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Heavy Metal Effects from Al-Dura Power Station on Plant *Citrus Aurantium* L.

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HEAVYMETALEFFECTSFROMALDURAPOWERSTATIONONPLANTCITRUSAURANTIUML

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I. Introduction

Heavy metals constitute an ill-defined group of inorganic chemical hazards, and those are most commonly found at contaminated sites such as chromium (Cr), lead (P b), zinc (Zn), arsenic (As), cadmium (Cd), copper (Cu) and nickel (Ni). Soils are the major sink for heavy metals released into the environment by many anthropogenic activities and unlike organic contaminants which are oxidized to carbon by microbial action. Most metals do not undergo microbial or chemical degradation, and their total concentration in soils persists for a long time after their introduction. Heavy metal contamination may pose hazards to the ecosystem through direct ingestion or contact with contaminated soil, the food chain, irrigation from contaminated ground water (Ling, *et al.* 2007).

Heavy metals such as copper and zinc are essential to maintain the metabolism of the plant and animals. However, others, such as Cd, Ni and Pb are all toxic elements. At higher concentrations, both groups of heavy metals can lead to poisoning. They accumulate rapidly and deplete slowly (Wuana. *et al.*, 2010).

An assessment of toxic concentrations and effects of heavy metals on plants is very complex because it depends on so many factors. Some of the most important factors are the proportions of related ions that are present in solution and their compounds. Although plants adapt easily to chemical stress, they may also be very sensitive to an excess of a particular heavy metal. Toxic concentrations of these heavy metals in plant tissues are very difficult to establish (Kabata-Pendias, 2011; Soydam *et al.,* 2011).

Citrus aurantium L.is sensitive to high concentrations of metals, toxicity causes retarded growth, leaf burn and yellowing starting near the tip, the yellowing is very bright, scattered over the leaves. In severe cases, gum spots appear on the lower leaf surfaces with leaf drop occurring prematurely. Leaf drop is heavy and dieback follows. Other symptoms can include twig dieback, feeder roots may also become darkened and exhibit restricted growth. Changes in chlorophyll content, lipid content and losses of polypeptides involved in photochemical activities and damage to DNA molecules (Futch and Tucker, 2000; Kabata_Pendias, 2011).

The aim of the present study is to determine the concentrations of some heavy metals in the areas near to Al-Daura thermal power station like(Nickel (Ni), Cadmium (Cd), Lead (Pb) and Copper (Cu)) their effects on the *Citrus aurantium* L. plant.

II. Materials and Methods

a) Sampling sites

Three sites with gradual distances from Al-Daura thermal power station (0.5, 1 and 2 Km, respectively) were chosen for sampling. In addition, one of unpolluted site was chosen for comparison as a control around 4 Km upwind from Al-Daura thermal power station. All samples were collected for two seasons; the first season was in December 2014 and the second season was in March 2015 (fig. 1).

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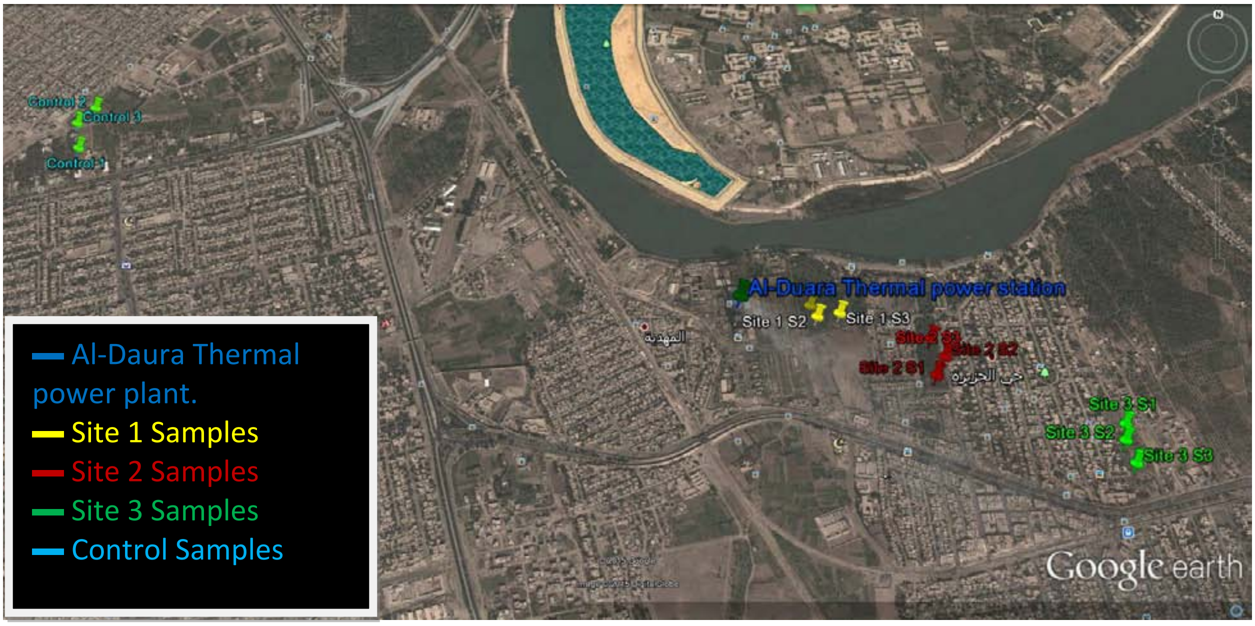

Figure 1 : The study area of Al-Daura thermal power station with 4 sampling stations

b) *Plant sampling*

Citrus aurantium L trees were selected in all three suspected polluted sites and for control site with large number and can be easily specified. Roots and leaves were used to measure the concentration of heavy metals in plants. For chemical analysis, three replicates of plant of leaves and roots were taken from each site in each season.

The chemical analysis of sour orange (*Citrusaurantium* L.) plants include measuring the concentration of heavy metals (Ni, Pb, Cd and Cu) using AAS device. The plant samples were prepared for measuring according to (APHA, 1998).

c) *Chlorophyll Content Analysis*

Chlorophyll pigment contents were determined according to (Goodwin, 1976):

d) *Protein Content Analysis*

Protein content was measured according to Biuret method and preparation of reagents was done according to Layne (1957).

III. RESULTS AND DISCUSSION

a) *Ni Concentration in Citrus aurantium L. (Leaves and Roots)*

The results in table 1 shows a significant increase in the concentrations of Ni in the leaves of *Citrus aurantium* L. in season 2 than season 1. While, no significant difference appeared in the concentrations of Ni in the root of *Citrus aurantium* L. (Table 2). At the same time, there were significant differences in Ni concentrations in *Citrus aurantium* L. among all sites. The concentration of Ni in leaves and roots in site 2 was higher than other sites.

The interaction (Seasons × sites) for the leaves and roots were significant, the higher values were in season 2 at site 2, while the lowest interaction values were in season 1 at the control sites.

Table 1 : Concentrations of Ni (µg/g) in the leaves of *Citrus aurantium* L. for the two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	5.74 ± 0.49	5.92 ± 0.84	5.83 ± 0.44 D
1 (0.5 Km distance)	28.19 ± 2.10	32.90 ± 1.15	30.545 ± 1.50 B
2 (1 Km distance)	34.88 ± 1.20	37.73 ± 1.90	36.305 ± 1.19 A
3 (2 Km distance)	12.55 ± 1.21	13.80 ± 1.09	13.175 ± 0.78 C
Mean ± SE	20.34 ± 3.57 B	22.587 ± 4.00 A	---
*LSD value: Site: 2.849 *, Season: 2.014 *, Interaction of Site × Season: 4.247 **			
(P>0.05) N.S: Not significant			

Table 2 : Concentrations of Ni (µg/g) in the root of *Citrus aurantium* L. for the two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	<u>3.84</u> ± 0.44	4.80 ± 0.36	4.32 ± 0.33 D
1 (0.5 Km distance)	19.60 ± 1.18	24.28 ± 1.79	21.94 ± 1.42 B
2 (1 Km distance)	25.06 ± 2.43	<u>27.43</u> ± 1.79	26.245 ± 1.45 A
3 (2 Km distance)	8.97 ± 0.82	9.12 ± 0.72	9.045 ± 0.49 C
Mean ± SE	14.37 ± 2.60 A	16.407 ± 2.95 A	---
*LSD value: Site: 2.931 *, Season: N.S , Interaction of Site × Season: 4.464 * (P>0.05) N.S: Not significant*			

b) Cd concentration

The results in tables 3 and 4 refer that there were no significant differences between two seasons in the concentrations of Cd in leaves and roots of *Citrus aurantium* L., whereas, there were significant differences among all sites, the highest concentrations were in site 2 (0.79 ± 0.04 for leaves and 1.065 ± 0.06 for roots) and the lowest concentrations were in the control site.

There were significant interactions between seasons and sites; higher concentration was in the site 2 of season 2, whereas and the lowest was in the control site in both seasons for leaves and roots.

Table 3 : Concentrations of Cd (µg/g) in the leaves of *Citrus aurantium* L. for the two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	<u>0.000</u> ± 0.00	<u>0.00</u> ± 0.00	0.00 ± 0.00 D
1 (0.5 Km distance)	0.620 ± 0.06	0.710 ± 0.06	0.665 ± 0.04 B
2 (1 Km distance)	0.730 ± 0.06	<u>0.850</u> ± 0.04	0.79 ± 0.04 A
3 (2 Km distance)	0.210 ± 0.04	0.270 ± 0.04	0.24 ± 0.04 C
Mean ± SE	0.39 ± 0.09 A	0.457 ± 0.10 A	---
*LSD value: Site: 0.093 *, Season: 0.065 * , Interaction of Site × Season:0.142 * (P>0.05) N.S: Not significant*			

Table 4 : Concentrations of Cd (µg/g) in the roots of *Citrus aurantium* L. for the two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	<u>0.066</u> ± 0.03	0.07 ± 0.01	0.068 ± 0.03 D
1 (0.5 Km distance)	0.780 ± 0.10	0.820 ± 0.03	0.80 ± 0.05 B
2 (1 Km distance)	0.960 ± 0.04	<u>1.170</u> ± 0.07	1.065 ± 0.06 A
3 (2 Km distance)	0.280 ± 0.03	0.322 ± 0.02	0.301 ± 0.02 C
Mean ± SE	0.67 ± 0.11 A	0.595 ± 0.13 A	----
*LSD value: Site: 0.120 *, Season: N.S, Interaction of Site × Season: 0.179 * (P>0.05), N.S: Not significant*			

c) Cu concentration

The results in table 5 showed no significant difference in the concentrations of Cu in the leaves between the two seasons, while a significant difference between seasons in roots were shown (Table 6). Also, a significant difference in the concentrations of Cu in leaves and roots for all three sites appeared when compared with the control. The highest concentrations of Cu in plants were in site 2 (50.015 ± 1.50 and 46.135± 2.15 µg/g for leaves and roots, respectively), in the same time there was no significant difference between site 1 and 2. The higher significant interaction for the leaves and roots was recorded during season 2 at site 2, while the lowest value was in season 1 at the control site.

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Table 5 : Concentrations of Cu (μg/g) in the leaves of *Citrus aurantium* L. for the two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	17.80 ± 0.71	18.46 ± 1.44	18.13 ± 0.73 C
1 (0.5 Km distance)	43.75 ± 2.99	48.46 ± 1.83	46.105 ± 1.89 A
2 (1 Km distance)	48.70 ± 2.41	51.33 ± 1.90	50.015 ± 1.50 A
3 (2 Km distance)	25.92 ± 2.95	28.31 ± 0.93	27.115 ± 1.48 B
Mean ± SE	34.042 ± 3.95 A	36.64 ± 4.19 A	---
*LSD value: Site: 4.37 *, Season: N.S, Interaction of Site × Season: 6.47 ** *(P<0.05) N.S: Notsignificant*			

Table 6 : Concentrations of Cu (μg/g) in the roots of *Citrus aurantium* L. for the two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	13.48 ± 0.82	14.80 ± 0.88	14.14 ± 0.61 C
1 (0.5 Km distance)	41.70 ± 1.62	44.62 ± 1.32	43.16 ± 1.14 A
2 (1 Km distance)	42.40 ± 2.74	49.87 ± 1.28	46.135 ± 2.15 A
3 (2 Km distance)	21.80 ± 1.128	22.10 ± 1.32	21.95 ± 0.82 B
Mean ± SE	29.845 ± 3.86 B	32.847 ± 4.47 A	---
*LSD value: Site: 3.21 *, Season: 2.27 *, Interaction of Site × Season: 4.81 ** *(P>0.05) N.S: Not significant*			

The results in tables 1,2,3,4,5 and 6 shows a significant difference in the concentrations of heavy metals (Ni, Cd and Cu) in leaves and roots of *Citrus aurantium* L. at all three sites when compared with the control and they were higher than the permissible values that were estimated 0.1–5 ; 0.05-0.2 and 5-30 μg/g for of Ni, Cd and Cu respectively (Kabata-Pendias, 2011).

The increases in the concentrations of heavy metal in the studied plant resulted from its growth in the polluted soil with heavy metals. Site 2 showed high concentrations of Ni, Cd and Cu in plant tissues. This result meets with the results of Doganlar and Atmaca (2011) who showed a high ability of Citrus plant to absorb the heavy metals from polluted soil, which may cause genotoxicity in plant and leads to a human health hazard.

d) Pb concentration

There was no significant difference between season 1 and 2 in the concentrations of Pb in the leaves of *Citrus aurantium* L. (Table 7). While there was an increase with a significant difference in the concentrations of Pb in the root of *Citrus aurantium* L. in season 2 when compared to season 1 (Table 8). Results indicated a significant difference in the concentrations of Pb in the leaves and roots of *Citrus aurantium* L. among sites; the highest concentration was in site 3. Also, the higher interaction value of Pb with a significant difference was in site 3 at season 2, while the lowest interaction value was in the control site at season 1. The plants in all sites for two seasons were within a permissible value, which was estimated at 5-10 μg/g for Pb in the plant, except the plant on site 3 that was higher from the permissible level (Kabata-Pendias, 2011).

Table 7 : Concentrations of Pb (μg/g) in the leaves of *Citrus aurantium* L. for the two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	6.60 ± 0.51	6.91 ± 0.21	6.755 ± 0.26 D
1 (0.5 Km distance)	9.78 ± 0.27	10.39 ± 1.04	10.085 ± 0.50 C
2 (1 Km distance)	11.20 ± 0.81	12.30 ± 0.79	11.75 ± 0.56 B
3 (2 Km distance)	14.21 ± 0.81	15.60 ± 0.65	14.905 ± 0.56 A
Mean ± SE	10.447 ± 0.86 A	11.3 ± 0.99 A	---
*LSD value: Site: 1.469 *, Season: N.S, Interaction of Site × Season: 2.23 ** *(P>0.05) N.S: Not significant*			

Table 8 : Concentrations of Pb (µg/g) in the roots of *Citrus aurantium* L. for two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	5.21 ± 0.56	5.34 ± 0.71	5.275 ± 0.41 C
1 (0.5 Km distance)	8.03 ± 0.45	8.22 ± 0.44	8.125 ± 0.28 B
2 (1 Km distance)	8.15 ± 0.32	9.94 ± 0.59	9.045 ± 0.50 AB
3 (2 Km distance)	9.28 ± 0.23	10.85 ± 0.83	10.065 ± 0.52 A
Mean ± SE	7.667 ± 0.48 B	8.587 ± 0.69 A	---
*LSD value: Site: 1.167 *, Season: 0.825 *, Interaction of Site × Season: 1.589 * (P>0.05), N.S: Not significant*			

The distributions of heavy metals concentrations in *Citrus aurantium* L. leaves and roots for all three sites and control site were shown in figures 2 and 3.

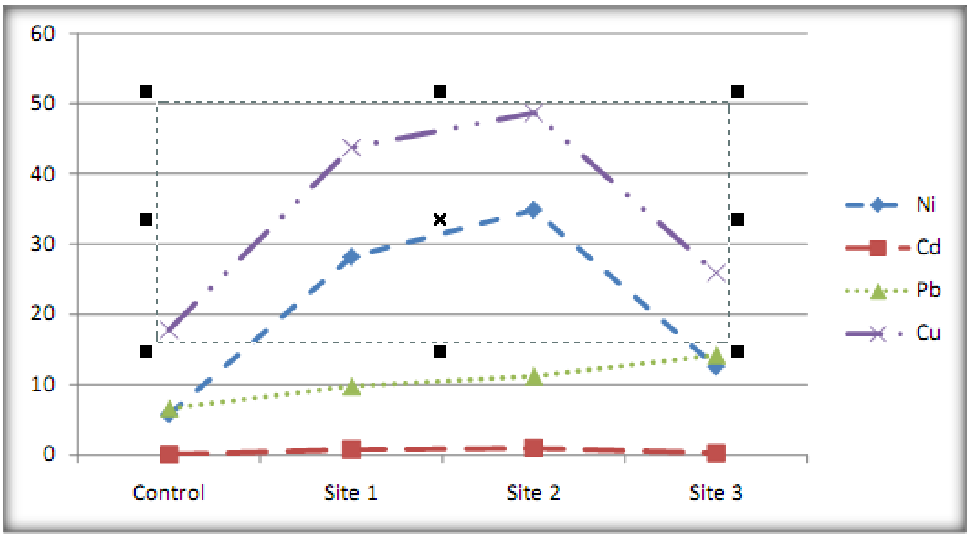

Figure 2 : Distributions of heavy metals concentrations in the leaves of *Citrus aurantium* L. in the three sites and control site

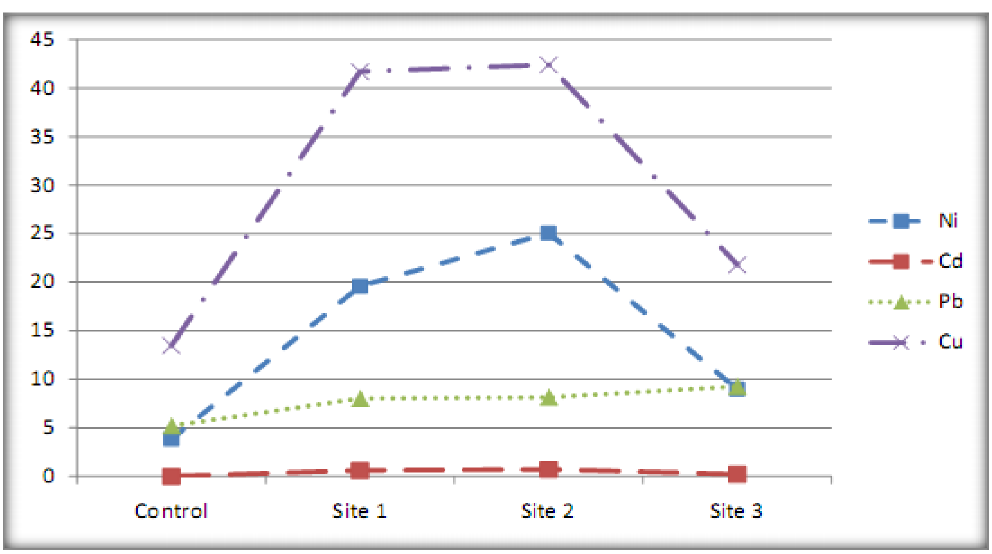

Figure 3 : Distributions of heavy metals concentration in the roots of *Citrus aurantium* L. in the three sites and control site

In this study, it has been found that there was a slight significant increase in most of the heavy metals in plant tissues in season 2 more than that in season 1. The amounts of rainfall throughout the seasons and increase the influence of ambient temperature have important effects on enhancing the response of Citrus trees to the uptake of metals(Table 9).

Table 9 : Weather of study area for two seasons

Season	Mean of Air Temperature (ºC)	Mean of Air Maximum Temperature (ºC)	Mean of Air Minimum Temperature (ºC)	Total Monthly Rainfall (mm)	Relative Humidity (%)	Mean of Wind Speed (m/s)
December (2014)	13.9	19.6	8.1	3.9	65	2.4
March (2015)	17.9	25.4	9.7	26.1	43	3

Iraqi Meteorological Organization, (2015).

In subtropical climates, Citrus plants show higher stem water potential when it is a warmer weather with more rain. In contrast, a reduction in stem water potential is found when it is a colder weather with less rain, in which plants may get fewer levels of some trace elements from the soil solution (Ribeiro and Eduardo, 2007).

Al-Safawe *et al.* (2014) found that the Citrus trees grow in the industrial areas and main streets were containing high concentrations of heavy metal in their tissues. While, Kabata-Pendias (2011) reported that in general, plants readily take up the heavy metals dissolved in soil solutions, and it also may absorb heavy metals by aboveground parts from aerial deposition.

e) Chlorophyll Content in the leaves of Citrus aurantium L.

The results in table 10 show that the chlorophyll content of *Citrus aurantium* L. leaves in season 2 were higher than season 1 with a significant difference. Ribeiro and Edurdo, (2007) stated that the photosynthetic activity of citrus trees was affected during the winter season when growing in a subtropical climate, under both low temperatures and drought.

A significant difference in interaction value of chlorophyll was recorded during season 2 at the control site that was 2631 ± 40.07 µg/g, while the lowest interaction value was in season 1 at site 2 that was 1986 ± 23.79 µg/g.

Table 10 : Chlorophyll Content (µg/g)in the leaves of Citrus aurantium L. for two seasons

Site	Season		Mean ± SE
	1 December 2014	2 March 2015	
Control	2504 ± 4.93	2631 ± 40.07	2567.5 ± 29.97 A
1 (0.5 Km distance)	2161 ± 22.64	2192 ± 19.05	2176.5 ± 14.94 C
2 (1 Km distance)	1986 ± 23.79	1993 ± 14.04	1989.5 ± 12.45 D
3 (2 Km distance)	2385 ± 21.96	2391 ± 10.50	2388 ± 10.96 B
Mean ± SE	2259 ± 60.90 B	2301 ± 69.9 A	---
*LSD value: Site: 46.58 *, Season: 32.94 *, Interaction of Site × Season: 54.46 ** *(P>0.05), N.S: Not significant*			

The chlorophyll content in the leaves of *Citrus aurantium* L. in the control sites was higher than the three sites with a significant difference, while the lower value was in site 2. This might be due to that chlorophyll synthesis is a breakdown in plants as an effect of an elevation of heavy metals in this site. Tables 1-8, which reported the increasing of the concentrations of heavy metals in site 2 compared with other sites; *i.e.* a decrease in the chlorophyll content was detected in parallel with an increasing in the pollution level. The results of this study agree with the results of Yurekli and Porgali (2006); Bhardwaj *et al.* (2009) and Doganlar *et al.* (2012) who found that the heavy metals cause a decrease in the content of photosynthetic pigment in many plant species including *Citrus aurantium* L. due to the peroxidation of the photosynthetic membrane and inhibition of chlorophyll biosynthesis enzymes.

Doganlar and Atimaca (2011) reported that the chlorophyll content was decreased 15 -66 % in industrial and urban street plants as a result of air pollutants and heavy metals such as Ni, Pb and Zn. Also, Peralta-Videa *et al.* (2004) and Kiran and Sahin (2005) stated that in addition to the effects of heavy metals on physiological and morphological characteristics of plants like inhibition of seed germination and root-shoot development; these elements caused a major biochemical changes such as the formation of reactive oxygen species (ROS) that resulted in altered levels of enzymatic and non-enzymatic antioxidants, and depression of plant proteins and pigment content.

f) Protein Content in the leaves and roots of Citrus aurantium L.

The results presented in tables 11 and 12 show that the protein content in the leaves and roots of *Citrus aurantium* L. was higher in season 2 than season 1 with a significant difference for. Touchette and Burkholder (2002) found that proteins in plants increased with the progression growing season. The higher interaction value with significant difference was found in season 2 at the control site, whereas the lowest value was found in season 1 at site 2.

In this study, the protein content of *Citrus aurantium* L. leaves and roots in all three sites were decreased compared with that in the control site. The higher decrease with a significant difference in protein content was in sites 1 and 2 compared to site 3 and the control site because of the increasing of pollutants from Al-Daura power station in these sites.

Table 11 : Protein content (μg/g) of the leaves of *Citrus aurantium* L. for the two seasons

Site	Season		Mean
	1 December 2014	2 March 2015	
Control	362.13 ± 3.22	379.29 ± 3.97	370.71 ± 4.35 A
1 (0.5 Km distance)	355.40 ± 4.21	364.90 ± 4.61	306.15 ± 2.91 C
2 (1 Km distance)	298.19 ± 2.72	309.13 ± 4.25	303.66 ± 5.67 C
3 (2 Km distance)	312.22 ± 3.23	323.80 ± 5.22	318.01 ± 3.77 B
Mean ± SE	331.98 ± 8.36 B	344.28 ± 7.69 A	---
*LSD value: Site: 9.26 *, Season: 6.553* , Interaction of Site × Season: 13.66 ** *(P>0.05) N.S: Not significant*			

Table 12 : Protein Content (μg/g) of the roots of *Citrus aurantium* L. for the two seasons

Site	Season		Mean
	1 December 2014	2 March 2015	
Control	268.58 ± 4.62	291.22 ± 5.81	279.9 ± 3.95 A
1 (0.5 Km distance)	251.50 ± 4.59	257.61 ± 3.67	254.55 ± 2.96 B
2 (1 Km distance)	243.44 ± 7.41	247.39 ± 4.28	245.415± 3.93 B
3 (2 Km distance)	267.21 ± 5.05	271.60 ± 4.52	269.405± 2.99 A
Mean ± SE	257.68± 4.74 B	266.95 ± 2.80 A	---
*LSD value: Site: 10.36 * , Season: 7.32 * , Interaction of Site × Season: 14.78 ** *(P>0.05), N.S: Not significant*			

The presence of high levels of heavy metal ions causes a wide range of cellular responses including changes in genes expression and synthesis of metal-detoxifying peptides (Yousefi, 2009). While, Bhardwaj *et al.* (2009) found that the protein content, total soluble sugar as well as photosynthetic pigments was decreased as concentrations of heavy metals increased in comparison with the control plants.

The use of composted sewage sludge which contains a high metals concentration could result in an increase in the metal accumulation in the plant leaves and roots. Such phenomenon has an effect on the plant development, biomass, protein and chlorophyll content (Kevresan *et al.,* 2001).

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