

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



H

EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
BOTANY



ISSN 2090-3812

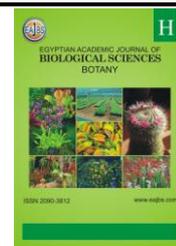
www.eajbs.com

Vol. 8 No.1 (2017)

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences , Department of Entomology ,Faculty of Sciences Ain Shams University .

The Botany Journal publishes original research papers and reviews from any botanical discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematic, morphology, evolution, control of herbs, arachnids, and general botany..

www.eajbs.eg.net



Non-enzymatic Antioxidants and Transaminases Constitute Homeostatic Mechanisms for Some Egyptian Vegetables Irrigated with Treated Wastewater

Zeinab Ahmed Khidr¹, Sawsan Abdel Rahman Abdel Rahman¹, Hedaya Ahmed Kamel² and Hend Mustafa Habib²

1- Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Cairo, Egypt).

2- Radioisotope Department, Nuclear Research Center Atomic Energy Authority (Cairo, Egypt).

ARTICLE INFO

Article History

Received: 3/1/2017

Accepted: 15/2/2017

Keywords:

leafy vegetables

wastewater

phenols

ascorbate

free thiols

GOT

GPT

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 wastewater; 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 oleracea* (spinach) family: Amaranthaceae and *Petroselinum 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.

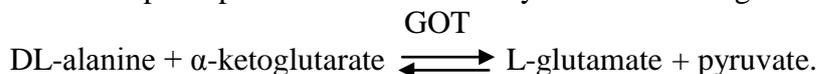
Transaminases (GOT and GPT): The activity of enzymes; glutamic oxaloacetic transaminase [GOT, EC 2.6.1.1] or aspartate transaminase (AST) and glutamic pyruvic transaminase [GPT, EC 2.6.1.2] or alanine transaminase (ALT) were determined according to Reitman and Frankel (1957).

The principle of this method catalysis the following reaction:



The oxalacetate formed may be assayed by the formation of 2, 4- dinitrophenylhydrazine 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:



The pyruvate formed may be assayed by the formation of 2, 4- dinitrophenylhydrazine 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 possess 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 (Dučić *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 ($\mu\text{g} / \text{g}$ fresh wt.) and L-ascorbic acid ($\mu\text{g} / \text{ml}$) in studied vegetables.

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

Data are mean of three replicates, \pm 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 (25 $\mu\text{mol} / \text{l}$) 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 (μ M/ g fresh wt.) in studied vegetables.

Plant	Proline (μ g/ g fresh wt.)			Free thiols (μ M/ g fresh wt.)		
	Control	Treated	Chang (%)	Control	Treated	Chang (%)
Cabbage	1709.87 \pm 67.07 a	2742.75 \pm 66.91a*	60.4	0.03 \pm 0.00 c	0.02 \pm 0.00 e*	-33.3
Garden rocket	784.00 \pm 3.21 d	55.33 \pm 1.33 d *	-92.9	0.28 \pm 0.01 b	1.32 \pm 0.00 b*	371.4
Lettuce	225.12 \pm 2.78 e	197.39 \pm 10.10 c	-12.3	0.34 \pm 0.00 b	1.43 \pm 0.01 a*	320.6
Parsley	1178.66 \pm 18.05 b	1560.00 \pm 8.72b*	32.3	0.33 \pm 0.02 b	0.16 \pm 0.00 d*	-51.5
Spinach	899.67 \pm 34.31 c	7.17 \pm 0.44 d*	-99.2	2.29 \pm 0.13 a	0.83 \pm 0.02 c*	-63.7

Data are mean of three replicates, \pm 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 phytochelatin (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 μ M 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 \pm 0.60 c	52.83 \pm 3.92 e*	31.0	40.00 \pm 1.80 b	43.50 \pm 1.32 a	8.7
Garden rocket	64.00 \pm 0.87c	112.33 \pm 12.94 d*	75.5	48.57 \pm 18.44 b	57.17 \pm 1.01 c	17.7
Lettuce	203.33 \pm 3.087 a	328.67 \pm 0.88 a*	61.6	167.33 \pm 2.92 a	354.17 \pm 1.42 b*	111.6
Parsley	225.67 \pm 18.68 a	284.17 \pm 1.59 b*	25.9	176.67 \pm 10.27 a	106.00 \pm 3.77 c*	-40.0
Spinach	109.83 \pm 3.18 b	205.67 \pm 2.20 c*	87.3	63.17 \pm 5.46 b	57.67 \pm 1.88 c	-8.7

Data are mean of three replicates, \pm 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 μ M 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 metallothioneins. 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 H₂O₂-scavenging, phenolic/ ASC/ POD system. So, depending on species of the plant and nature of the stress, the plant will respond in different ways.

REFERENCES

- Aly, M. A. S.; Ezz El arab, A.; Megahed, Y. M.; Hamad, H. B. and Abdel galil, F.M. (2002). Some toxicological studies of deltamethrin in rats. Arab J. of Nucl. Sci. and Applic., 35(1): 319- 329.
- Amarteifio, J.O.; Tibe, O. and Njogu, R. M. (2006). The mineral composition of bambara ground nut (*Vigna subterranean* L.) grown in southern Africa. African Journal of Biotechnology, 5: 2408- 2411.
- Bahorun, T.; Ramma, A. L.; Crozier, A. and Aruoma, O. I. (2004). Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J. Sci. Food Agric., 84: 1553 –1561.
- Bajaj, K. L. and Kaur, G. (1980). Spectrophotometric determination of L- ascorbic acid in vegetables and fruits. Analyst., 106: 117-120.
- Barman, S. C. and Lal, M. M. (1994). Accumulation of heavy metals (Zn, Cu, Cd and Pb) in soils and cultivated vegetables and weeds grown in industrially polluted fields. Journal of Environmental Biology, 15: 107- 115.
- Belkadhi, A.; Djebali, W.; Hédiji, H. and Chaïbi, W. (2016). Cellular and signaling mechanisms supporting cadmium tolerance in salicylic acid treated seedlings. Plant Science Today, 3(1): 41-47.
- Bohnert, H. J.; Nelson, D. E. and Jensen, R. G. (1995). Adaptations to environmental stress. The Plant Cell, 7: 1099-1111.
- Bortoli, C. G.; Simontacchi, M.; Montaldi, R. and Puntarulo, S. (1997). Oxidants and antioxidants during aging of *Chrysanthemum* petals. Plant Sci., 129: 157- 165.
- Choudhary, M.; Jetley, U. K.; Khan, M. A.; Zutshi, S. and Fatma, T. (2007). Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. Ecotoxicology and Environmental Safety, 66 (2): 204- 209.
- Dresler, S. and Maksymiec, W. (2013). Capillary zone electrophoresis for determination of reduced and oxidised ascorbate and glutathione in roots and leaf segments of *Zea mays* plants exposed to Cd and Cu. Acta Sci. Pol., Hortorum Cultus 12(6): 143-155.

- Dučić, T.; Maksimović, V. and Radotić, K. (2008). Oxalate oxidase and non-enzymatic compounds of the antioxidative system in young *Serbian spruce* plants exposed to cadmium stress. Archives of Biological Sciences (Belgrade), 60(1): 67-76.
- Dytham, C. (1999). Choosing and Using Statistics, A Biologist's Guide, Blackwell Science Ltd., London, UK.
- Eitenmiller, R. R. and Landen, W.O. (1999). Vitamin Analysis for the Health and Food Sciences. Department of food science and technology university of Georgia. CRC press LLC. 223- 236.
- El-Beltagi, H. S. and Mohamed, A. A. (2010). Changes in non protein thiols, some antioxidant enzymes activity and ultrastructural alteration in radish plant (*Raphanus sativus* L.) grown under lead toxicity. Notulae Botanicae, Horti Agrobotanici, Cluj-Napoca, 38 (3): 76- 85.
- El-Khatib, A. A. (2003). The response of some common Egyptian plants to ozone and their use as biomonitors. Environmental Pollution, 124: 419- 428.
- Ellman, G. L. (1959). Tissue sulfhydryl groups. Archives Biochem. Biophys., 82: 70-77.
- Gajewska, E.; Wielanek, M.; Bergier, K. and Sklodowska, M. (2009). Nickel-induced depression of nitrogen assimilation in wheat roots. Acta Physiologia Plantarum, 31 (6): 1291-1300.
- Gardner, P. T.; White, T. A. C.; Mcphail, D. B. and Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoid and phenolics to the antioxidant potential of fruit juices. Food Chemistry, 68: 471- 474.
- Guo, Z.; Tan, H.; Zhu, Z.; Lu, S. and Zhou, B. (2005). Effect of intermediates on ascorbic acid and oxalate biosynthesis of rice and in relation to its stress resistance. Plant Physiology and Biochemistry, 43(10-11): 955- 962.
- Gupta, S.; Satpati, S.; Nayek, S. and Garai, D. (2010). Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes. Environ. Monit. Assess., 165: 169-177.
- Hayat, S.; Hayat, Q.; Alyemeni, M.N.; Wani, A.S.; Pichtel, J. and Ahmad, A. (2012). A review: role of proline under changing environments. Lands Bioscience. Plant Signaling and Behavior 7:(11) 1456 -1466.
- Hubalek, J.; Hradecky, J.; Adam, V.; Krystofova, O.; Huska, D.; Masarik, M.; Trnkova, L.; Horna, A.; Klosova, K.; Adamek, M.; Zehnalek, J. and Kizek, R. (2007). Spectrometric and voltammetric analysis of urease - nickel nanoelectrode as an electrochemical sensor. Sensors, 7: 1238-1255.
- Jozefczak, M.; Bohler, S.; Schat, H.; Horemans, N.; Guisez, Y.; Remans, T.; Vangronsveld, J.; Cuypers, A. (2015). Both the concentration and redox state of glutathione and ascorbate influence the sensitivity of Arabidopsis to cadmium. Annals of Botany, 116(4): 601–612.
- Khan, N. A.; Anjum, N. A.; Nazar, R. and Iqbal, N. (2009). Increased activity of ATP-sulfurylase and increased contents of cysteine and glutathione reduce high cadmium-induced oxidative stress in mustard cultivar with high photosynthetic potential. Russian Journal of Plant Physiology, 56 (5): 670-677.
- Khidr, Z. A. and Nour El-Din, N. M. (2005). Correlation between sulfhydryl and disulfide groups and cadmium accumulation in two newly bred lines of bread and durum wheat. Women's College Ann. Rev., 24 : 27- 44.
- Kiziloglu, F. M.; Turan, M.; Sahin, U.; Angin, I.; Anapali, O. and Okuroglu, M. (2006). Effects of wastewater irrigation on soil and cabbage-plant (*Brassica oleracea* var. capitata cv. yalova-1) chemical properties. Hydrological Processes, 20: 1- 4.
- Krystofova, O.; Shestivska, V.; Galiova, M.; Novotny, K.; Kaiser, J.; Zehnalek, J.; Babula, P.; Opatrilova, R.; Adam, V. and Kizek, R. (2009). Sunflower plants as bioindicators of environmental pollution with lead (ii) ions. Sensors, 9: 5040-5058.
- Kubo, I.; Fijita, K.; Kubo, A.; Nehi, K. and Gura, T; (2004). Antibacterial activity of coriander volatile compounds against *Salmonella choleraesuis*. J. of Agric. and Food Chem., 52: 3329- 3332.

- Kumar, A.; Vajpayee, P.; Ali, M. B.; Tripathi, R. D.; Singh, N.; Rai, U. N. and Singh, S. N. (2002). Biochemical responses of *Cassia siamea* Lamk grown on coal combustion residue (fly-ash), Bull. Environ. Contam. Toxicol., 68: 675- 683.
- Larrosa, M.; Liorach, R.; Espin, J. C. and Tomás- Baraberán, F. A. (2002). Increase of antioxidant activity of tomato juice upon functionalization with vegetable by product extracts. Lebensm- Wiss. U- Technol., 35: 532-542.
- Malec, P.; Maleva, M. G.; Prasad, M. N. V. and Strzalka, K. (2010). Responses of *Lemna trisulca* L. (Duckweed) exposed to low doses of cadmium: thiols, metal binding complexes, and photosynthetic pigments as sensitive biomarkers of ecotoxicity. Protoplasma, 240 (1-4): 69- 74.
- Malik, R. S.; Ramkala; Gupta, S. P. and Dahiya, S. S. (2004). Background levels of micronutrients and heavy metals in sewage- irrigated soils and crops in Haryana. Indian Journal of Agricultural Sciences, 74 (3): 156- 158.
- Mapanda, F.; Mangwayana, E. N.; Nyamangara, J. and Giller, K.E. (2005). The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agric. Ecosyst. Environ., 107: 151-165.
- Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish J. of Environ. Stud., 15 (4): 523-530.
- Noctor, G.; Arisi, A. M.; Jouanin, L.; Kunert, K. J.; Rennenberg, H. and Foyer, C. H. (1998). Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. J. Exp. Bot., 49: 623- 647.
- Osler, M.; Heitmann, B.L.; Gerdes, L.U.; Jorgensen, L.M. and Scroll, M. (2001). Dietary patterns and mortality in Danish men and women: a perspective observational study. British Journal of Nutrition, 85: 219- 225.
- Rai, V.; Vajpayee, P.; Singh, S. N. and Mehrotra, S. (2004). Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. Plant Sci., 167: 1159-1169.
- Raju, S. M. M.; Varakumar, S.; Lakshminarayana, R.; Krishnakantha, T.P. and Baskaran, V. (2007). Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. Food Chemistry, 101: 1598- 1605.
- Rastgoo, L. and Alemzadeh, A. (2011). Biochemical responses of Gouan (*Aeluropus littoralis*) to heavy metals stress. Australian Journal of Crop Science, 5 (4): 375- 383.
- Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin. Path., 28: 56- 63.
- Shyamala, B. N.; Gupta, S.; Lakshmi, A.J. and Prakash, J. (2005). Leafy vegetable extracts- antioxidant activity and effect of storage stability of heated oils. Innovative Food Sci. and Emerging Techno., 6: 239- 245.
- Singh, T. N; Paleg, L.G and Aspinall, D. (1973). Stress metabolism, nitrogen metabolism and growth in the barley plant during water stress. J. Biol. Sci., 26: 45 - 56.
- Singh, A.; Agrawal S, and Rathore, D. (2005). Amelioration of Indian urban air pollution phytotoxicity in *Beta vulgaris* L. by modifying NPK nutrients. Environmental Pollution, 134: 385-395.
- Singh, S. and Sinha, S. (2005). Accumulation of metals and its effects in *Brassica juncea* L. Czern (cv. Rohini) grown on various amendments of tannery waste. Ecotoxicology and Environment Safety, 62: 118-127.
- Singh, R. P. and Agrawal, M. (2010). Biochemical and physiological responses of rice (*Oryza sativa* L.) grown on different sewage sludge amendments rates. Bulletin of Environmental Contamination and Toxicology, 84 (5): 606-612.
- Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic and reagents. Am J Enol Vitic., 16: 144-153.
- Tantrey, M. S and Agnihotri, R. K. (2010). Chlorophyll and proline content of gram (*Cicer arietinum* L.) under cadmium and mercury treatments. Research Journal of Agricultural Sciences, 1(2): 119- 122.

- Vitecek, J.; Petrlova, J.; Adam, V.; Havel, L.; Kramer, K.J.; Babula, P. and Kizek, R. (2007). A fluorimetric sensor for detection of one living cell. *Sensors*, 7: 222- 238.
- Wagner, G.J. (1984). Characterization of a cadmium-binding complex of cabbage leaves. *Plant Physiol.*, 76: 797-805.
- Warrilow, A. G. S. and Hawkesford, M. J. (1998). Separation, subcellular location and influence of sulphur nutrition on isoforms of cysteine synthase in spinach. *J. Exp. Bot.*, 49: 1625- 1636.
- Yadav, R. K.; Chaturvedi, R. K.; Dubey, S. K.; Joshi, P.K. and Minhas, P. S. (2003). Potentials and hazards associated with sewage irrigation in Haryana. *Indian Journal of Agricultural Sciences*, 73 (5): 249- 255.
- Yamasaki, H. and Grace, S. C. (1998). EPR detection of phytophenoxyl radicals stabilized by zinc ions: evidence for the redox coupling of plant phenolics with ascorbate in the H₂O₂- peroxidase system. *FEBS lett.*, 422: 377-380.
- Yong, H. G.; Shao, W. Y.; Ci, S. C.; De, D. J. and Xun, S. Z. (2010). The effect of multiple heavy metals on ascorbate, glutathione and related enzymes in two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Oceanological and Hydrobiological Studies*, 39 (1): 11- 25.
- Zengin, F. K. and Munzuroglu, O. (2005). Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) Seedlings. *Acta Biologica Cracoviensia Series Botanica*, 47(2): 157- 164.

ARABIC SUMMARY

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

زينب احمد خضر^١ - سوسن عبدالرحمن عبدالرحمن^١ - هداية احمد كامل^٢ - هند مصطفى حبيب^٢

١- قسم النبات - كلية العلوم بنات - جامعة الأزهر

٢- قسم النظائر المشعة - هيئة الطاقة الذرية

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 بمياه 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 بمياه الصرف الصحي المعالجة. لوحظ إنخفاض معنوي في تركيز الفينولات الكلية في نباتات الكرنب، البقدونس والسبانخ بمقدار ١٥,٢%، ٢٧,٤% و ٢٧,١% على الترتيب، بينما لوحظ زيادة معنوية في نبات الخس بمقدار ٧٨,٣% عنها في النباتات الكنترول. حدث انخفاض معنوي في تركيز حمض الاسكوربيك في نباتات الجرجير، البقدونس والسبانخ المعالجة بمقدار ٣٦%، ٢٨,٨% و ٣٥,١% على الترتيب وذلك نتيجة الري بمياه الصرف المعالجة. وعلى العكس، لوحظ زيادة معنوية في تركيز حمض الاسكوربيك بمقدار ٢٠٢,٧% في نبات الخس المعالج عنها في الكنترول.

أدى الري بمياه الصرف المعالجة الى زيادة معنوية للبرولين في كل من نباتي الكرنب والبقدونس بينما أدى إلى انخفاض معنوي في نباتي الجرجير والسبانخ.

أظهرت النتائج ان الري بمياه الصرف المعالجة أدى إلى انخفاض معنوي في تركيز المركبات الكبريتية في كل من نباتات السبانخ، البقدونس والكرنب بمقدار ٦٣,٧%، ٥١,٥% و ٣٣,٣% على الترتيب، بينما حدثت زيادة معنوية لمركبات الكبريت الحر في نباتات الجرجير و الخس .

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