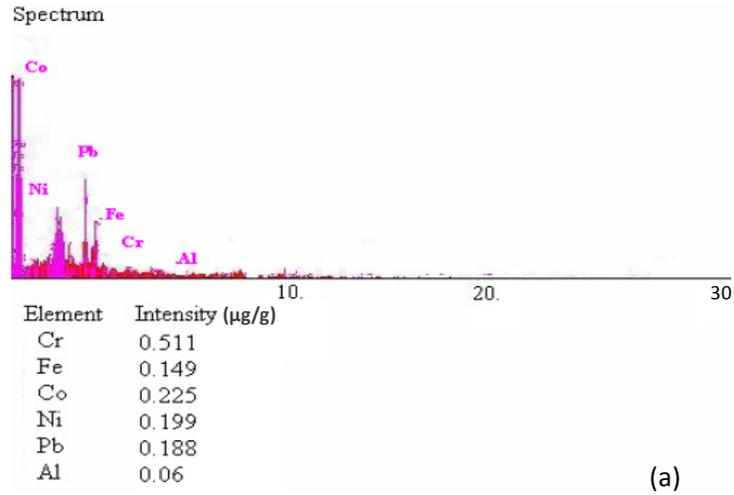


(c)



(d)

Figure 2. Concentrations of some heavy metals determined with elementary analysis; a) Heavy metal concentrations of plant samples at 300 m distance from the factory; b) heavy metal concentrations of plant samples at 3 km distance from the factory; c) heavy metal concentrations of plant samples at 5 km distance from the factory; d) Heavy metal concentrations of plant samples at 7 km distance from the factory.



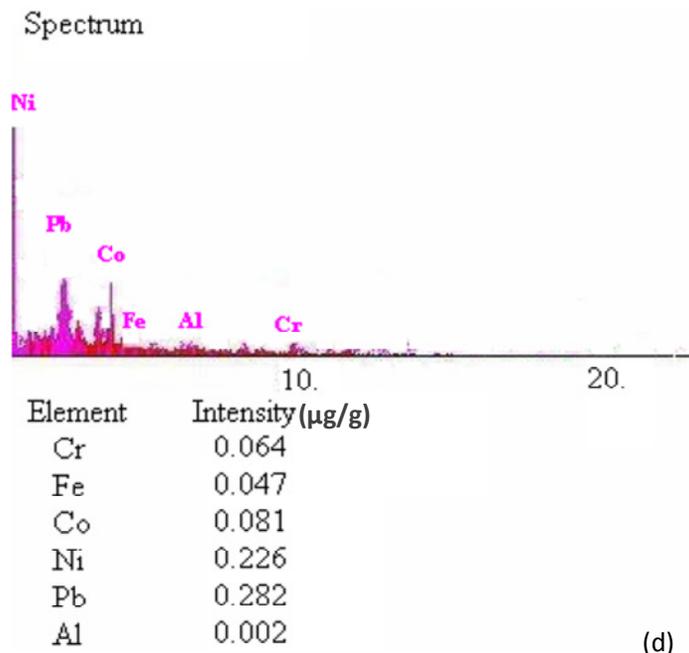


Figure 3. Heavy metal concentrations accumulated in soil were determined with elementary analysis; a) Heavy metal concentrations in soil at 300 m distance from the factory; b) heavy metal concentrations in soil at 3 km distance from the factory; c) heavy metal concentrations in soil at 5 km distance from the factory; d) heavy metal concentrations in soil at 7 km distance from the factory.

Table 3. Multiple linear correlation (Pearson 2-tailed) between heavy metal concentrations and lengths of leaves of *A. cepa*.

Correlations metal concentrations/ tissue growth	Zn	Cr	Co	Pb	Fe	Al
Pearson correlation sig. (2-tailed)	-0.332	-0.352	-0.938	-0.559	-0.832	-0.056

to test toxic effects of trace metals as environmental pollutants sample is a simpler and ecotoxicological sample.

The common onion *A. cepa* is gathered in places polluted with heavy metals from inhabitants for cooking and eating in large quantities. In this study, heavy metals causing air pollution in Ereğli was determined to accumulate easily during onion development. Our results revealed that heavy metal concentrations in this plant consumed as a vegetable in large quantities at Ereğli were very high levels. The two wind directions main at all regions are north-north west north-west north. By spreading to the region with factors such as location of iron-steel factory and wind direction, heavy metals affect their accumulation in tissues, plants and soil. Concentrations of heavy metals accumulated in plant samples at 300 m far from iron-steel factory were observed to be higher than concentrations of heavy metals accumulated in plant samples taken from other

sampling points. Cr stress causes marked reduction in growth and photosynthetic traits (Ali et al., 2011). The Cr concentration at 3 km away from the factory was 0.511 mg /g level while the amount of 5 km away from it decreased to the 0.058 µg/g levels (Table 1). Uyar et al. (2008) Cr levels of *Hypnum cupressiforme* in this area were observed to increase significantly at compared with European values. Combined stress of Cr and Al causes further reduction in growth and photosynthetic parameters When Al is dissolved with effect of rain water, It causes the change of the water quality by mixing with drinking and soil waters. Al absorbed by plant tissue is toxic to the plants and people (Ali et al., 2011).

The accumulation capacity in the tissues indicated that this plant is a good bioindicator in the determination of air pollution. Cd concentration in leafy vegetables are absorbed more easily than other plants. Heavy metals such as Cd and Hg probably by initiating production of active oxygen radicals induce formation of pulmonary

fibrosis and lung cancer (Sekeroglu et al., 2008; Kelleher et al., 2000). In this study, Hg concentrations in four sample points at different distances from the factory commonly are at very high levels (Tables 1 and 2). Atmospheric mercury concentrations are in the value of 0.00001 ppm in the places being not industrial activity. Sardans and Penuelas (2010) obtained results about Hg concentration and in the soils in the moss *H. cupressiforme* and *Quercus ilex* at non-industrial areas. They found that Hg concentration was higher in the tree *Q. ilex* (0.0024 ppm) than *H. cupressiforme* (0.0012 ppm) in non-industrial areas and the soil (0.258 ppm).

In this study, the average concentration of Hg accumulated in onion plants was determined to be 7.072 mg/g levels in leaves and 89.69 µg/g levels in soil. During burning of fossil fuels, the coal or lignite, besides industrial activity in this region, mercury is spread to the atmosphere at significant amounts.

The amount of Fe in plants concerns with releasing into air from iron metal industry. Fe leads to the formation of necrotic lesions and to the formation of an injured plant leaves (Prabu, 2009). Uyar et al. (2008) revealed there was a relationship between the Fe concentrations and the sampling points for *H. cupressiforme*. Fe concentration at distances of 500 to 1000 m was determined to be in very high levels, was the average 3360 (µg/g dry weight) concentration. This value is quite high compared to European values. Acute toxicity levels of Fe are as inducer of liver damage, heart disease, cancer and diabetes (Valko et al., 2005; Dogan et al., 2007).

Heavy metals such as Zn, Ni, Co, Fe, Cr, Cd and Hg resulted from industrial activities cause pulmonary and systemic toxicity, also can change membrane structure and cell division, can inhibit water and nutrient flow in plants (Staykova et al., 2005; Elik, 2002). Cu was at the average 0.3405 µg/g concentrations in the leaves of onion plants and at 4.255 µg/g concentrations in soil. (Table 2). Geremias et al. (2010) were reported 0.1 to 0.10 mg/ml levels of copper reduced the development of the onion leaves. In this study, concentrations of elements of such as copper and nickel due to industrial activity were found to be higher (Table 1). While Zn values in the samples taken from the first sampling point was the highest levels, its values in samples grown on the other sampling points were lower (Figure 2a).

A. cepa shows significant effects even at the lower copper concentrations in terms of growth inhibition. The leaves showed a significant accumulation at the dose of 0.5 µg/ml and a lower growth (Geremias et al., 2010). In this study, Cu accumulation was at the higher doses. Cu concentrations in onions were at 0,340 µg/g levels.

Pb and Ni in roots of *Zea mays* was determined to selectively suppress cell division to induce errors at DNA and RNA synthesis, by binding to DNA and some cell wall proteins (Kozhevnikova et al., 2009). Inhibition of mitotic index and appearance of chromosomal aberrations, micronuclei and binucleate cells were also observed with

other studies. The known products of reactions between DNA, iron and other metals are not yet fully elucidated but include single and double-strand breaks, oxidatively modified based (Valko et al., 2005).

Few studies have been carried out to evaluate the toxic potential of these contaminants to the biota. Practical advantages in the use of this species include the sensibility, reproducibility and rapidity of results as well as the need of small volumes of samples and low cost. Our results on bioaccumulation and toxicity of heavy metals in *A. cepa* represent an important base for future application of such bioassays to evaluate the content of chemicals.

Eregli Iron-steel factory and atmosphere factors in terms of the defined distances as defined differently from each of four sampling points were measured with different concentrations of heavy metals (Li et al., 2007; Gaweda et al., 2009). Therefore, further comprehensive studies and regular surveys are recommended to carefully monitor heavy metal and trace element content to assure quality and safety of human resided in this region. Inhabitants worry about what is going on around them. Public should gather to consume this plant in place only slightly polluted with heavy metals and areas where there is no ecological hazard. This reflects the current situation. Excessive cell death and cell proliferation is closely related to exposure to carcinogenic metals. Heavy metals in humans may induce the formation of apoptosis (Valko et al., 2005; Uyar et al., 2008; Staykova et al., 2005; Wallenborn et al., 2009). Future research needs include development of biologic markers of metal-induced immunologic disease, detailed characterization of human exposure, examination of gene alleles that might confer risk, and association of exposure data with that of genetic susceptibility. The heavy metals offer a unique opportunity to examine the relationship between environmental exposure/dose and human genetic susceptibility.

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