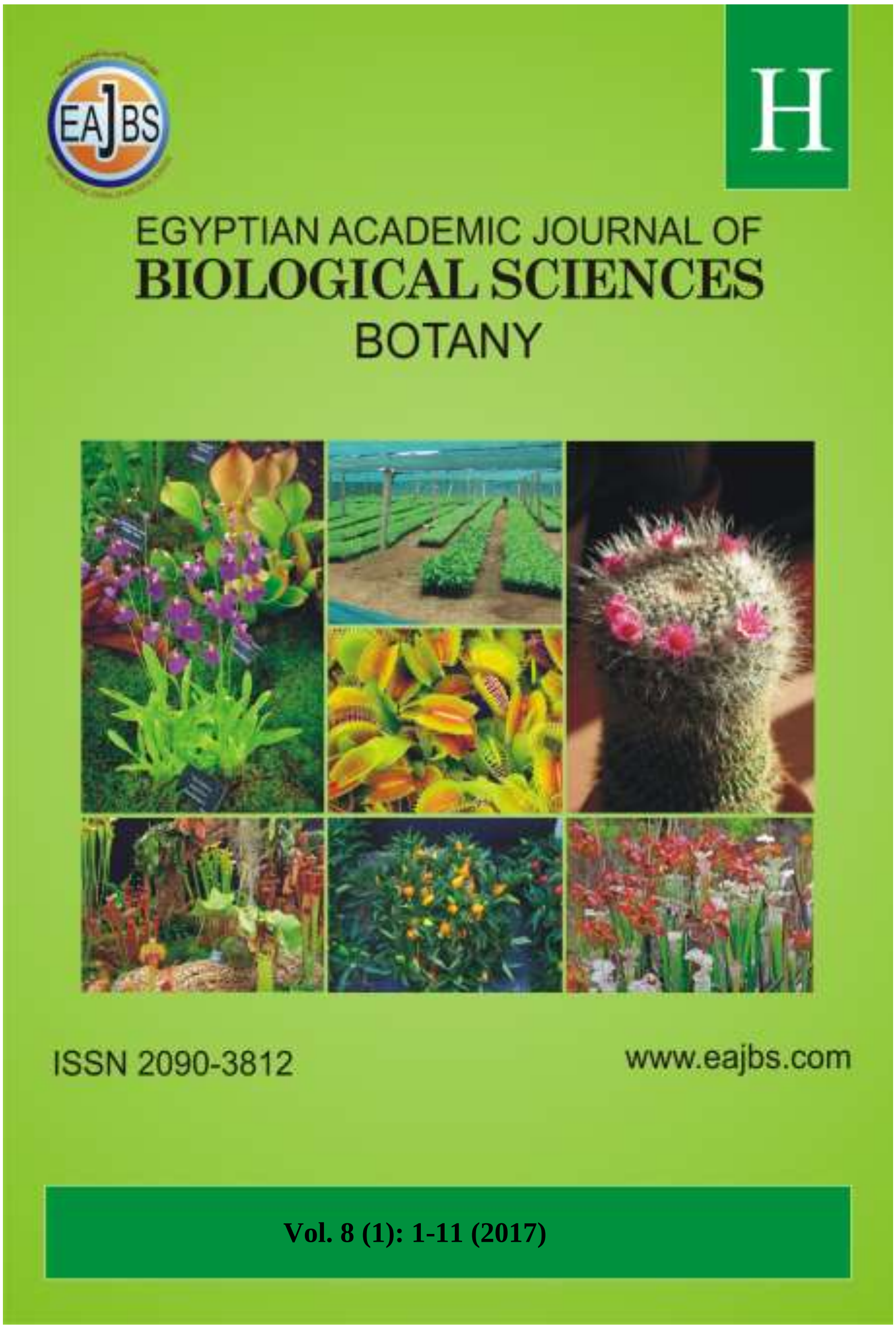

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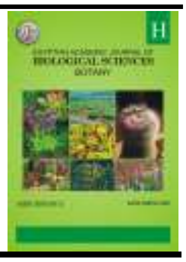

Non-enzymatic antioxidants and transaminases constitute homeostatic mechanisms for some Egyptian vegetables irrigated with treated wastewater

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ABSTRACT

To evaluate the effect of irrigation with treated wastewater on some Egyptian leafy vegetables, some non-enzymatic antioxidants and transaminases were determined. This study was conducted at wastewater irrigated site (Arab Al-Olyqat), 30 kilometers from Cairo, while 3 kilometers was the distance between control (Nile River canal, fresh water, irrigated site; Shibeen Al-Kanater) and treated wastewater irrigated site. Concentrations of total phenols and L-ascorbic acid were significantly decreased in parsley and spinach. Total phenols of cabbage and L-ascorbic acid of garden rocket were significantly decreased, while those of lettuce were significantly increased compared to their controls due to irrigation with treated wastewater. Irrigation with treated wastewater resulted in significant increases of proline in cabbage and parsley and significant decreases in garden rocket and spinach. Free thiol compounds showed significant decreases in spinach, parsley and cabbage, while significant increases in garden rocket and lettuce were recorded. Vegetables irrigated with treated wastewater showed significant increases in the activity of GOT or (AST) compared to their controls. Activity of GPT increased significantly in lettuce by 111.6 % and decreased in parsley by 40.0% due to irrigation with treated wastewater.

INTRODUCTION

To face the growing demand for irrigation water, non-conventional resources are often used (Mapanda *et al*., 2005). The demand for water is increasing due to population growth and urbanization, hence making these resources very scarce. Along with the high usage of water comes the problem of large quantities of wastewater (Amarteifio *et al*., 2006). Uncontrolled use of wastewater for irrigation can result in accumulation of salts and heavy metals in soil and may affect plant growth adversely. Thus contamination of food-chain through uptake by plants may occur (Malik *et al*., 2004). At the sewage treatment plants (STPs), the influent is "purified" to a certain quality before being released into the environment (Kiziloglu *et al*., 2006).

The major advantage of conventional STPs seems to be reduction of only the organic loads, while these remain ineffective in reducing the levels of soluble metals and other ions, except those might be retained as a part of sludge generated in these STPs (Yadav *et al*., 2003).

Plants possess homeostatic mechanisms that allow them to keep correct concentrations of essential metal ions in cellular compartments and to minimize the damaging effect of an excess of nonessential ones (Michalak, 2006). Cellular antioxidants (thiols, carotenoids, ascorbate, proline, etc.) may also play an important role in inducing resistance in plants to metals by protecting labile macromolecules against attack by free radicals which are formed during various metabolic reactions leading to oxidative stress (Kumar *et al*., 2002; Belkadhi *et al*., 2016). Green leafy vegetables (GLVs) hold an important place in well-balanced diets (Osler *et al*., 2001). They have long been recognized as the cheapest and most abundant potential sources of vitamins and minerals. The reports offer information on medicinal properties and therapeutic value of GLVs like anti-diabetic, anti-histaminic, anti-carcinogenic and hypolipidemic- antibacterial activity (Kubo *et al*., 2004; Shyamala *et al*., 2005). They are rich sources of antioxidant vitamins and pigments such as vitamin C, E and β-carotene. Phenolic agents are a major class of antioxidants that are found in plant foods, at very high concentration (Larrosa *et al*., 2002; Raju *et al*., 2007). Ascorbic acid can serve as a free radical scavenger (El-Khatib, 2003; Dresler and Maksymiec, 2013).

In reviewing the available literature, it showed lack of information about transaminases in vegetables; glutamic oxaloacetic transaminase (GOT) or aspartate transaminase [AST, EC 2.6.1.1] and glutamic pyruvic transaminase (GPT) or alanine transaminase [ALT, EC 2.6.1.2]. However, in the experimental animals, these enzymes were greatly affected as a measure of liver function disorder (Aly *et al*., 2002). The release of these enzymes from the injured tissues depends not only upon the abundance of the enzyme but also on its location within the cell and the firmness with which it is held. In plants, transaminases participate very effectively in transformations of nitrogen compounds. They are important for the synthesis of amino acids from oxo-acids in the citrate cycle and for other crucial biochemical pathways. They also play key roles in the synthesis of secondary metabolites as well as chlorophyll (Krystofova *et al*., 2009). This work aimed to evaluate the adaptive mechanisms of some leafy vegetables irrigated with treated wastewat; some non-enzymatic antioxidants and activity of certain transaminases were estimated.

MATERIALS AND METHODS

Materials:

Study sites: This study was conducted at wastewater irrigated site (Arab Al-Olyqat), 30 kilometers from Cairo, while 3 kilometers was the distance between control (Nile River canal, fresh water, irrigated site; Shibeen Al-Kanater) and treated wastewater irrigated site. Al- Gabal Al- Asfar sewage treatment plant (STP) of 1.2 million cubic meters per day capacity was (installed in 1998) the source for treated wastewater irrigation. Large- scale production of vegetables was conducted in this area, largely, to supply markets in the city.

Plants: Vegetables used in this study were *Brassica oleracea var. capitata* (cabbage) family: Brassicaceae, *Eruca sativa* (garden rocket) family: Brassicaceae, *Lactuca sativa var. longifolia* (Romaine lettuce) family: Asteraceae, *Spinacia oleraca* (spinach) family: Amaranthaceae and *Petroselium crispum* (parsley) family:

Apiaceae. The Egyptian cultivar Baladi was used for all the studied vegetables. Seeds were purchased from Agricultural Research Center, Giza, Egypt. They were planted and harvested in the winter (within the period from October to February). Thirty days after sowing (DAS), both cabbage and lettuce were irrigated four and five times, respectively. For spinach and parsley, first irrigation was seven DAS. They received four and six irrigations, respectively. Garden rocket irrigated three times, fifteen DAS. For all plant species, foliar spraying with nileflor (nutrient solution) was applied 15 DAS. Samples of vegetables were harvested at marketable stages of development. They were put in polyethylene bags in the field, and were transferred to the laboratory in ice box for sample processing.

Methods:

Non- enzymatic antioxidants:

Total phenol: Extraction of total phenols was carried out according to Bahorun *et al*. (2004). Total phenols were determined by the method of Singleton and Rossi (1965) using the Foline-Ciocalteu reagent. Results were expressed in µg gallic acid g^{-1} fresh weight. L-Ascorbic acid (vitamin C) was extracted as described by Eitenmiller and Landen (1999) and estimated according to the method of Bajaj and Kaur (1980). The concentration of ascorbic acid was then determined by references to the calibration graph and was expressed as µg / ml. Free proline was estimated according to the method of Singh *et al*. (1973). The proline concentration was estimated from a standard curve constructed using authentic proline in a concentration range of 50-500 m M. The results were expressed as µg /g fresh weight. Free thiols were determined according to the method of Ellman (1959). The results were expressed as µM/g fresh weight.

Transaminases (GOT and GPT): The activity of enzymes; glutamic oxaloacetic transaminase [GOT, EC 2.6.1.1] or aspartate transaminase (AST) and glutamic pyruvic transaminase [GPT, EC 2.6.1.2] or alanine transaminase (ALT) were determined according to Reitman and Frankel (1957).

The principle of this method catalysis the following reaction:

$$\text{L-aspartate} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{GOT}} \text{L-glutamate} + \text{oxalacetate.}$$

The oxalacetate formed may be assayed by the formation of 2, 4- dinitrophenyl-hydrazine which yields a reddish brown color. A standard curve was made for GOT (AST) calculation ranging from 15 to 60 Unit/ ml.

The principle of this method catalysis the following reaction:

$$\text{DL-alanine} + \alpha\text{-ketoglutarate} \xrightleftharpoons{\text{GPT}} \text{L-glutamate} + \text{pyruvate.}$$

The pyruvate formed may be assayed by the formation of 2, 4- dinitrophenyl-hydrazine which yields a reddish brown color in alkaline medium. A standard curve was made for GPT (ALT) calculation ranging from 10 to 55 Unit/ ml.

Statistical analysis: The data were subjected to one way ANOVA to compare each plant with the remaining plants (for the same treatment) using SPSS software version 10 (SPSS, Richmond VA, USA) as described by Dytham (1999). Significant differences, between control and treated wastewater irrigated plants, were found at $p > 0.05$ by Tukey's test (t-test).

RESULTS AND DISCUSSION

Non- enzymatic antioxidants:

Total phenols

Polyphenols are quantitatively the main dietary antioxidants and posses higher, *in vitro*, antioxidant capacity than vitamins and carotenoids (Gardner *et al*., 2000).

There was a significant variation in total phenol concentrations among the studied vegetables under control conditions. Also, there were significant differences in the concentration of total phenols in plants irrigated with treated wastewater with the exception of that between lettuce and parsley. Spinach was the most abundant with phenolic compounds under both conditions. Irrigation with treated wastewater resulted in a significant decrease in the concentration of total phenols in each of cabbage, parsley and spinach, while that of lettuce was significantly increased compared to their controls (Table 1). Decline in the concentration of phenols in tested vegetables could be totally or partially due to its consumption while acting as antioxidant.

The trend of increasing and decreasing of phenols in tested vegetables was approximately similar to that of ascorbic acid. It can be concluded that phenols act with ascorbic acid and peroxidase in the H_2O_2- scavenging, phenolic/ ASC/ POD system. This is supported by the results of Michalak (2006). Also, metal ions may influence the nature of plant phenolics, *in vivo*, by altering the life time of phenoxyl radicals. The toxicity of metals such as Al^{3+}, Cd^{2+} and Zn^{2+} retained in the root apoplast could be explained in this way (Yamasaki and Grace, 1998). Singh and Agrawal (2010) stated that phenol content decreased in rice (*Oryza sativa*) plants grown at different rates of sewage sludge amendment. They concluded that this might be a good option as rice plant has adequate heavy metal tolerance mechanism shown by increased rate of photosynthesis and chlorophyll content, decreased phenols and various antioxidant levels. The results of Gupta *et al*. (2010) showed slight increases in phenol content in the studied plants; colocasia (*Colocasia esculentum),* mustard *(Brassica nigra),* and radish (*Raphanus sativus*). An increase in phenol concentration was commonly observed after exposure of plants to polluted environments (Duĉić *et al*., 2008). Also, there have been many reports about induced accumulation of phenolic compounds in plants treated with high concentrations of metals (Michalak, 2006). Rastgoo and Alemzadeh (2011) stated that different concentrations (50 and 100 micro M) of Cd, Co, Pb and Ag for one week significantly increased the amount of phenolic compounds in *Aeluropus littoralis* plants due to inductive oxidative stress and heavy metal toxicity because of the accumulation of reactive oxygen species in the treated plants.

L- Ascorbic acid (vitamin C)

Ascorbic acid plays an important role in δ-tocopherol regeneration, which has been reported to act as the primary antioxidant (Bortoli *et al*., 1997). It is an ubiquitous soluble antioxidant in photosynthetic organisms, and the most important reducing substrate for H_2O_2 detoxification (Singh *et al*., 2005; Jozefczak *et al*., 2015). Concentrations of L-ascorbic acid were significantly decreased in garden rocket, parsley and spinach due to irrigation with treated wastewater. On the contrary, L-ascorbic acid was significantly increased in lettuce irrigated with treated wastewater compared to the control (Table 1). Decline in the concentration of ascorbic acid in tested vegetables could be totally or partially due to its consumption while acting as antioxidant to limit lipid peroxidation (Rai *et al*., 2004). Yong *et al*. (2010) studied the effect of multiple heavy metal stress on ascorbate in the leaves, stems and roots of

Kandelia candel and *Bruguiera gymnorrhiza*. Initial increase and subsequent decrease in response to heavy metal stress was occurred. On the contrary, Guo *et al*. (2005) found that ascorbic acid increased marginally in all wastewater-irrigated rice plants than control. Ascorbic acid as an antioxidant plays an important role in protection against physiological stress. Heavy metals bring about a varied response in plants (El-Beltagi and Mohamed, 2010; Singh and Agrawal, 2010). The effect of toxicants varies from species to species depending upon several factors (Barman and Lal, 1994).

Table 1: Effect of irrigation with treated wastewater on total phenols (µg / g fresh wt.) and L-ascorbic acid (µg / ml) in studied vegetables.

Plant	Phenols (µg / g fresh wt.)			L-Ascorbic acid (µg / ml)		
	Control	Treated	Chang (%)	Control	Treated	Chang (%)
Cabbage	6.86 ± 0.21 e	5.82 ± 0.29 d*	-15.2	42.43 ± 2.61 c	37.67 ± 0.45 d	-11.2
Garden rocket	15.23 ± 0.49 c	13.70 ± 0.78 c	-10.0	288.00 ± 1.74 a	184.38 ± 1.66a*	-36.0
Lettuce	10.37 ± 0.19 d	18.49 ± 0.22 b*	78.3	29.45 ± 1.70 c	89.14 ± 0.66 c*	202.7
Parsley	23.55 ± 0.52 b	17.10 ± 0.95 b*	-27.4	188.49 ± 13.7b	134.11± 9.91b*	-28.8
Spinach	33.80 ± 1.43 a	24.64 ± 0.15 a*	-27.1	286.09 ± 2.01 a	185.52 ± 2.02a*	-35.1

Data are mean of three replicates, ± standard error
Means in the same column having the same letter are not significantly differed at 5%.
*Significant differences, between control and treated wastewater irrigated plants, were found at $p< 0.05$ by Tukey's test (t- test)

Free proline

Proline concentrations were significantly differed among control plants. Cabbage possessed the highest value. Plants irrigated with treated wastewater also recorded significant differences in their proline concentration, except that for garden rocket and spinach. Irrigation with treated wastewater resulted in significant increases of proline in cabbage and parsley and significant decreases in garden rocket and spinach. Lettuce showed non-significant decrease of proline concentration compared to control plants (Table 2). Sharp reduction of proline concentration in treated garden rocket and spinach might be due to the damage by high levels of heavy metals and excessive salts to the defense system of these plants (Noctor *et al*., 1998), leading to an increase in proline degradation or /and a reduction in proline biosynthesis. In this study, consumption of glutamate by GOT for other amino acid production may restrict proline biosynthesis in such plants. These results are supported by Tantrey and Agnihotri (2010) who stated that nitrogen application reduced proline content in gram plants.

At higher concentrations (25µmol /l) proline was reduced to 15% and 14% compared to the control under Cd and Hg treatments. Significant increases of proline in cabbage and parsley were supported by the results of Zengin and Munzuroglu (2005). Rai *et al*. (2004) reported that many environmental stresses have been reported to increase the level of proline in plants, such as heavy metals, UV radiation, temperature, salinity and drought. Higher accumulation of proline in chromium-treated plants of *Ocimum tenuiflorum* has been observed which might be attributed to the strategies adopted by plants to cope with chromium toxicity as proline has multiple functions, such as, osmoticum, scavenger of free radicals, protector role of cytoplasmic enzymes, source of nitrogen and carbon for post stress growth, stabilizer of membranes, machinery for protein synthesis and a sink for energy to regulate redox potential (Hayat *et al*., 2012).

Bohnert *et al*. (1995) reported that the accumulation of proline may be due to increased synthesis from glutamate, lower rate of protein oxidation and slowed incorporation of proline into protein. Increase in protein content under heavy metal stress in *Spirulina platensis* indicated that it may contain a larger proportion of proline. Binding with metal ions due to the chelating ability of proline can also be a defense mechanism for survival (Choudhary *et al*., 2007).

Free thiols

Although many metals are essential, all metals are toxic at higher concentrations, because they cause oxidative stress by formation of free radicals. Antioxidants like cysteine and non-protein thiol (sulfhydryl) play an important role in detoxification of toxic metal ions (Singh and Sinha, 2005). With respect to control plants, there were no significant changes in free thiol concentration of garden rocket, lettuce and parsley, while a significant change in the free thiols of either cabbage or spinach was detected when compared to each other or with the previously mentioned plants. There were significant changes in the concentration of free thiols among the treated plants. Cabbage seemed to be the least compared to the other vegetables. Irrigation with treated wastewater resulted in a significant decrease in free thiol concentration of spinach, parsley and cabbage, while caused a significant increase in free thiol compounds of garden rocket and lettuce (Table 2).

Table 2: Effect of irrigation with treated wastewater on proline (µ g/ g fresh wt.) and free thiols (µM/ g fresh wt.) in studied vegetables.

Plant	GOT (U / ml)			GPT (U / ml)		
	Control	Treated	Chang (%)	Control	Treated	Chang (%)
Cabbage	17.09 ± 67.07a	2742.75 ± 66.91a*	60.4	0.03 ± 0.00 c	0.02 ± 0.00e*	-33.3
Garden rocket	784.00 ± 3.21d	55. 33 ± 1.33 d*	-92.9	0.28 ± 0.01 b	1.32 ± 0.00b*	371.4
Lettuce	225.12 ± 2.78e	196.39 ± 10.10 c	-12.3	0.34 ± 0.00b	1.43 ± 0.01a*	320.6
Parsley	1178.66 ± 18.05b	1560.00 ± 8.82 b*	32.3	0.33 ± 0.02b	0.16 ± 0.00d*	-51.5
Spinach	899.67 ± 34.31c	7.17 ± 0.44d*	-99.2	2.29 ± 0.13 a	0.83 ± 0.02 c*	-63.7

Data are mean of three replicates, ± standard error

Means in the same column having the same letter are not significantly differed at 5%.

*Significant differences, between control and treated wastewater irrigated plants, were found at $p< 0.05$ by Tukey's test (t- test).

In characterization of a cadmium binding complex of cabbage leaves, Wagner (1984) reported that it had high acidic and low hydrophobic residue and lacking in disulfide bonds. Khidr and Nour El-Din (2005) revealed that under 20 ppm Cd treatment, the percentage of increase in free-SH of wheat flour reached 73.9%, while that of S-S bond was only 9.7%, indicating reduction of S-S into reactive -SH for Cd-binding. The thiol group of cysteine is strongly nucleophilic, a feature which defines its functions for stress responses (Warrilow and Hawkesford, 1998). The reconversion of oxidized thiol group of cysteine to the reduced form is essential in redox potential of control, important in all stress responses. Within the plant cell heavy metals may trigger the production of oligopeptide ligands known as phytochelatins (PCs) and metallothioneins (MTs). These peptides bind and form stable complexes with the heavy metal and thus neutralize the toxicity of the metal ions. Khan *et al*. (2009)

stated that plants adopt several mechanisms of Cd detoxification, such as accumulation of sulfur-rich compounds, like glutathione (GSH) and its precursor cysteine (Jozefczak *et al*., 2015). Malec *et al*. (2010) investigated the physiological responses of *Lemna trisulca* L. (Duckweed) to the lowest (1 micro mol) and to the highest (100 micro mol) Cd concentration. The results showed that total soluble thiols drastically increased. On the other hand, chromium significantly reduced the non-protein thiol (NP-SH) contents of *Ocimum tenuiflorum* leaves. The maximum inhibition in NP-SH (60.87%) was recorded when plants were treated with 100 µM chromium for 72 h (Rai *et al*., 2004).

Transaminases:

There is still not much available information about the significance of some commonly analyzed enzymes as markers of stress reactions in plants. Aminotransferases can participate in plant stress reactions (Hubalek *et al*., 2007; Vitecek *et al*., 2007). Thus, we focused our attention on the activities of GOT and GPT. Vegetables irrigated with treated wastewater showed significant increases in the activity of GOT or (AST) compared to their controls. The highest percentage of increase was recorded by spinach, while the lowest one was that for parsley. Increase in the activity of GOT may suggest its involvement in supplying the plants with this amino acid under stress. On the other hand, there was no significant variation in the activity of GPT or (ALT) between control and treated plants except those for lettuce and parsley. Activity of GPT increased in lettuce by 111.6 % and decreased in parsley by 40.0% due to irrigation with treated wastewater (Table 3).

Table 3: Effect of irrigation with treated wastewater on GOT and GPT (U / ml) in studied vegetables.

Plant	GOT (U / ml)			GPT (U / ml)		
	Control	Treated	Chang (%)	Control	Treated	Chang (%)
Cabbage	40.33 ± 0.60 c	52.83 ± 3.92 e*	31.0	40.00 ± 1.80 b	43.50 ± 1.32 a	8.7
Garden rocket	64.00 ± 0.87c	112.33 ± 12.94 d*	75.5	48.57 ± 18.44 b	57.17 ± 1.01 c	17.7
Lettuce	203.33 ± 3.087 a	328.67 ± 0.88 a*	61.6	167.33 ± 2.92 a	354.17 ± 1.42 b*	111.6
Parsley	225.67 ± 18.68 a	284.17 ± 1.59 b*	25.9	176.67 ± 10.27 a	106.00 ± 3.77 c*	-40.0
Spinach	109.83 ± 3.18 b	205.67 ± 2.20 c*	87.3	63.17 ± 5.46 b	57.67 ± 1.88 c	-8.7

Data are mean of three replicates, ± standard error

Means in the same column having the same letter are not significantly differed at 5%.

*Significant differences, between control and treated wastewater irrigated plants, were found at $p < 0.05$ by Tukey's test (t- test).

These results are in agreement with the results of Krystofova *et al*. (2009) who found that AST and ALT activities, in roots of sunflower plants (*Helianthus annuus*) treated with 0, 10, 50, 100 and 500 µM Pb-EDTA for eight days, were increased during the experiments in comparison with control plants. This increase corresponds well with the higher metabolic activity. Our results are contradictory to the results of Gajewska *et al*. (2009) who indicated that, starting from the fourth day the activity of GOT remained unaltered after Ni application (50 and 100 micro M) but that of GPT showed a considerable enhancement in the wheat roots. Increase in the glutamate-producing activity of GPT may suggest its involvement in supplying the wheat roots with this amino acid under Ni stress.

CONCLUSION

Glutamate is the precursor of proline, the substrate for GOT and GPT and a constituent of short chain peptides; phytochelatins and metalothioneins. So, a great induction for one of them may lead to a reduction in one or more of the others. Concentration of proline was significantly decreased by 99.2 and 92.9 %, while activity of GOT was significantly increased by 87.3 and 75.5% in both spinach and garden rocket, respectively. As the concentration of proline was significantly increased by 60.4 and 32.4 % in both cabbage and parsley, the percent of increase in their GOT activity was only 31.0 and 25.9 %, respectively. Lettuce belongs to one of the heavy metals hyper-accumulating families; Astraceae.

It possessed several defense mechanisms; the only significant increase in total phenols, L- ascorbic acid and GPT as well as the second and third percent of increase in free thiol and GOT, respectively was recorded in that plant. It achieved 20.9% increment in photosynthetic capacity and hence significant increase in vegetative yield (data not shown). Lettuce can accumulate heavy metals in plant tissues without showing toxicity symptoms.

All plant species, except lettuce, showed reduction in total phenols and L-ascorbic acid. It can be concluded that phenols act with ascorbic acid and peroxidase in the H_2O_2-scavenging, phenolic/ ASC/ POD system. So, depending on species of the plant and nature of the stress, the plant will respond in different ways.

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ARABIC SUMMARY

مضادات الاكسدة غير الإنزيمية والإنزيمات الناقلة لمجموعة الأمين تُشكّل آليات توازنية لبعض الخضروات المصرية المروية بمياه صرف معالجة

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نفذت هذه الدراسة في موقعين الأول بقرية عرب العليقات و يروي بمياه الصرف المعالجة بمحطة الجبل الأصفر ويبعد حوالي ٣٠ كيلومتر عن القاهرة والثاني بمدينة شبين القناطر ويروي بمياة النيل(كنترول) ويبعد عن الموقع الأول حوالي ٣ كيلومتر. وكان الهدف من الدراسة هو بيان التأثيرات الفسيولوجية في بعض الخضروات (الكرنب – الجرجير- الخس- البقدونس – السبانخ) كنتيجة للري بمياة الصرف الصحي المعالجه.

لوحظ إنخفاض معنوى فى تركيز الفينولات الكلية فى نباتات الكرنب، البقدونس والسبانخ بمقدار ١٥,٢%، ٢٧,٤% و٢٧,١% على الترتيب، بينما لوحظ زيادة معنوية فى نبات الخس بمقدار ٧٨,٣% عنها فى النباتات الكنترول. حدث انخفاض معنوى فى تركيز حمض الاسكوربيك فى نباتات الجرجير، البقدونس والسبانخ المعالجة بمقدار٣٦%، ٢٨,٨% و ٣٥,١% على الترتيب وذلك نتيجة الرى بمياه الصرف المعالجه. وعلى العكس، لوحظ زيادة معنوية فى تركيز حمض الاسكوربيك بمقدار ٢٠٢,٧% فى نبات الخس المعالج عنها فى الكنترول.

ادى الرى بمياه الصرف المعالجة الى زيادة معنوية للبرولين فى كل من نباتي الكرنب والبقدونس بينما أدى إلى انخفاض معنوى فى نباتي الجرجير والسبانخ.

أظهرت النتائج ان الرى بمياه الصرف المعالجة أدى إلى انخفاض معنوى فى تركيز المركبات الكبريتية فى كل من نباتات السبانخ، البقدونس والكرنب بمقدار ٦٣,٧%، ٥١,٥% و٣٣,٣% على الترتيب، بينما حدثت زيادة معنوية لمركبات الكبريت الحر فى نباتات الجرجير و الخس .

أدى رى الخضروات بمياه الصرف المعالجة إلى زيادة معنوية فى نشاط انزيم GOT عنها فى الكنترول وقد زاد نشاط انزيم GPT معنويا فى نبات الخس ونقص معنويا فى البقدونس.