Journal of Stress Physiology & Biochemistry, Vol. 9 No. 4 2013, pp. 145-162 ISSN 1997-0838

ORIGINAL ARTICLE

Soil Contamination With Heavy Metals and Its Effect on Growth, Yield and Physiological Responses of Vegetable Crop Plants (Turnip and Lettuce)

Raifa Ahmed Hassanein[1], Hanan Ahmed Hashem[1]*,

Mohamed Hashem El-Deep[2], Ahmed Shouman[3]

[1] *Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt.*
[2] *Department of Horticulture, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*
[3] *Department of Basic Science, Institute of Environmental Studies and Research, Cairo, Egypt.*

Tel.: +201141234444
*E-Mail: **Hashem.hanan@gmail.com**

Received July 2, 2013

The present study was conducted to investigate the impact of irrigation with industrial wastewater on soil and plant. For these purpose turnip and lettuce plants were cultivated in soil irrigated with wastewater then heavy metals content of the soil, plant growth, yield and the subsequent changes in biochemical constituents of plant were examined. Irrigation with wastewater was found to load the soil with heavy metals (Pb, Co, Ni and Cd) that were not detected in soil before irrigation. The magnitude of Cd in soils after irrigation with industrial wastewater exceeds the maximum allowable limit (3 mg Kg^{-1}). Both turnip and lettuce exhibited significant decreases in leaf area, fresh weight and dry weight of shoots and roots as well as all the measured yield components in response to wastewater irrigation. The magnitude of decrease was positively correlated with the amounts of heavy metals detected in the soil and the inhibitory effect on turnip was much more pronounced than in lettuce. Furthermore, heavy metals accumulation in soil resulted in an oxidative damage to turnip and lettuce as indicated by the significant increase in lipid peroxidation and H_2O_2 levels in both plants comparing to control values. The significant increases in putrescine in lettuce and turnip shoots and roots and spermidine in lettuce roots as well as total phenolics and flavonoids in plants cultivated in soil enriched with heavy metals are believed to be defense mechanisms in turnip and lettuce plants to counteract the oxidative stress resulted from heavy metals contamination generated from irrigation with wastewater.

Key words: Brassica napus, contamination, flavonoid, Lactuca sativa, polyamines

ORIGINAL ARTICLE

Soil Contamination With Heavy Metals and Its Effect on Growth, Yield and Physiological Responses of Vegetable Crop Plants (Turnip and Lettuce)

Raifa Ahmed Hassanein[1], Hanan Ahmed Hashem[1]*,

Mohamed Hashem El-Deep[2], Ahmed Shouman[3]

[1] *Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt.*
[2] *Department of Horticulture, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*
[3] *Department of Basic Science, Institute of Environmental Studies and Research, Cairo, Egypt.*

Tel.: +201141234444
*E-Mail: ***Hashem.hanan@gmail.com***

Received July 2, 2013

The present study was conducted to investigate the impact of irrigation with industrial wastewater on soil and plant. For these purpose turnip and lettuce plants were cultivated in soil irrigated with wastewater then heavy metals content of the soil, plant growth, yield and the subsequent changes in biochemical constituents of plant were examined. Irrigation with wastewater was found to load the soil with heavy metals (Pb, Co, Ni and Cd) that were not detected in soil before irrigation. The magnitude of Cd in soils after irrigation with industrial wastewater exceeds the maximum allowable limit (3 mg Kg^{-1}). Both turnip and lettuce exhibited significant decreases in leaf area, fresh weight and dry weight of shoots and roots as well as all the measured yield components in response to wastewater irrigation. The magnitude of decrease was positively correlated with the amounts of heavy metals detected in the soil and the inhibitory effect on turnip was much more pronounced than in lettuce. Furthermore, heavy metals accumulation in soil resulted in an oxidative damage to turnip and lettuce as indicated by the significant increase in lipid peroxidation and H_2O_2 levels in both plants comparing to control values. The significant increases in putrescine in lettuce and turnip shoots and roots and spermidine in lettuce roots as well as total phenolics and flavonoids in plants cultivated in soil enriched with heavy metals are believed to be defense mechanisms in turnip and lettuce plants to counteract the oxidative stress resulted from heavy metals contamination generated from irrigation with wastewater.

Key words: Brassica napus, contamination, flavonoid, Lactuca sativa, polyamines

Increasing scarcity of freshwater resources is driving many countries in the arid and semi-arid regions to use marginal quality water for agriculture and related activities. Although the nutrients present in the wastewater is considered as beneficial to agriculture, yet this wastewater may also carry appreciable amounts of trace toxic metals and its long term application on agricultural lands contributes significantly to the buildup of elevated concentration of toxic metals in irrigated soil and plants (Rattan *et al.,* 2005). The contaminants present in the wastewater pose health risks directly to agricultural workers and indirectly to consumers of the wastewater grown product (fodder crops, greens and vegetables), as the long term application of the wastewater may result in the accumulation of toxic compounds such as heavy metals in soil and plants. In this way the heavy metals enter the food chain of animals and human and cause health hazards (Chandran *et al*., 2012). So, it is essential to monitor food quality, given that plant uptake is one of the main pathways through which heavy metals enter the food chain (Antonious and Kochhar, 2009).

Heavy metal accumulation is known to produce significant physiological and biochemical responses in vascular plants (Mangabeira *et al*., 2001). As stated by Preeti and Tripathi (2011), there is a direct relationship between chemical characteristics of soil, heavy metals' concentration and morphological and biochemical responses of plants. The effect of heavy metals toxic influence on plants is large, a strong and fast inhibition of growth processes of the above- and underground parts, as well as the decreased activity of the photosynthetic apparatus are reported (Lin *et al*., 2005). The accumulation of heavy metals in plant tissues might cause reduction in physiological and biochemical activities of plants resulting in lower biomass and yield (Scoccianti *et al.,* 2006). Yield had also significant and negative relationship with the concentrations of Cd, Cu, Pb, Zn and Cr in root and shoot (Sutapa and Bhattacharyya, 2008).

One of the major consequences of heavy metal action is enhanced production of reactive oxygen species (ROS) which usually damages the cellular components such as membranes and nucleic acids (Drążkiewicz *et al*., 2004). Formation of lipid peroxides may be a prolonged consequence of heavy metal-induced oxidative stress and may act as an activation signal for plant defense genes through increase of the octadecanoid pathways (Maksymiec, 2007). It has been known that ROS-induced lipid peroxidation of membranes is the reflection of stress-induced damage at the cellular level (Karenlampi *et al*., 2000). Therefore the level of malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids produced during peroxidation of membranes lipid is used as an indicator of oxidative damage (Metwally *et al*., 2003).

Hydrogen peroxide (H_2O_2), a kind of natural reactive oxygen species, is generated with various environmental and developmental stimuli. It has been approved to act as a new signal molecule and played important roles in many physiological processes such as cell expansion, development, stomatal closure and programmed cell death (Chao *et al*., 2008). Increased level of H_2O_2 occurred after Cu, Cd (Drążkiewicz *et al*., 2004) and Hg (Cho and Park, 2000) treatment. Increased accumulation of H_2O_2, usually connected with changes in the cellular redox status, alerts the plant cell against environmental stresses (Foyer and Noctor, 2003), and may enhance the plant's antioxidant response through calcium signaling in the expression of glutathione transferase gene (Rentel and Knight, 2004).

Polyphenols are involved in the defense mechanism of plants and their levels are enhanced as a response to biotic and abiotic stresses (Dudjak *et al*. 2004). Many authors provide evidence of induction of phenolic metabolism as a response to metal stress (Michalak, 2006; Singh and Malik, 2011). Phenolic compounds are shown to have strong antioxidant activity in plants growing under heavy metal stress. It has been suggested that their antioxidant act resides chiefly in their chemical structure. Phenols are oxidized by peroxidase and contribute in scavenging H_2O_2 (Singh and Malik, 2011). In addition, it has been reported that the antioxidant mechanism of flavonoids, polyphenolic compounds that are ubiquitous in nature, may come from the interaction between transition-metal ions and flavonoids to produce complexes that keep the metal ions from their participation in free-radical generation (Miller *et al*. 1996). At the same time, as natural metal chelators, flavonoids show a significant function on the bio-utilization of metal and anti metal-toxicosis (Chen *et al*. 1990). They form complexes with metals (Viswanathan *et al*. 2000), as was shown for rutin and transitions metals complexes (Bai *et al*. 2004).

Polyamines (PAs) (putrescine, spermidine and spermine) are group of phytohormone-like aliphatic amine natural compounds with aliphatic nitrogen structure and present in almost all living organisms including plants. Polyamines are considered as one of the reserves of carbon and nitrogen in plant tissues (Lefevre *et al.,* 2001). Evidences showed that polyamines are involved in many physiological processes, such as cell growth and development and respond to stress tolerance to various environmental factors. In many cases the relationship of plant stress tolerance was noted with the production of conjugated and bound polyamines as well as stimulation of polyamine oxidation (Gill and Tuteja, 2010).

The disposal of industrial effluent into water is a major problem that affects soil and plants. The objective of the present study was to highlight the harmful / deleterious effects of industrial wastewater on soil characteristics, plant growth and productivity. In addition, the changes in some stress related compounds such as phenolic compounds and polyamines were also studied.

MATERIALS AND METHODS

Plant materials

Seeds of lettuce *(Lactuca sativa* L.*)* and turnip (*Brassica napus* L.), were obtained from the Horticulture Institute Research Center, Giza, Egypt. These vegetables were selected because they differ in edible parts, which are the leaves in lettuce plant, and the tuberous roots in turnip plant.

Soil

Virgin loam soil taken from El Nobaria region was used. Soil was collected at a depth of 0–30 cm.

Irrigation water

Different samples of industrial wastewater were collected from the El-Amia drain in Egypt. The study area extends between the Behera Governorate in south and Alexandria Governorate in north, exactly between Kafr El-Dawar south and Abu Qir north, through which El-Amia drain has been passed. The drain contains waste from the industrial factories in Kafr El- Dawar area and Abu Qir area, which include companies involved in spinning and weaving, artificial silk making, and in production of pigments, fertilizers, paper, pesticides, plastics and petroleum. The first sample was collected 1 km after the beginning of the drain (T_1), the second sample was collected 10 km after the beginning of the drain (T_2) and the third sample was collected 19 km after the beginning of the drain (T_3); the total length of the drain is about 20 km.

Growth conditions

Seeds of lettuce and turnip were surfacesterilized with 0.001 M $HgCl_2$ solution for 3 min and washed thoroughly with several changes of sterile distilled water. Ten seeds were sown in each pot containing 20 kg of loam soil (40 cm in diameter, 25 cm deep) at a 3 cm depth. Pots were irrigated with fresh water in the first week. After that, pots of the two crops under investigation (lettuce and turnip) were divided into four groups of 10 pots each. The first group continued to be irrigated by fresh water (control); the second, third and fourth groups were irrigated by wastewater collected from the sampling sites 1, 10, and 19 km into the drain, and are referred to as T_1, T_2 and T_3, respectively. Pots were maintained in a greenhouse under natural lighting with an 8 h photoperiod and average 25°C/10°C ± 3°C day/night temperatures. Samples were taken before the flowering stage (70 days after sowing) to measure growth criteria (area of leaves per plant, fresh and dry weights of shoot and roots) and to determine the changes in polyamines (putrescine, spermine and spermidine) and certain secondary metabolites with antioxidant activity (total phenols and flavonoids) in response to irrigation with industrial wastewater. The area of leaves per plant (cm^2) were determined by multiplying length x width x 0.75 (Quarrie and Jones, 1979). In addition, 100 days old plants were harvested to measure yield components in both turnip and lettuce. Finally, heavy metal accumulation in the soil was investigated at the end of the experimental period.

Soil analysis

The soils were evaluated for texture, pH, EC (electrical conductivity), SP (saturation percentage), soluble ions and heavy metals content (Fe, Mn, Zn, Cu, Pb, Cd and Ni). Soil texture was determined using the texture triangle diagram, which described by Soil Survey Staff (2004). The soil pH and electrical conductivity was measured in a 1:2.5 soil water suspension using a glass electrode (Bochman bench type pH –meter) and electrical conductivity meter, respectively. Soluble ions of the soil were determined according to the methods described by Richards (1954). Available heavy metals in the soil were determined by extracting the soil with 0.01 M diethylenetriamine penta acetic acid (DTPA) as described by Lindsay and Norvell (1978).

Plant analysis

Lipid peroxidation (malondialdehyde content)

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content using the method of Hodges *et al.* (1999). The MDA content was calculated using its absorption coefficient of 155 $nmol^{-1}$ cm^{-1} and expressed as nmol (MDA) g^{-1} FW.

Hydrogen peroxide assay

Hydrogen peroxide content was determined by measuring the absorbance of titanium-hydroperoxide complex (Mukherjee and Choudhury, 1983). The acetone extract of the tissue was reacted with titanium reagent and ammonium to form hydroperoxide-titanium complex. The complex was dissolved in 1 M sulfuric acid and absorbance of the supernatant was measured at 415 nm against blank. Concentration of hydrogen peroxide was determined using the standard curve plotted with known concentration of hydrogen peroxide, and expressed as µmol g^{-1} FW.

Extraction and estimation of polyamines

Putrescine (Put), spermine (Spm) and spermidine (Spd) were extracted and determined in both shoots and roots of turnip and lettuce according to Mietz and Karmas (1977), Maijala and Eerola (1993), and Ayesh *et al*. (2002) as follows:

Extraction: Ten grams of plant tissue were blended with 50 ml of 5% trichloroacetic acid (TCA) for 3 min using a warming blender then filtered. Ten ml of each extract were transferred into a culture tube with 4g NaCl and 1ml of 50% of NaOH then shacked and extracted three times by 5ml n-butanol/chloroform (1:1 v/v) stoppered and shacked vigorously for 3 min. Centrifugation for 5 min at 3000 rpm were carried out and the upper layer was transferred to 50 ml separating funnel. To the combined organic extracts (upper layer), 15ml of n-heptane were added and extracted three times with 1ml portions of 0.2N HCl, the HCl layers were collected in a glass stoppered tube. The solution was evaporated just to dryness using water bath at 95ºC with the aid of a gentle current of air.

***Formation of dansylamines*:** One hundred µl of each stock standard solution were dried under vacuum. About 0.5ml of saturated $NaHCO_3$ solution was added to the residue of the sample extract (or the standard). One ml of dansyl chloride solution was added and mixed-thoroughly. The reaction mixture was incubated at 55°C for 45 min. The extraction of dansylated biogenic amines was carried out using three times of 5ml portions of diethylether, stoppered, shacked carefully for 1 min. The combined ether extracts were carefully evaporated at 35°C in dry bath with the aid of air current. The obtained dry film was dissolved in 1ml methanol.

Determination of dansylamines by TLC (Thin Layer Chromatography): One–dimensional TLC carried out the chromatographic separation to separate the studied dansylamines. The plate was developed using chloroform: benzene: triethylamine (6: 4.5: 1), then plate was dried at room temperature until the excess of solvent disappeared. The resulting zones were examined and marked under long ultraviolet wavelength (365nm). The marked areas were determined using CS-9000 dual wavelength flying spot scanning densitometer (SHIMADZU) using wavelength 254nm. Standard curve of each dansylamine was used to calculate the concentrations of biogenic amines in the tested samples.

Phenolic compounds

***Total phenols*:** Total phenols were determined in leaves according to the method described by Malik and Singh (1980) using the Folin-Ciocalteau reagent. The absorbance was read at 650 nm and the values were expressed as µg gallic acid equivalent (GAE) g^{-1} FW.

Flavonoids: Total flavonoid content was determined using the Dowd method as adopted by Arvouet-Grand *et al.* (1994). The absorbance was read at 415 nm and the results were expressed as µg g^{-1} FW.

RESULTS

Accumulation of heavy metals in the soil

Available heavy metals were measured in the soil before and at the end of the experiment as illustrated in tables (2 and 3), respectively. The obtained data showed significant increases in Fe and Cu contents and non significant increase in Zn in soil irrigated with industrial wastewater as compared with the control soil irrigated with fresh water. In addition, considerable amounts of other heavy metals (Pb, Cd, Co and Ni) that were not detected in soil at the beginning of the experiment or at the end of the experiment in soil irrigated with fresh water were found in soil irrigated with industrial wastewater. The accumulated amounts were in the following order: Pb (ranged from 6.3-7.9 mg kg^{-1}) ≥ Ni (6.2-7.9 mg kg^{-1}) > Co (4.7-7.1 mg kg^{-1}) > Cd (3.8-5.3 mg kg^{-1}). The detected amounts of Pb, Cd, Co and Ni were positively correlated with the distance of the used wastewater from the beginning of the drain. Thus heavy metals concentrations in soil irrigated with wastewater collected from 1 km from the beginning of El-Amia drain < those in soil irrigated with wastewater from 10 km of the drain < in soil irrigated with wastewater 19 km of the drain. Interestingly the accumulated amounts of Cu, Pb, Cd, Co and Ni in response to irrigation with wastewater (collected from 19 km from the beginning of the drain) in soil cultivated with turnip plants were much higher than those detected in soil cultivated with lettuce plants by 11%, 13%, 4%, 4.4% and 5%, respectively.

Plant growth and yield

Tables 4 and 5 presented the results of lettuce and turnip growth and yield as affected by irrigation with wastewater. Both plants exhibited significant decreases in leaf area, fresh weight and dry weight of shoots and roots; the magnitude of decrease was positively correlated with the amounts of heavy metals detected in the soil, with highest reduction in plants irrigated with wastewater collected 19 km from the beginning of El-Amia drain. The inhibition in turnip growth was calculated by 57.6%, 68.5%, 31%, 81.9% and 57.8% and in lettuce growth by 71%, 14.6%, 33%, 19.45% and 56% in leaf area, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight, respectively as compared with control plants irrigated with tap water. Weight of tuberous roots (fresh yield) of turnip was reduced to 78.3%, 73.4% and 69.07% of the control value in plants irrigated with wastewater collected from 1, 10 and 19 km from the drain, respectively. Whereas, fresh weight of shoots (represented the fresh yield) of lettuce was reduced to 94.1%, 88.38% and 85.37% of the control value in plants irrigated with wastewater collected from 1, 10 and 19 km from the drain, respectively.

The present work showed that irrigation with industrial wastewater has a negative impact on all the measured yield components. In this respect, number of leaves per plant of lettuce plants and tuberous root circumference of turnip plants were inhibited by 2.4%, 16.7% and 38.1% in case of number of leaves per lettuce plant and 22.7%, 54.5% and 54.5% in case of turnip root circumference in response to irrigation with wastewater collected from 1, 10 and 19 km of the drain, respectively as compared with control plants irrigated with fresh water. Moreover, number of seeds per plants was significantly reduced by increasing soil contamination to reach the highest reduction (44.1% in case of turnip and 55.5% in case of lettuce) in plant grown in soil with maximum heavy metals content; irrigated with wastewater from 19 km of the drain. Seed index (weight of 1000 seeds) of turnip plants was much more affected by irrigation by wastewater than lettuce plants, with reduction calculated by 55.1% in plants irrigated with wastewater; 19 km of the drain compared to corresponding control irrigated with fresh water.

Lipid peroxidation and hydrogen peroxide content

Change in the level of lipid peroxidation (as indicated by MDA content) and hydrogen peroxide in lettuce and turnip plants in response to wastewater irrigation are shown in Fig. 1. Both MDA and hydrogen peroxide contents exhibit a significant increase in lettuce and turnip plants due to irrigation using wastewater. The magnitude of increase was almost the same in the two studied plants; lettuce and turnip with the highest values of MDA and H_2O_2 were detected in plants irrigated with wastewater collected 19 km from the beginning of El-Amia drain and were calculated as

1.8- and 1.7 - fold the value of the control plants irrigated with fresh water, in both turnip and lettuce plants.

Changes in polyamines content

The results indicated that all the examined wastewater samples (1, 10 and 19 km from the beginning of the drain) caused significant increase in putrescine and significant decreases in both spermidine and spermine contents in lettuce shoot and turnip shoot and root comparing with control plants irrigated with fresh water. Consequently total polyamines of turnip shoot and root was slightly reduced whereas, in lettuce shoot total polyamines content increased as putrescine comprises 66% of total polyamines content. In contrast to turnip, in lettuce root tissue spermidine, in addition to putrescine, was increased significantly by 1.2, 1.5 and 1.8-fold the value of the control in response to irrigation using wastewater 1, 10 and 19 km from the beginning of the drain, respectively. Worth to mention here that the changes in the total polyamine contents in shoots and roots tissue of turnip and lettuce plants were not significant in response to irrigation with wastewater as compared with total polyamine contents of the control plants. The significant changes were observed in polyamine fractions (putescine, spermine and spermidine). The ratio of Spd + spm / put showed a significant reduction in turnip shoots and roots and lettuce shoots, the reduction was positively correlated with the level of heavy metals detected in the soil and were calculated by 8.6%, 51.88% of the control value in turnip shoot and root, respectively and 8.3% of the control value in case of lettuce shoot.

Changes in phenolic compounds

Irrigation with industrial wastewater caused significant increases in phenolic compounds and flavonoids content in both lettuce and turnip shoots as compared with control values. The highest level of phenolic compounds and flavonoids were detected in turnip and lettuce plants irrigated with wastewater collected from 19 km from the beginning of El-Amia drain and were estimated by 137% in case of phenolic compounds in lettuce and turnip and 109% and 117% in case of flavonoids in turnip and lettuce, respectively as compared with control plants irrigated by fresh water.

Table 1. Physio-chemical properties of the soil.

Soil characteristic	**Values**
S.P (%)	24
pH	7.82
E.C (Mmohs)	1.72
$CaCo_3$ (%)	0.31
Mechanical analysis (%)	
Sand (%)	10
Silt (%)	70.5
Clay (%)	19.5
Clay + Silt (%)	90
Soil texture	Silt Clay Loam
Soluble ions (meq/l)	
Calcium	2.7
Magnesium	1.35
Sodium	8.95
Potassium	0.9
Carbonate	---
Bicarbonate	3.50
Chloride	9.75
Sulphate	0.65

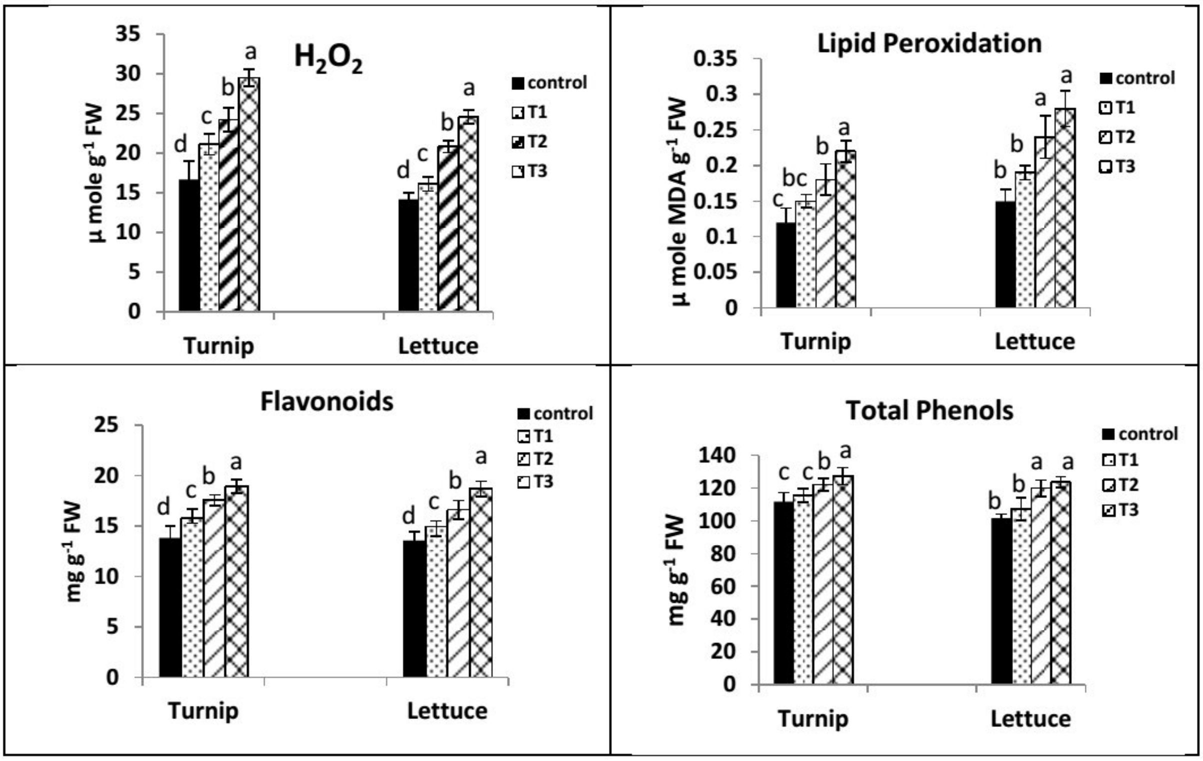

Figure 1. Changes in H_2O_2**,** lipid peroxidation (malondialdehyde content), flavonoids and total phenols content of turnip *(Brassica napus* L.*)* and lettuce *(Lactuca sativa* L.*)* irrigated with industrial wastewater. Control: Plants irrigated with fresh water, T_1: plants irrigated using water collected 1 km from beginning of the drain, T_2: plants irrigated using water collected after 10 km from beginning of the drain, T_3: plants irrigated using water collected 19 km from beginning of the drain. For each plant; columns with the same lower-case letters are not significantly different ($p < 0.05$).

Table 2. Heavy metal ions content in the soil at the beginning of the experiment (before irrigation with wastewater).

Heavy metal	Value (mg Kg^{-1})
Fe	1.66
Mn	0.33
Zn	0.31
Cu	0.16
Pb	Nd
Cd	Nd
Co	Nd
Ni	Nd

Nd = Not detected

Table 3. Heavy metal ions content in the soil cultivated with turnip or lettuce plants and irrigated with industrial wastewater for 100 days.

Treatment	Heavy metals (mg Kg^{-1})							
	Concentrations of essential micronutrients				Concentrations of metals			
	Fe	Mn	Zn	Cu	Pb	Cd	Co	Ni
Turnip plant								
Control	28.9 b	23.2 b	18.7 a	5.4 c	Nd	Nd	Nd	Nd
T_1	29.0 ab	23.8 ab	19.0 a	6.8 b	7.0 c	4.1 c	6.3 b	6.2 b
T_2	29.8 a	24.5 ab	19.2 a	7.8 ab	7.4 b	4.9 b	6.8 a	6.3 b
T_3	30.1 a	25.00 a	20.1 a	8.0 a	7.9 a	5.3a	7.1 a	7.9 a
LSD ($p < 0.05$)	2.02	1.66	1.53	0.35	0.36	0.23	0.31	0.23
Lettuce plant								
Control	30.9 c	23.7 a	18.8 a	5.4 c	Nd	Nd	Nd	Nd
T_1	31.8 bc	24.5 a	18.9 a	5.8 bc	6.3 b	3.8 c	4.7 b	6.8 b
T_2	33.5 ab	24.9 a	19.4 a	6.0 b	6.7 a	4.4 b	5.0 b	7.0 b
T_3	34.3 a	25.1 a	20.3 a	7.2 a	7.0 a	5.1a	6.8 a	7.5 a
LSD ($p < 0.05$)	2.26	1.96	1.54	0.38	0.37	0.22	0.31	0.21

Control: Plants irrigated with fresh water, T_1: plants irrigated with water collected 1 km from beginning of the drain, T_2: plants irrigated with water collected after 10 km from beginning of the drain, T_3: plants irrigated with water collected 19 km from beginning of the drain. Values within a column with the same lowercase letters are not significantly different ($p < 0.05$).

LSD = Least Significant Difference.

Table 4. Effect of industrial wastewater irrigation on growth parameters of turnip plant (*Brassica napus* L.) and lettuce plant (*Lactuca sativa* L.) before the flowering stage (70 days after sowing).

Turnip plant					
Treatment / Character	Area of leaves per plant (cm^2)	Shoot system Fresh Weight (g)	Fresh yield (weight of tuberous root) (g)	Shoot system dry Weight (g)	Root system dry Weight (g)
Control	131.1 a	339.8 a	193.4 a	192.3 a	112.8 a
T_1	101.49 b	171.0 b	151.4 b	93.1 b	69.3 b
T_2	84.68 b	138.3 c	142.0 c	65.7 c	58.3 c
T_3	55.467 c	107.3 d	133.6 d	34.6 d	47.6 d
LSD ($p < 0.05$)	19.570	6.8372	6.4813	6.4865	7.7611
Lettuce plant					
Treatment / Character	Fresh yield		Root system Fresh Weight (g)	Shoot system dry Weight (g)	Root system dry Weight (g)
	Area of leaves per plant (cm^2)	Shoot system Fresh Weight (g)			
Control	142.63 a	411.85 a	94.15 a	345.88 a	53.30 a
T_1	96.32 b	387.35 b	81.38 b	313.750 b	41.98 b
T_2	66.73 c	364.00 c	68.93 c	292.73 c	28.75 c
T_3	40.03 d	351.60 d	63.18 d	278.63 d	23.43 c
LSD ($p < 0.05$)	18.513	11.665	6.32	9.9131	7.2171

Control: Plants irrigated with fresh water, T_1: plants irrigated with water collected 1 km from beginning of the drain, T_2: plants irrigated with water collected after 10 km from beginning of the drain, T_3: plants irrigated with water collected 19 km from beginning of the drain. Values within a column with the same lowercase letters are not significantly different ($p < 0.05$).

Table 5. Effect of industrial wastewater irrigation on yield components of turnip *(Brassica napus* L.*)* and Lettuce *(Lactuea sativa* L.) plants (after 100 days from sowing).

Turnip plant										
Treatment / Character	Root circumference (cm)	Pods number per plant	Pod fresh weight (g)	Pod dry weight (g)	Pods fresh weight/ plant (g)	Pods dry weight /plant (g)	Seeds number per pod	Seeds number per plant	Seeds weight per plant (g)	Seed index (Wt. of 1000 seeds,g)
Control	22.5 a	230.0 a	0.1450a	0.075a	76.31a	35.590a	15.0a	3450 a	10.179 a	2.95 a
T_1	17.5 a	190.0 ab	0.1350a	0.070a	56.79ab	28.020ab	12.5ab	2375 ab	4.188 b	1.763 b
T_2	10.0 b	189.5 ab	0.1250a	0.060a	41.11b	19.500b	10.5ab	1989.75 b	2.860 c	1.437 c
T_3	10.0 b	179.0 b	0.0950a	0.050a	36.67b	19.355b	8.50b	1521.5 c	2.015 c	1.324 c
LSD ($p <$ 0.05)	5.889	49.56	0.1128	0.0687	28.017	11.318	5.1007	252.756	3.461	0.13
Lettuce plant										
Treatment / Character	Number of leaves per plant	Pods number per plant	Pod fresh weight (g)	Pod dry weight (g)	Pods fresh weight (g)	Pods dry weight (g)	Seeds number in pod	Seeds number in plant	Seeds weight in plant (g)	Seed index (Wt. of 1000 seeds,g)
Control	10.5 a	602.50 a	0.108 a	0.0600 a	68.83 a	56,67 a	34.25 a	20635.62 a	22.16 a	1.07 b
T_1	10.25 a	555.00 ab	0.080 ab	0.0475 ab	62.67 ab	50.54 ab	32.25 ab	17898.75 b	19.23 a	1.744 a
T_2	8.75 a	532.50 b	0.070 b	0.0450 ab	57.46 bc	45.88 bc	28.25 bc	15043.12 c	13.80 b	0.917 b
T_3	6.5 b	472.00 c	0.045 b	0.0225 b	51.85 c	38.51c	24.25 c	11446.00 d	7.87 c	0.688 c
LSD ($p <$ 0.05)	2.245	49.978	0.0371	0.0297	6.7608	7.898	4.0518	202.501	3.75	0.18

Control: Plants irrigated with fresh water, T_1: plants irrigated with water collected 1 km from beginning of the drain, T_2: plants irrigated with water collected after 10 km from beginning of the drain, T_3: plants irrigated with water collected 19 km from beginning of the drain. Values within a column with the same lowercase letters are not significantly different ($p <$ 0.05).

Table 6. Effect of industrial wastewater irrigation on polyamines contents (mg kg^{-1}) of turnip plant (*Brassica napus* L.) and lettuce plant (*Lactuca sativa* L.) before the flowering stage (70 days after sowing).

Turnip plant										
Treatment	Shoot					Root				
	Putrescine (Put)	Spermidine (Spd)	Spermine (Spm)	$\frac{Spd + Spm}{Put}$	Total polyamines	Putrescine (Put)	Spermidine (Spd)	Spermine (Spm)	$\frac{Spd + Spm}{Put}$	Total polyamines
Control	42.3 c	12.4 a	12.3 a	0.584 a	67 a	58.8 d	19.6 a	22.7 a	0.719 a	101.1 a
T_1	46.8 c	8.5 b	9.6 b	0.387 b	64.9 a	61.3 c	18.0 a	20.1 b	0.622 a	99.4 a
T_2	53.8 b	5.3 c	5.1 c	0.193 c	64.2 a	67.2 b	16.2 b	17.8 c	0.506 b	101.2 a
T_3	61.7 a	1.0 d	2.1 d	0.0502 d	64.8 a	72.6 a	12.9 c	14.2 d	0.373 c	99.7 a
LSD ($p <$ 0.05)	5.08	0.99	1.89	0.03	7.9	3.6	1.76	2.23	0.11	7.59
Lettuce plant										
Control	20.9 c	7.0 a	3.8 a	0.517 a	31.7 b	59.8 b	8.4 d	8.6 a	0.284 a	76.8 a
T_1	40.1 b	5.2 b	3.0 b	0.2045 b	48.3 a	60.1 b	10.3 c	7.3 b	0.293 a	77.7 a
T_2	42.8 b	3.3 c	1.7 c	0.117 c	47.8 a	60.3 b	12.6 b	3.5 c	0.267 a	76.4 a
T_3	47.6 a	1.4 d	0.65 d	0.043 d	49.65 a	62.9 a	15.6 a	1.4 d	0.27 a	79.9 a
LSD ($p <$ 0.05)	4.43	0.47	0.55	0.02	5.45	0.81	1.88	0.89	0.034	3.58

Control: Plants irrigated with fresh water, T_1: plants irrigated with water collected 1 km from beginning of the drain, T_2: plants irrigated with water collected after 10 km from beginning of the drain, T_3: plants irrigated with water collected 19 km from beginning of the drain. Values within a column with the same lowercase letters are not significantly different ($p <$ 0.05).

DISCUSSION

Heavy metal in soil

As shown in table (3) considerable amounts of heavy metals were found in soil irrigated with wastewater samples collected from different sites of El-Amia drain (1, 10 and 19 km from the beginning of the drain) as compared with control soil irrigated with tap water. Therefore, irrigation with wastewater was believed to be responsible for soil enrichment with heavy metals (Pb, Co, Ni and Cd) that were not detected before irrigation with wastewater (table 2). The magnitude of Cd in soils irrigated with industrial wastewater exceed the maximum permissible limit of 3 mg kg^{-1} (Kabata-Pendias and Pendias, 2001) by 1.4, 1.6 and 1.77 – fold the maximum allowable limit in case of soil cultivated with turnip and 1.3, 1.46 and 1.7 – fold in case of soil cultivated with lettuce and irrigated with wastewater collected from 1, 10 and 19 km from the beginning of the drain, respectively. On the other hand, Pb, Co and Ni in soils did not exceed the maximum allowable limits of 100, 50 and 50 mg kg^{-1}, respectively (Kabata-Pendias and Pendias, 2001). It has been reported that cadmium is a highly mobile metal, easily absorbed by the plants through root surface and moves to wood tissue and transfers to upper parts of plants (Itanna, 2002). In this respect, Muhammad *et al*., (2008) found that there is a direct relation between the levels of presence of cadmium in the root zone and its absorption by plant. The present study therefore reflects the harmful effect of irrigation with industrial wastewater as a potential source for soil pollution with heavy metals and hence threatens the food production and human health.

Generally, the level of heavy metals concentrations in the soil were in the order of distance of the used irrigation wastewater from the beginning of El-Amia drain: heavy metals in soil irrigated with wastewater 1 km from the beginning of the drain < soil irrigated with wastewater 10 km from the beginning of the drain < soil irrigated with wastewater 19 km from the drain. This could be attributed to increased accumulation of toxic metals by increasing the industrial effluent disposal along the drain length.

The observed increase in the accumulated amounts of Cu, Pb, Cd, Co and Ni in response to irrigation with wastewater in soils cultivated with turnip plants comparing to those detected in soils cultivated with lettuce plants may reflect the increased potential of lettuce plants to absorb and accumulate heavy metals than turnip plants. The metals availability for plants is controlled by their requests for micronutrients and their capacity to absorb and eliminate toxic elements. This availability is different, depending on plant species and their adaptation to the environment conditions (Smical *et al*., 2008). Herbs absorb less metal than fast growing plants such as lettuce and spinach. When the growing takes place on the same type of soil, the cadmium accumulation in different species decreases in the following order: Grains < Root <Vegetables < Leaf vegetables (Oros, 2001).

Plant growth and yield

Irrigation with industrial wastewater significantly reduced lettuce and turnip growth, the reduction was positively correlated with the detected level of heavy metals in the soil. The inhibition in leaf area, fresh and dry weights of shoots and roots reach its highest value in lettuce and turnip plants irrigated with wastewater collected from 19 km from the beginning of the drain. The reduction in growth in the studied plants due to industrial effluents was because of the accumulation of Pb, Cd, Co and Ni in the soil, especially Cd which found to exceed the maximum allowable limits. In this respect, Scoccianti *et al*. (2006) and Bini *et al*. (2012) stated that the negative effect of wastewater (mostly polluted with heavy metals) on root and shoot fresh weight may probably due to that these metals decrease the root water uptake and relative water content, damage plant roots and inhibited uptake of nutrient, thus inhibiting normal plant growth.

According to Sandalio *et al*. (2001) the reduction of plant growth by Cd toxicity could be the direct consequence of the decreased uptake of nutrient elements, inhibition of various enzyme activities, and induction of oxidative stress including alterations in enzymes of the antioxidant defense system.

The current study showed that irrigation with industrial wastewater exerted significant negative influence on all the measured yield components (pods number per plant, pods fresh and dry weights per plants, seeds number and weight per plant and seed index) in both turnip and lettuce plants. This could be attributed to the detected amounts of heavy metals in the soil as a result of irrigation with industrial wastewater. Similar results were obtained by Begum *et al*. (2011) working on rice plant who suggested that markedly reduced grain yield with industrial wastewater irrigation was remarkable since this water had contained high concentration of toxic elements like Cu, Mn, Cl, and Cr.

Comparing lettuce and turnip plants growth and yield in response to cultivation in heavy metal contaminated soil, it was found that the reduction in lettuce fresh yield (fresh weight of shoots and numbers of leaves) and seed index (weight of 1000 seeds) was less than that observed in fresh yield (weight of tuberous root and root circumference) and seed index of turnip plants cultivated under the same heavy metal stress condition. These results along with the detected reduction in heavy metal contents of soil cultivated with lettuce plants comparing to that cultivated with turnip plants suggested that lettuce is more appropriate than turnip to be cultivated in soil irrigated with wastewater conditioned that the level of heavy metals in the plants should be monitored not to exceed the permissible doses for human hence cause hazard to consumers.

Hydrogen peroxide content and membrane lipid peroxidation

The intoxication with pollutant metals induces oxidative stress because they are involved in several different types of ROS-generating mechanisms (Stohs and Bagchi, 1995). Lipids, a key component of cell membranes, are sensitive to oxidation processes generated by free radicals. In biological systems, the presence of oxidation products such as malondialdehyde is directly related to the beginning of peroxidation of unsaturated fatty acids constituting cellular membranes (Turton *et al*., 1997). Therefore, in the present work, malondialdehyde and H_2O_2 levels have been used as an indicator of damage on the studied plants by heavy metal pollutants.

Significantly higher malondialdehyde and H_2O_2 content were detected in turnip and lettuce plants irrigated with wastewater as compared with control plants irrigated with tap water. The present data reflect a positive correlation of heavy metals content in the soil with the level of MDA and H_2O_2 in plant. The magnitude of the increase was almost the same in the two studied plants; indicating that turnip and lettuce exhibit equal degree of damage at cellular level when growing in a comparable conditions.

Polyamines and phenolic compounds

PAs are an integral component of plant stress management. Variations in PA contents have been associated with heavy metals stress (Kuznetsov *et al*., 2009). The strong antioxidant character of PAs is proven (Ha *et al*., 1998). Alsokari and Aldesuquy (2011) found that Spm, Spd or their interaction play an important role in increasing the tolerance of wheat plants to waste water treatment by decreasing the accumulation of Cd, Pb, Cu, Ni and Zn contents in root and consequently in shoot as compared with their corresponding control values. The repairing effect induced by PAs may be due to PAs: (1) increase the production of phytochelatins (PCs) particularly in root; (2) increase the cell wall and vacuolar storage of these heavy metals; (3) acted as an efficient antioxidants and free radical scavengers under this stress increase the root exudates into the soil (biosphere) (Ferreira *et al*., 2002).

The present work revealed significant increases in putrescine and significant decreases in both spermidine and spermine contents in lettuce shoot and turnip shoot and root in response to irrigation with industrial wastewater as compared with control plants irrigated with tap water. These results revealed selective synthesis of low molecular weight PA, Put, than high molecular weight Spm and Spd in response to soil contamination with multi-metals (Fe, Cu, Pb, Cd, Co and Ni). Similarly, enhanced accumulation of putrescine had been reported in rice leaves subjected to copper stress (Lin and Kao, 1999) and sunflower leaf discs under cadmium stress (Groppa *et al.,* 2008). The significant increase in Spd, of more amine groups than low molecular weight Put, in lettuce root that were not detected in turnip suggested that lettuce exhibited a faster scavenging of oxidant or free radicals generated under heavy metal stress. The strong antioxidant character of high molecular weight PAs than low molecular weight PAs had been recorded by Ha *et al.* (1998). This finding could explain why lettuce growth and yield was less affected than turnip by soil contamination with heavy metals.

Phenolics, especially flavonoids and phenylopropanoids, are oxidized by peroxidase, and act in H_2O_2- scavenging, phenolic/ ASC (ascorbic acid) / POX (peroxidase) system. Their antioxidant action resides mainly in their chemical structure. There is some evidence of induction of phenolic metabolism in plants as a response to multiple stresses (including heavy metal stress) (Michalak, 2006). Flavonoids in low concentrations are capable of displaying functional roles of extraordinary significance in plant-environment interactions (Fini *et al*., 2011).

In the present work irrigation with industrial wastewater caused significant increases in phenolic compounds and flavonoids content in both lettuce and turnip leaves as compared with control values. The highest induction was detected in turnip and lettuce plants cultivated in the soil with the highest load of heavy metals. Similarly, the induction of phenolic compound biosynthesis was observed in wheat in response to nickel toxicity (Diáz *et al*., 2001) and in maize in response to aluminum (Winkel-Shirley, 2002). *Phaseolus vulgaris* exposed to Cd^{2+} accumulate soluble and insoluble phenolics and *Phyllantus tenellus* leaves contain more phenolics than control plants after being sprayed with copper sulphate (Diáz *et al*., 2001).

Comparing the two studied plants turnip and lettuce, the current data showed that the value of increase in phenolic compounds was almost the same in turnip and lettuce plants cultivated under comparable condition comparing to control plants. On the other hand, the increase in flavonoids contents in lettuce leaves was much more than those detected in turnip leaves as compared with the corresponding control value. This could be attributed to the increased biosynthesis of flavonoids in lettuce leaves or because flavonoids are efficient metal chelators (Bai *et al*., 2004; Brunetti *et al.,* 2013), we believe that many flavonoid structures may be bounded with transition metals in chelate complexes, and this is a reason of a decrease of free flavonoids in turnip leaves comparing to lettuce leaves.

CONCLUSIONS

Rapid industrialization and urbanization in developing countries like Egypt, has resulted in large – scale pollution of the environment. The risk assessment of El-Amia drain in Alexandria - Egypt indicated that soil irrigation with water collected from different distances along the drain resulted in the enrichment of metals in soil and has a negative impact on growth and yield of lettuce (a leafy vegetable) and turnip (a root vegetable) irrigated with this water. Moreover, the intoxication of soil with heavy metals induces oxidative stress on plants as indicated by the increased levels of lipid peroxidation and H_2O_2 in both plants under investigation. Consequently, compounds are known to exhibit antioxidant activities like polyamines and phenolic compounds were increased. We believed that lettuce is more suitable than turnip to be cultivated in these circumstances of soil pollution with heavy metals as the present data revealed that lettuce growth and yield was less affected by soil contamination. However, due to its potential to absorb and accumulate heavy metals the endogenous level of heavy metals especially in the edible part should be monitored to avoid health hazards. As the present detected harmful effect of irrigation with industrial wastewater on soil was happened after only 100 days of irrigation, which is the duration of the experiment, we strongly recommended a periodical assessment to be done to soil irrigated with wastewater for prolonged period and we believe that the use of industrial wastewater in such form, on agricultural lands is not suitable without proper treatment.

REFERENCES

Alsokari, S.S. and Aldesuquy, H.S. (2011) Synergistic effect of polyamines and waste water on leaf turgidity, heavy metals accumulation in relation to grain yield. *J. Appl. Sci. Res.,* **7**(3), 376-384.

Antonious, G. and Kochhar, T. (2009) Mobility of heavy metals from soil into hot peper fruits: a field study. *Bull. Environ. Contam. Toxicol.,* **82**, 59-63.

Arvouet-Grand, A., Vennat, B., Pourrat, A. and Legret, P. (1994) Standardization d'un extrait de propolis et identification des principaux constituants. *J. Pharm. Belg.,* **49**, 462–468.

Ayesh, A.M., Rowayshed, G.H. and Deabes, M.M. (2002) Inhibitory effect of some food additives on biogenic amines formation in beef sausages trails. *Egypt. J. Nut*., **17**, 197-227.

Bai, Y., Song, F., Chen, M., Xing, J., Liu, Z. and Liu, S. (2004) Characterization of the rutin-metal complex by electrospray ionisation tandem mass spectrometry. *Anal. Sci.,* **20**, 1147–1151.

Begum, R.A., Zaman, M.W., Mondol, A.T., Islam, M.S. and Hossain, K.F. (2011) Effects of textile industrial waste water and uptake of nutrients on the yield of rice. *Bangladesh J. Agri. Res.*, **36**(2), 319-331.

Bini, C., Wahsha, M., Fontana, S., Maleci, L. (2012)

Effects of heavy metals on morphological characteristics of *Taraxacum officinale* Web growing on mine soils in NE Italy. *J. Geogr. Explor.,* **123**, 101-108.

Brunetti, C., Ferdinando, M., Fini, A., Pollastri, S. and Tattini, M. (2013) Flavonoids as antioxidants and developmental regulators: relative significance in plants and humans. *Int. J. Mol. Sci.*, **14,** 3540-3555; doi:10.3390/ijms14023540

Chandran, S., Niranjana, V. and Benny**,** J. (2012) Accumulation of heavy metals in wastewater irrigated crops in Madurai, India, *J. Environ. Res. Develop.,* **6** (3), 432-438.

Chao, Y., Zhang, M., Tian, S., Lu, L. and Yang, X. (2008) Differential generation of hydrogen peroxide upon exposure to zinc and cadmium in the hyperaccumulating plant species (*Sedum alfredii* Hance). *J Zhejiang Univ. Sci. B.*, **9**(3), 243–249.

Chen, Y.T., Zheng, R.L., Jia Z.J. and Ju Y. (1990) Flavonoids as superoxide scavengers and antioxidants. *Free Radica. Biol. Med*., **9**, 19–21.

Cho, U-H. and Park, J-O. (2000) Mercury-induced oxidative stress in tomato seedlings. *Plant Sci.,* **156**, 1-9.

Diáz, J., Bernal, A., Pomar, F. and Merino, F. (2001) Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annum* L.) seedlings in response to copper stress and its relation to lignification. *Plant Sci.,* **161**, 179.

Drążkiewicz, M., Skórzyńska-Polit, E. and Krupa, Z. (2004) Copper-induced oxidative stress and antioxidant defense in *Arabidopsis thaliana*. *BioMet.*, **17**, 379-387.

Dudjak, J., Lachman, J., Miholová, D., Kolihová, D. and Pivec V. (2004) Effect of cadmium on polyphenol content in young barley plants (*Hordeum vulgare* L.). *Plant Soil Environ.,* **50**, 471–477.

Ferreira, R.R., Fornazeir, R.F., Vitoria, A.P., Lea, A.P. and Azevedo, R.A. (2002) Changes in antioxidant enzymes activities in soybean under cadmium stress. *Plant Nutr*., **25,** 327-342.

Fini, A., Brunetti, C., Di Ferdinando, M., Ferrini, F. and Tattini, M. (2011) Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. *Plant Signal Behav.,* **6**(5), 709-711.

Foyer, Ch. and Noctor, G. (2003) Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant.,* **119**, 355-364.

Gill, S.S. and Tuteja, N. (2010) Polyamines and abiotic stress tolerance in plants. *Plant Signal Behav*., **5** (1): 26–33.

Groppa, M.D., Benavides, M.P. (2008) Polyamines and abiotic stress: recent advances. *Amino Acids,* **34**, 35-45.

Ha, H.C., Sirisoma, N.S., Kuppusamy, P., Zweier, J.L., Woster, P.M. and Casero RA (1998) The natural polyamine spermine functions directly as a free radical scavenger. *Proc. Natl. Acad. Sci. USA,* **95**, 11140-11145.

Hodges, D.M., Delong, J.M., Forney, C., Prange, P.K. (1999) Improving the thiobarbituric acid. Reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compound. *Planta,* **207**, 604-611.

Itanna, F. (2002) Metals in leafy vegetables grown in Addis Ababa and toxicological implications. *Ethiop. I. Health Dev*., **16**(3), 295-302.

Kabata-Pendias, A., Pendias, H. (2001) Trace elements on soil and plants, 3rd Ed., CRC, Boca

Raton.

Karenlampi, S., Schat, H., Vangronsveld, J., Verkleij, J.A.C., Lelie, D.V.D., Mergeay, M. and Tervahauta, A.I. (2000) Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environ. Pollut*., **107**, 225–231.

Kuznetsov, V.V., Stetsenko, L.A., Shevyakova, N.I. (2009) Exogenous cadaverine induces oxidative burst and reduces cadaverine conjugate content in the common ice plant. *J. Plant. Physiol*., **166**(1), 40-51.

Lefevre, I., Gratia, E. and Lutts, S. (2001) Discrimination between the ionic and osmotic components of salt stress in relation to free polyamine level in rice (*Oryza sativa*). *Plant Sci.,* **16**, 943–952.

Lin, C.C. and Kao, C.H. (1999) Excess copper induces an accumulation of putrescine in rice leaves. *Bot. Bull. Acad. Sinica*, **40**, 213-218.

Lin, Ch-Ch., Chen, L-M. and Liu, Z-H. (2005) Rapid effect of copper on lignin biosynthesis in soybean roots. *Plant Sci*., **168**, 855-861.

Lindsay, W. L. and Norvell, W. A. (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Amer. J.,* **42**, 421-428.

Maijala, R.L. and Eerola, S.H. (1993) Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. *Meat Sci*., **35**, 387-395.

Maksymiec, W. (2007) Signaling responses in plants to heavy metal stress. *Acta Physiol. Plant*., **29**, 177-187.

Malik, C.P. and Singh, M.B. (1980) Plant enzymology and histo – Enzymology: A text manual. Kalyani Publishers, New Delhi, p 53.

Mangabeira, P., Almeida, A.A., Mielke, M., Gomes, F.P., Mushrifah, I., Escaig, F., Laffray, D., Severo, M.I., Oliveira, A.H. and Galle, P. (2001) Ultrastructural investigations and electron probe X-ray microanalysis of chromium-treated plants. Proc. VI ICOBTE, Guelph, pp. 555 (abstract).

Metwally, A., Finkemeier, I., Georgi, M. and Dietz, K.J. (2003) Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiol.,* **132**, 272-281.

Michalak, A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish J. Environ. Study,* **15** (4), 523-530.

Mietz, J.L. and Karmas, E. (1977) Chemical quality index of conned tuna as determined by HPLC. *J. Food Sci.,* **42**, 155-158.

Miller, N.J., Castellucio, C., Tijburg, L. and Rice-Evans, C. (1996) The antioxidant properties of theaflavins and their gallate esters – radical scavengers or metal chelators? *FEBS Lett.,* **392**, 40–44.

Muhammad, F, Farooq, A. and Umer, R. (2008). Appraisal of Heavy Metal Contents in different vegetables grown in the vicinity of an industrial area. *Pak. J. Bot.,* **40**(5), 2099-2106.

Mukherjee, S.P. and Choudhury, M.A. (1983) Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant.*, **58,** 166-170.

Oros, V. (2001) *Heavy Metals Biomonitoring*, (in Romanian), North University Baia Mare, Romania.

Preeti, P. and Tripathi, A.K. (2011) Effect of heavy metals on morphological and biochemical characteristics of *Albizia procera* (Roxb.) Benth. Seedlings. *Inter. J. Environ. Sci.,* **1**, 5.

Quarrie, S.A. and Jones, H.G. (1979) Genotype variation in leaf water potential, stomatal conductance and abscisic acid concentration in spring wheat subjected to artificial drought stress. *Ann. Bot.*, **44**, 323-332.

Rattan, R.K., Datta S.P., Chhonkar, P.K., Suribabu, K. and Singh, A.K. (2005) Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater: A case study. *Agric. Ecosyst. Environ.,* **109** (3-4), 310-322.

Rentel, M. and Knight, M.R. (2004) Oxidative stress-induced calcium signaling in *Arabidopsis. Plant Physiol.,* **135**, 1471-1479.

Richards, R.L. (1954) Diagnosis and improvement of saline and alkali soils. In Richards, R.L. (ed), *Agriculture Hand Book No.60*, U.S Government Printing Office, Washington, USA.

Sandalio, L.M., Dalurzo, H.C., Gomez, M., Romero-Puertas, M.C. and Rio, L.A.D. (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.*, **52,** 2115–2126.

Scoccianti, V., Crinelli, R., Tirillini, B., Mancinelli, V., Speranza, A. (2006) Uptake and toxicity of Cr (III) in celery seedlings. *Chemosphere*, **64,** 1695-1703.

Singh, Y. and Malik, C.P. (2011) Phenols and their antioxidant activity in *Brassica juncea* seedlings growing under HgCl2 stress. *J. Microbiol. Biotech. Res.,***1** (4), 124-130.

Smical, A-I, Hotea, V., Oros, V., Juhasz, J. and Pop, E. (2008) Studies on transfer and bioaccumulation of heavy metals from soil into lettuce. *Environ. Eng. Manag. J.,* **7**(5), 609-615.

Soil Survey Staff (2004) Soil survey laboratory methods manual. Burt, R. (ed.).Soil Survey Invest. Rep. (SSIR) 42, Version 4.0., USDA, Natural Resources Conservation Service.

Stohs, S.J. and Bagchi, D. (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Rad. Biol. Med*., **18,** 321-336.

Sutapa, B. and Bhattacharyya, A. K., (2008) Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. *Chemosphere*, **70** (7), 1264-1272.

Turton, H.E., Dawes, I.W. and Grant, C.M. (1997) *Saccharomyces cerevisiae* exhibits a yAP-1-mediated adaptive response to malondialdehyde. *J. Bacteriol.* **179**, 1096–1101.

Viswanathan, P., Sriram, V., Yogeeswaran, G. (2000) Sensitive spectrophotometric assay for 3-hydroxy-substituted flavonoids, based on their binding with molybdenum, antiomony, or bismuth. *J. Agri. Food Chem*., **48**, 2802–2806.

Winkel-Shirley, B. (2002) Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant Biol*., **5**, 218-223.