IRRIGATION WITH INDUSTRIAL WASTEWATER ACTIVATES ANTIOXIDANT SYSTEM AND OSMOPROTECTANT ACCUMULATION IN LETTUCE, TURNIP AND TOMATO PLANTS.

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ABSTRACT

We focused on the impact of industrial factories on the water quality of the El-Amia drain in Egypt and the effect of irrigation with industrial wastewater on the growth, cell membranes, photosynthetic pigment content, the antioxidant system and selected osmoprotectants (proline, total amino nitrogen and soluble sugars) in three crop plants: turnip, tomato and lettuce. For this purpose, water samples were collected 1, 10 and 19 km from the beginning of the drain and used for irrigation, with fresh water as a control. We found that industrial wastewater contained significant amounts of heavy metals (Pb, Cd, Ni and Co). The crop plants showed a significant decrease in leaf area, fresh weight and dry weight of shoots and roots, accompanied by a marked reduction in photosynthetic pigment content and damage to cell membranes, as indicated by increased electrolyte leakage and a lower membrane stability index. Significant increases in the activities of antioxidant enzymes and in the glutathione, proline, soluble sugar and total amino nitrogen content in response to irrigation with wastewater may be defense mechanisms induced in response to heavy metal stress.

Keywords
Brassica napus, *Lactuca sativa*, *Solanum lycopersicum*, Antioxidant, Soluble sugar.

Abbreviations

Ascorbate (ASC), ascorbate peroxidase (APX), catalase (CAT), dry weight (DW), fresh weight (FW), peroxidase (POD), reactive oxygen species (ROS), reduced glutathione (GSH), superoxide dismutase (SOD).

INTRODUCTION

Increasing pollution due to heavy metals has become a serious environmental concern (Alkorta et al. 2004). Heavy metal contamination in agricultural environments can come from atmospheric fall-out, pesticide formulations, contamination by chemical fertilizers, and irrigation with water of poor quality (Marcovecchio et al. 2007). Irrigation with wastewater results in build-up of heavy metals in the soil that could restrict soil function, resulting in toxicity of plants and contamination of the food chain, affecting food quality and safety (Singh et al. 2010).

Consumption of plants containing accumulated heavy metals is an important pathway for the entry of toxic heavy metals in the human body. Heavy metal-contaminated food can seriously deplete some essential nutrients in the body, resulting in reduced immunological defense, intrauterine growth retardation and disabilities associated with malnutrition (Arora et al. 2008). Moreover, evidence of severe poisoning caused by some metallic compounds and the proven carcinogenicity of some metal ions has fostered intensive research into the uptake and translocation patterns in food crops (Olowoyo et al. 2012).

Application of industrial effluent decreases germination percentage, root and shoot length, and fresh weight of seedlings (Nagajyoti et al. 2008). Recently, Bini et al. (2012), working on *Taraxacum officinale*, found a clear correlation between heavy metal content in soil and plants, as well as morphological alterations (reduced leaf thickness, changes in cellular organization), slowing of plant development and decrease in biomass, which were also reported in studies on *Hordeum vulgare* (Argese et al. 2001), *Brassica campestris* and *Apium graveolens* (Yang et al. 2011; Zong et al. 2007).

The photosynthetic apparatus is one of the target sites of heavy metal action in plants (Krupa 1999). In this respect, Oancea et al. (2005), working on tomato plants, showed that both growth and photosynthetic pigments are affected by the presence of heavy metals**.** In addition, Dos Santos et al. (2012) demonstrated that *Gracilaria domingensis* plants exposed to Cd show chloroplast alteration, especially degeneration of thylakoids and a decrease in the concentration of photosynthetic pigments, such as chlorophyll *a* and phycobiliproteins.

Interestingly, significant decreases in amounts of photopigments were found in *Avicennia marina* treated with Cu and Zn at concentrations lower than those inducing visible toxicity; thus, photosynthetic pigments may be sensitive biological indicators of Cu and Zn stress in this plant (MacFarlane and Burchett 2001).

Heavy metals adversely affect the cell membrane by causing changes in membrane lipid and protein structure, resulting in loss of membrane integrity and selective permeability. Following membrane damage, leakage of electrolytes from the cell can occur (Cuny et al. 2002). Thus, measurements of cell membrane stability and electrolyte leakage have been widely utilized to study effects of stress on plants.

One mechanism by which many plants respond to and apparently detoxify heavy metals is the production of proline (Shah and Dubey 1998; Verma 1999). The accumulation of proline in stressed plants is associated with reduced damage to membranes and proteins (Shah and Dubey 1998; Verma 1999). Proline synthesis has been implicated in the alleviation of cytoplasmic acidosis and may maintain NADP/NADPH ratios at values compatible with metabolism (Hare and Cress 1997).

Soluble sugars are involved in the responses to a number of stresses; in this respect, Couée et al. (2006) found that they act as nutrients and metabolite signaling molecules and can cause important modifications to gene expression and proteomic patterns. Sugar signaling and sugar-modulated gene expression are related to the control of oxidative stress (Barros et al. 2004). Some organic solutes in plants (such as proline and soluble sugars) act as osmoprotectants in adaptation to heavy metal stress (Zhang and Huang 2000; Yang et al. 2005). Chai et al. (2012) detected increased contents of proline and soluble sugar in *Spartina alterniflora* subjected to Cd stress.

One of the major consequences of heavy metals on plants is the enhanced production of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) (Li and Staden 1998), which damage cell membranes, nucleic acids and chloroplast pigments (Fang and Kao 2000; Tewari et al. 2002). Removal of

ROS is strictly controlled by an array of non-enzymatic and enzymatic antioxidant mechanisms in plants. Enzymatic ROS scavenging includes catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), ascorbate peroxidase (APX), and non-enzymatic scavengers, e.g. glutathione (GSH), carotenoids, and ascorbate (ASC) (Xiang and Oliver 1998; Srivastava et al. 2009). Enzymes of the detoxification machinery can serve as important markers of environmental pollution (Filho et al. 2001) and a good correlation with pollutant levels strengthens their utility as biomarkers (Fernandes et al. 2002). It is well documented that heavy metal stress leads to sharp changes in the activities of certain enzymes like SOD, CAT, APX and POD (Sai Kachou et al. 2009).

From the perspective of the alarming increase in water pollution due to mixing with industrial effluent, we have investigated the effect of industrial factories on contaminating water with toxic metals and the impact of irrigation using industrial wastewater on growth and photosynthetic pigment content of some vegetable crop plants; fruits in the case of tomato, leaves in the case of lettuce and roots in the case of turnip plants in order to establish advice regarding consumption of vegetables irrigated by wastewater or grown in soils contaminated by heavy metals. In addition, we investigated the effect of heavy metal-contaminated water on some osmoprotectants and the effectiveness of the antioxidant system as biomarkers of metal pollution and strategic defense mechanisms in plants.

MATERIAL AND METHODS

Plant materials

Seeds of the three vegetable crops under investigation, tomato (*Solanum lycopersicum),* lettuce *(Lactuca sativa)* and turnip (*Brassica napus*), were obtained from the Horticulture Institute Research Center, Giza, Egypt. These vegetables were selected because they differ in which parts are edible, which for tomato is the fruit, for lettuce, the leaves, and for turnip, the roots.

Soil

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

Irrigation water

Industrial wastewater was collected from the El-Amia drain. The El-Amia drain gets passed water containing waste from the industrial factories in the Kafr El-Dawar area and the 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 each crop plant under investigation were surface-sterilized 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 (40 cm in diameter, 25 cm deep) at a 3 cm depth containing 20 kg of loam soil. Pots were irrigated with fresh water in the first week. After that, pots of each crop under investigation (tomato, 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 respectively referred to as T_1, T_2 and T_3. 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 at the vegetative stage (60 days after sowing for tomato plants and 30 days after sowing for lettuce and turnip plants) to measure growth criteria and determine photosynthetic pigment content. Other samples were taken before the flowering stage (100 days after sowing for tomato and 70 days after sowing for lettuce and turnip) to assess electrolyte leakage, the membrane stability index, and components of the antioxidant system in leaves (antioxidant enzymes including CAT, POD, SOD, APX and antioxidant compounds including reduced GSH and ASC). In addition, the content of potentially effective osmoprotectants (soluble sugars, proline and total amino acids) was measured in both shoots and roots.

Water analysis

Water samples were collected from three selected sites (1, 10 and 19 km from the beginning of the drain) in chemically resistant plastic bottles to avoid adherence of solutes and suspended particles to the container wall. The water samples were digested with concentrated HNO_3 at 80°C until the solution become clear (APHA, 1985). The Fe, Mn, Zn, Cu, Pb, Cd and Ni content of the water samples was determined with a Model SOLAAR 969 atomic absorption spectrophotometer (Unicam).

Plant analysis

Electrolyte leakage (EL)

The total inorganic ion leakage from the leaves was measured by the method described by Sullivan and Ross (1979) according to the following equation:

$$\text{Electrolyte leakage (\%)} = \frac{EC_b - EC_a}{EC_c} \times 100$$

Where EC_a = electrical conductivity at 45°C, EC_b = electrical conductivity at 55°C and EC_c = electrical conductivity at 100°C.

Membrane stability index (MSI)

The membrane stability index (MSI) was estimated using the formula described by Premchandra et al. (1990) and modified by Sairam (1994):

$$MSI = [1 - (C_1/C_2)] \times 100$$

Where C_1= electrical conductivity at 40°C and C_2= electrical conductivity at 100°C.

Photosynthetic pigments

The amounts of the photosynthetic pigments chlorophyll *a* (chl a), chlorophyll *b* (chl b) and carotenoids in fresh leaves were determined as described by Metzner et al. (1965). The concentration of each pigment (as μg/ml) was calculated using the following equations:

$$\text{Chl } a = 10.3\ E_{663} - 0.918\ E_{644}$$

Chl *b* = $19.7\ E_{644} – 3.87\ E_{663}$

Carotenoids = $4.2\ E_{452.5}$ – (0.0264 chl *a* + 0.4260 chl *b*)

Finally, the pigment contents were expressed as μg g^{-1} dry weight (DW) of leaves.

Estimation of soluble sugar content

Soluble sugar was extracted from air-dried leaf tissue (1 g) with 80% ethanol according to the method described by Homme et al. (1992) and determined by the anthrone sulfuric acid method described by Scott and Melvin (1956). Data were calculated as mg 100 g^{-1} DW of tissue.

Estimation of proline content

Free proline was extracted and determined in fresh leaves in accordance with the method of Bates et al. (1973). Proline concentration was determined and calculated as mg 100 g^{-1} DW of tissue.

Total amino nitrogen content

The plant tissue was extracted according to the method of Yemm and Willis (1956). The absorbance was measured directly at 580 nm (Muting and Kaiser, 1963). Total amino nitrogen content was calculated as mg g^{-1} DW of tissue.

Analysis of antioxidant system

Preparation of samples for enzyme extraction followed the method described by Mukherjee and Choudhurri (1983). SOD (EC 1.15.1.1) activity was measured in accordance with the method of Dhindsa et al. (1981). One unit of SOD activity was defined as the amount of enzyme that caused half the maximum inhibition of nitroblue tetrazolium reduction to blue formazan at 560 nm under the experimental conditions. CAT (EC 1.11.1.6) activity was estimated by the decrease in absorbance at 240 nm over 1 min as a consequence of H_2O_2 consumption (Aebi 1983). POD (EC 1.11.1.7) activity was determined using guaiacol as substrate (Malik and Singh 1980). The increase in absorbance as a result of dehydrogenation of guaiacol was monitored at 470 nm (Klapheck et al. 1990). APX (EC 1.11.1.11) activity was assayed in accordance with the method of Asada (1992) by measuring the decrease in absorbance at 290 nm over 1 min as a result of oxidation of ASC using a Spectronic 601 UV spectrophotometer.

The activities of CAT, POD, SOD and APX were expressed as enzyme units per gram fresh weight (U/g FW).

GSH was extracted and measured by the method adopted by Tanaka et al. (1985) and expressed as U/g FW. Reduced ASC was quantified by the bipyridyl method (Knorzer et al. 1996) and expressed as nmol/mg FW.

Statistical evaluation of the data

The experiment utilized a completely randomized design. Mean values were calculated from measurements of five replicates and standard deviations of the means were calculated. All data were subjected to Duncan’s multiple range test to discriminate significance (defined as $p < 0.05$). All data were analyzed statistically by one-way analysis of variance using the SPSS program (version 18.0).

RESULTS AND DISCUSSION

Industrial factories had a negative impact on the quality of drain water (Table 1). This was illustrated by the significant amounts detected of some heavy metals (Pb, Cd, Ni and Co) and the observed increase in amounts of other metals (Cu, Zn, Mn) compared with fresh water. A positive trend between metal concentration and the site of water sampling was observed, with the amount of metal in the samples increasing from 1 to 19 km from the beginning of the drain.

Table(1) Heavy metals contents in irrigation water of samples collected at different distance from (Elamia)drain.

Heavy metals mg/L	Control	Water samples		
		T_1	T_2	T_3
Fe	7.50	9.3	10.17	12.82
Mn	4.20	4.6	5.23	8.90
Zn	2.13	3.1	4.90	6.01
Cu	0.14	1.3	2.84	3.99
pb	nd	0.90	1.13	2.98
Cd	nd	0.31	0.33	0.71
Ni	nd	0.68	0.70	1.02
CO	nd	0.41	0.82	0.91

Control: fresh water, T_1: wastewater sample collected 1 km from the beginning of the drain,T_2: wastewater sample collected after 10 km from the beginning of the drain, T_3: wastewater sample collected after 19 km from the beginning of the drain. Nd= not detected

Significant decreases in area of leaves and in the FW and DW of shoots and roots were detected in the three studied plants (Table 2); the decreases were positively correlated with the amounts of heavy metals detected in the irrigation water.

Similar results were reported in studies on *H. vulgare* (Argese et al., 2001), *B. campestris* and *A. graveolens* (Yang et al. 2011; Zong et al. 2007). The morphological responses are probably due to high metal concentrations that damaged plant roots and inhibited uptake of nutrients, thus inhibiting normal plant growth. The magnitude of reduction differed among plants, with the maximum inhibition of the shoot system detected in turnip plants and calculated as 64.314% inhibition of the FW and 76.965% of the DW of control plants. On the other hand, the shoot system of lettuce was less affected, with only 14.35% and 18.846% inhibition in FW and DW, respectively, of controls. For the root system, the maximum decrease in DW was detected in tomato plants, followed by turnip and then lettuce, which showed the minimum inhibition in root DW in response to heavy metals. This could be attributable to these three plants having varying rates of uptake and transport of heavy metals and thus differing in their tolerance to heavy metals (Yang et al. 1995).

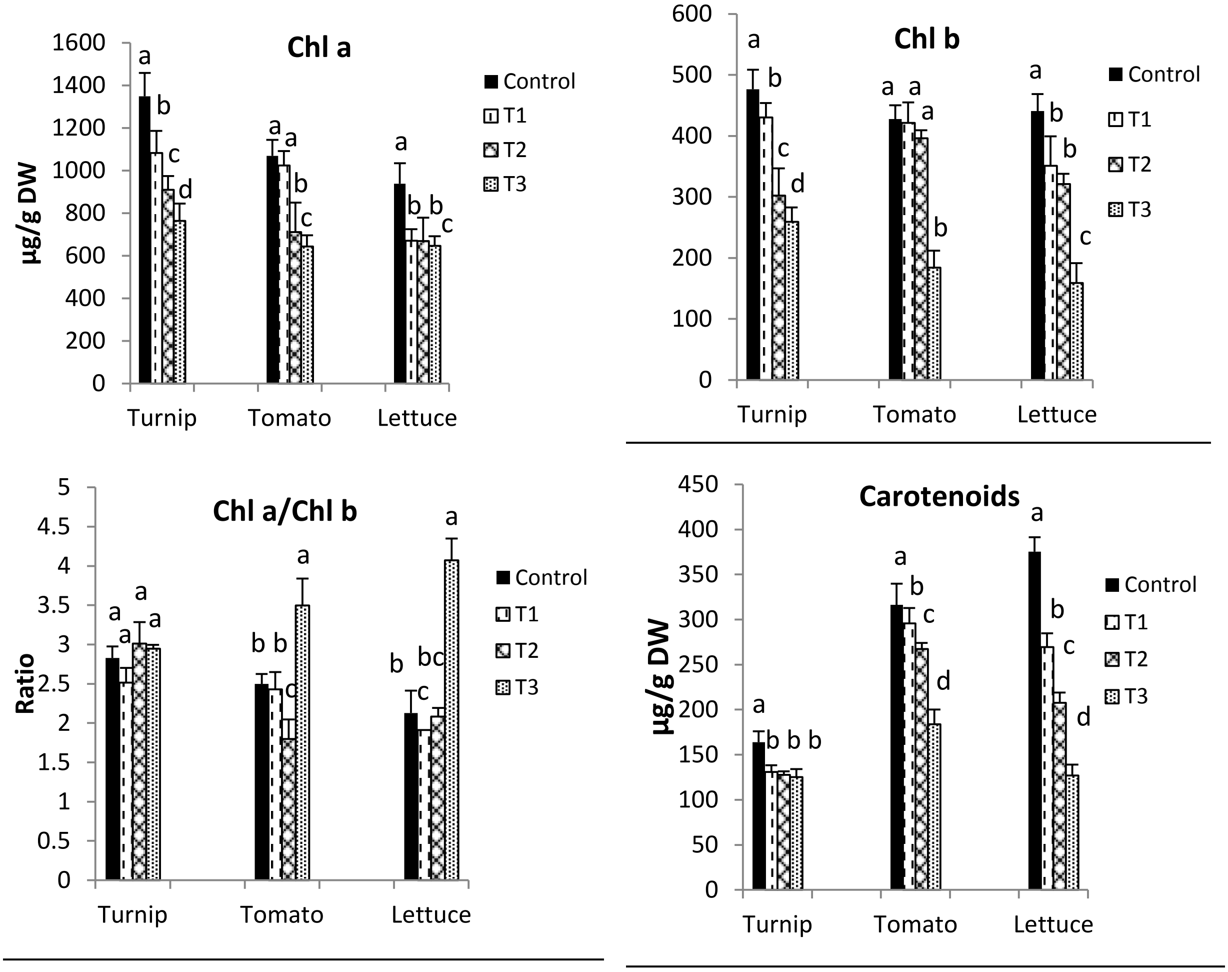
Table 2. Effect of industrial wastewater irrigation on growth parameters of turnip plants (*Brassica napus*), tomato plants *(Solanum lycopersicum*) and lettuce plants (*Lactuca sativa*) in the vegetative growth stage

Turnip plants (*Brassica napus*)

Treatment / Character	Number of leaves	Leaf Area (cm^2)	Shoot system Fresh Weight (g)	Root system Fresh Weight (g)	Shoot system dry Weight (g)	Root system dry Weight (g)
Control	11.4a	162.08a	350.0a	200.0a	199.7a	120.4a
T1	11.0a	134.3a	181.2b	159.0b	102.3b	79.5b
T2	9.8b	133.6a	149.8c	154.0bc	76.7c	66.0c
T3	7.2c	80.4b	124.9d	150.0c	46.0d	54.7d
L.S.D value at 5% level	1.1015	30.398	6.3668	5.2572	5.9575	5.4975
Tomato*(Lyeopersicon esculentum*)						
Control	11.25a	3578a	296.13a	82.5a	211.3a	33.4a
T_1	11.0a	32.23a	276.85b	78.9ab	165.2b	25.5b
T_2	10.75a	29.60a	253.55c	70.90b	146.5c	17.6c
T_3	9.5a	18.05b	215.60d	62.15c	109.6d	13.9c
L.S.D valu at 5% level	2.2895	11.075	16.624	8.5435	7.6275	5.8851
lettuce plant *(Lactuea sativa)*						
Control	11.50a	185.50a	416.38a	96.13a	348.63a	54.58a
*T1	11.00a	154.55b	391.13b	84.10b	317.05b	43.83b
**T2	9.75ab	105.43c	369.00c	71.50c	296.53c	31.50c
***T3	8.50b	69.68d	356.63d	66.00c	282.93d	26.68c
LSD at 5% level	1.8202	26.94	11.035	6.7442	9.8227	7.0079

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 ($p < 0.05$).

In accordance with the growth responses, the studied plants showed a significant and progressive decrease in chl a, chl b, carotenoid and total pigment contents with increase in heavy metal content in the irrigation water (Figure1). The reduction in photosynthetic pigment content may be a phytotoxicity symptom due to heavy metal accumulation. Similar results have been obtained by several workers (Singh et al. 2012; Preeti and Tripathi 2011). In lettuce and tomato, chl b was affected more than chl a by increasing amounts of heavy metals in irrigation water, which resulted in a marked increase in the ch a/ch b ratio in plants treated with water of the highest heavy metal content, compared with fresh water or less-contaminated industrial wastewater. Similar results were obtained by Stiborova et al. (1986). On the other hand, in turnip, both chl a and chl b content were affected almost to the same degree, resulting in no significant change in the ch a/chl b ratio with respect to control plants. In addition, turnip leaves in control plants and those exposed to different levels of pollution contained the lowest content of carotenoids and the highest ratio of total chl/carotenoids of the three types of plants. In this respect, Jampeetong and Brix (2009) concluded that lower chl a+b/carotenoid ratios at high salinity indicate stress and damage to the photosynthetic apparatus.


Chl a
µg/g DW
1600
1400
1200
1000
800
600
400
200
0
Turnip
Tomato
Lettuce
■ Control
◫ T1
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a b c d
a a b c
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Chl b
600
500
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300
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100
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Chl a/Chl b
Ratio
5
4.5
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1
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0
a a a a
b b c a
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Carotenoids
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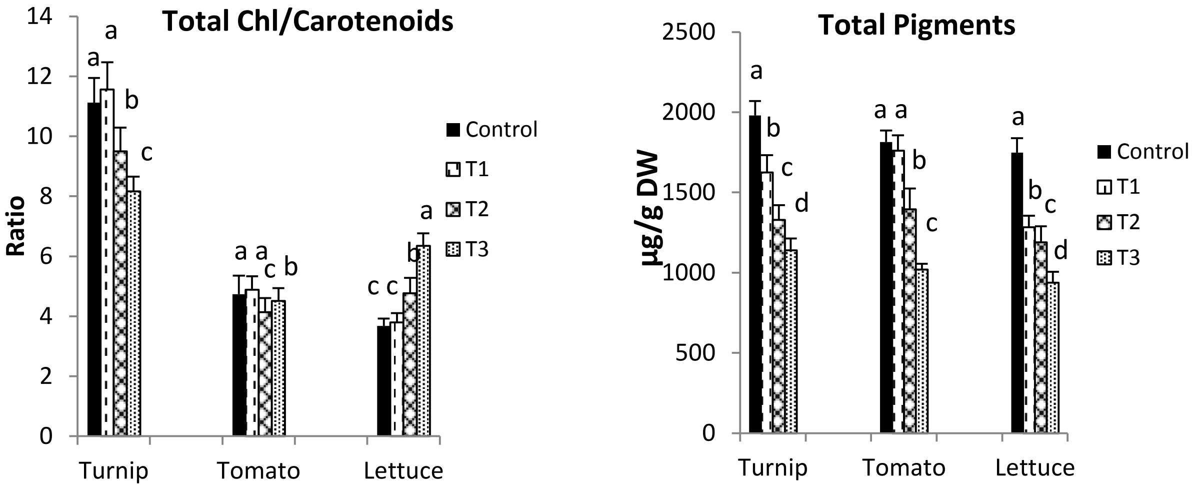
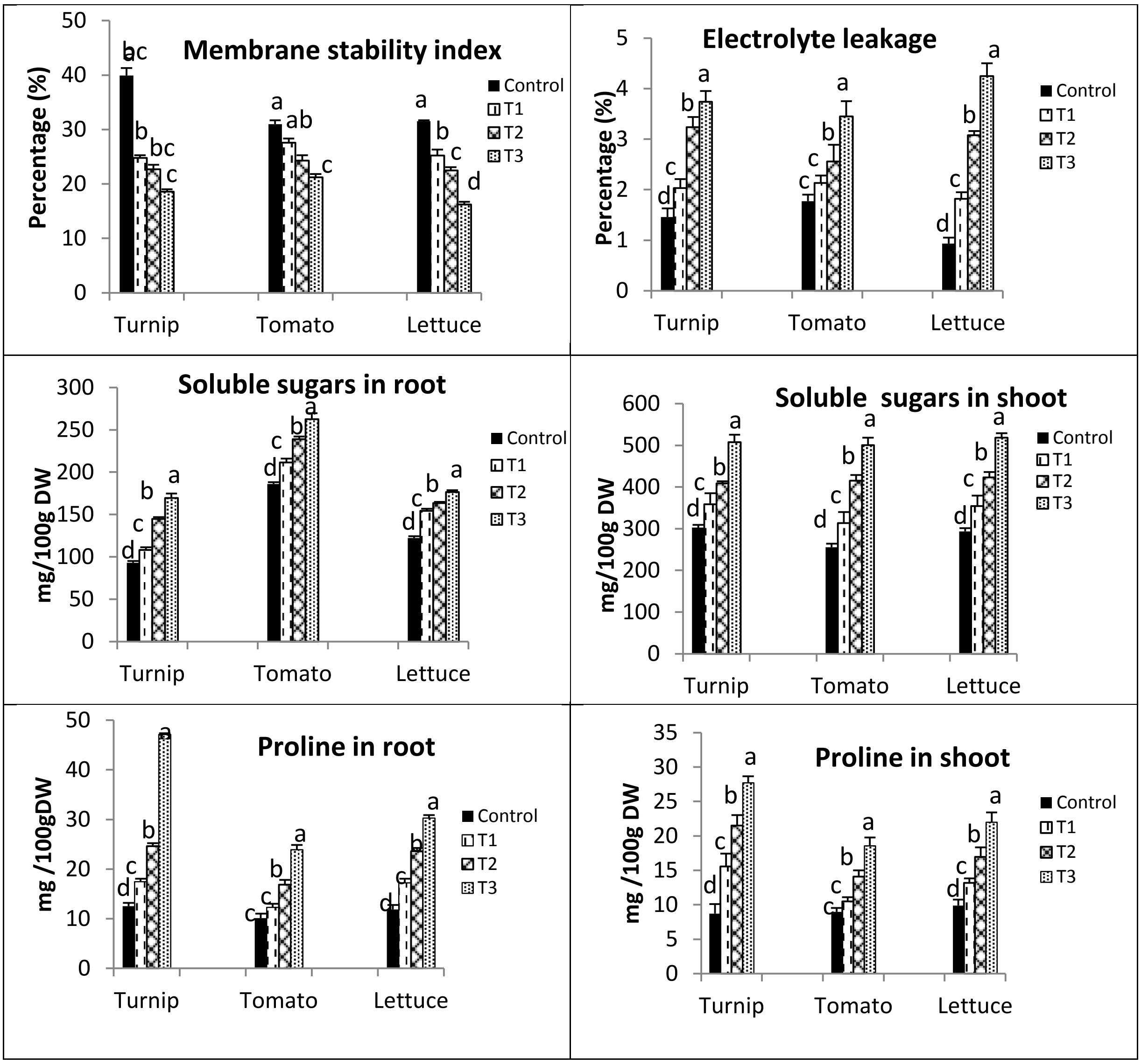


Figure 1. Effect of wastewater irrigation on photosynthetic pigment contents of turnip plant *(Brassica napus),* tomato plant (*Solanum lycopersicum*) and lettuce *(Lactuca sativa).* Control: Plants irrigated with fresh water, T_1: plants irrigated using water collected 1 km from beginning of the drain, 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 ($p < 0.05$).


Membrane stability index
Percentage (%)
0
10
20
30
40
50
Turnip
Tomato
Lettuce
a
bc
b
bc
c
a
ab
c
a
b
c
d
Control
T1
T2
T3
Electrolyte leakage
Percentage (%)
0
1
2
3
4
5
Turnip
Tomato
Lettuce
Soluble sugars in root
mg/100g DW
0
50
100
150
200
250
300
Soluble sugars in shoot
mg/100g DW
0
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200
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Proline in root
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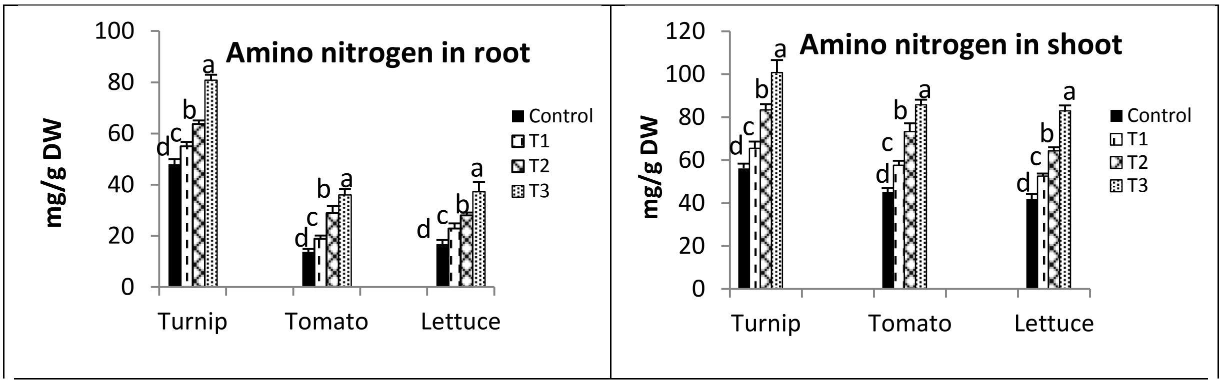

Figure 2. Effect of wastewater irrigation on electrolyte leakage, membrane stability index, soluble sugars, proline and total amino nitrogen contents of turnip, tomato and lettuce plants. Control: Plants irrigated with fresh water, T_1: plants irrigated using water collected 1 km from beginning of the 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 ($p < 0.05$).

Membrane damage can be evaluated indirectly by measuring solute leakage from cells (Ekmekci et al. 2007) and the MSI (Ali et al. 2008). Our results revealed that heavy metals adversely affect the cell membrane of the studied plants, as indicated by the significant decrease in their membrane stability index and the dramatic increase in electrolyte leakage (Figure 2). The deleterious effect of wastewater on the membrane stability index was much more pronounced in turnip plants irrigated with water collected 19 km from the drain and was estimated as 46.6% the value of the controls. The highest electrolyte leakage was detected in lettuce irrigated with water collected 19 km from the drain and was calculated as 4.57-fold the value of the control plants

Heavy metals promote the formation of active oxygen forms in cells (Seregin and Ivanov, 2001). In response to oxidative stress, an increase in antioxidant enzyme activities neutralizes free radicals and peroxides. In the present study, a significant increase in SOD, CAT, POD and APX activities were detected in response to irrigation with wastewater. The magnitude of such an increase was directly proportional to the detected amounts of heavy metals in the wastewater (Table 3). In addition, a significant amount of GSH accumulated in the plants in response to all treatments, with the highest accumulation in plants irrigated with wastewater collected 19 km from the drain; relative to controls of the same species, the accumulation was highest in tomato (130.56%), intermediate in lettuce (122.56%), and lowest in turnip (119.31%). The induction of the synthesis of glutathione – a substrate in the synthesis of phytochelatins – was observed in plants in response to heavy metals (Yadav 2010). In contrast, the amount of detected ASC decreased in plants irrigated with contaminated wastewater relative to control plants. Madhava Rao and Sresty (2000) also reported that the ASC content of roots and shoots of two pigeon pea cultivars showed a significant negative correlation with increasing concentrations of metal ions (Zn and Ni). Such a decrease in ASC content along with the significant increase in the activities of antioxidant enzymes led us to postulate that a constitutively high antioxidant capacity or increase in the levels of one or more antioxidants could prevent oxidative damage and improve resistance to oxidative stress (Sharma et al. 2012).

Table3. Effect of wastewater irrigation on antioxidant system of turnip plants (*Brassica napus*), tomato plants (*Solanum lycopersicum*) and lettuce plants (*Lactuca sativa*).

Turnip (*Brassica napus*)						
Treatment	Antioxidant enzymes				Antioxidant compounds	
	SOD (unit/g FW)	CAT (unit/g FW)	POD (unit/g FW)	APX (unit/g FW)	GSH (unit/g FW)	ASC (nmol/mg FW)
Control	381.11d	201.00d	37.11d	46.12d	118.21d	2.89a
T_1	508.31c	361.71c	46.19c	68.10c	124.24c	2.18b
T_2	712.14b	503.73b	63.37b	84.36b	133.55b	1.72c
T3	1082.71a	700.16a	73.16a	116.71a	141.04a	1.14d
LSD ($p < 0.05$)	1.6	1.4	1.5	1.5	1.5	0.459
Tomato (*Solanum lycopersicum*)						
Control	222.18d	672.1d	29.70d	8.61d	126.62d	3.16
T_1	401.50c	866.7c	48.71c	14.31c	137.87c	2.27b
T_2	512.73b	971.3b	73.44b	24.70b	149.26b	1.99c
T3	723.14a	1173.9a	92.61a	38.13a	165.32a	1.30d
LSD ($p < 0.05$)	1.4	1.5	1.3	1.5	1.6	0.487
Lettuce (*Lactuca sativa*)						
Control	107.13d	312.70d	51.71d	16.71d	121.52c	3.03a
T_1	292.16c	490.16c	73.16c	22.14c	132.38b	2.23b
T_2	403.91b	700.61b	90.33b	34.11b	142.13a	1.90c
T3	619.12a	903.33a	140.31a	46.91a	148.94a	1.22d
LSD ($p < 0.05$)	1.6	1.4	1.5	1.3	1.5	0.475

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 ($p < 0.05$).

We observed significant increases in proline, total amino nitrogen and soluble sugars in both shoots and roots of plants treated with wastewater as compared with control plants. The magnitude of the increase was directly proportional to the level of heavy metals in the water (Figure 2), which can be regarded as an important adaptive response of plants to overcome metal toxicity**.** Proline, sugar and other organic solutes are believed to improve metal tolerance by contributing to osmosis and preserving enzyme activity in the presence of toxic ions (Singh 2012).

CONCLUSIONS

We found that industrial factories adversely affected the water of the El-Amia drain; hence, the tomato, lettuce and turnip plants grown in water obtained near the drain demonstrated harmful effects on their growth, cell membrane and photosynthetic pigments. In addition, the biochemical defenses of these plants against heavy metals are, at least in part, through the synthesis of osmolytes and activation of the antioxidant defense system. The positive correlation of our measurements of the stimulation of the antioxidant system and osmoprotectant levels with the amounts of heavy metal in irrigation water proved that these two measurements are efficient biomarkers for the degree of water pollution with heavy metals.

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الري بمياه الصرف الصناعي يحفز انتاج مضادات الاكسده والحوافظ الازموزيه في نباتات الخس واللفت والطماطم.

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المستخلص

تناولت الدراسه تأثير الري بمياه الصرف الصناعي الناتج من مصرف العاميه الواقع بين منطقتى كفر الدوار (محافظة البحيرة)وأبو قير(محافظة الاسكندريه) على دالات النمو ، أغشية الخلايا، محتوى أصباغ البناء الضوئي ، و مضادات الأكسدة و بعض الحوافظ الأزموزيه (البرولين، المحتوي اليتروجيني الأميني والسكريات الذائبه) في ثلاثه محاصيل للخضر: اللفت، الخس و الطماطم. ولهذا الغرض، تم جمع عينات مياه من الكيلو 1 و 10 و 19 من بداية مصرف العاميه علما بأن طول المصرف حوالى 20 كم واستخدامها في الري، مع المياه النقيه كمجموعة ضابطه. وقد وجد أن مياه الصرف الصناعي تحتوي على كميات كبيرة من المعادن الثقيلة (الرصاص، الكادميوم، النيكل والكوبالت). كما ادي الري بمياه المصرف الي حدوث انخفاض كبير في مساحة الأوراق، الوزن الطازج والوزن الجاف للمجموع الجذري والخضري وقد كان ذلك متزامنا مع انخفاض ملحوظ في محتوى أصباغ البناء الضوئي وتدمير لأغشية الخلايا (استدل علي ذلك من انخفاض ثبات الغشاء وزياده تسربيه للايونات) وقد كان هذا أكثر وضوحا باستعمال الماء عند الكيلو 19 من بدايه المصرف. ايضا اوضحت نتائج الدراسه حدوث زياده معنويه في أنشطة الانزيمات المضادة للأكسدة ،ومحتوي كل من الجلوتاثيون، والبرولين و السكريات الذائبه و النيتروجين الكلي الأميني في النباتات التي تم ريها بمياه الصرف الصناعي مقارنه بالمجموعه الضابطه التي تم ريها بالماء العذب مما يدل علي انها بمثابه اليات دفاع مستحثه من النبات لمواجهه التعرض لاجهاد المعادن الثقيله.