Advances in Environmental Biology, 7(14) December 2013, Pages: 4741-4748

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Effects of Airborne Heavy Metal Pollution on Physiological and Biochemical Processes in Lettuce (*Lactuca sativa* L.Romaine) Plants

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ARTICLE INFO

Article history:
Received 15 November 2013
Received in revised form 14 January 2014
Accepted 20 January 2014
Available online 25 February 2014

Key words:
Photosynthetic rate (A);
Stomatal conductance (g_s);
airborne heavy metal pollution;
Lettuce (Lactuca sativa L. cv. Romaine) plants

ABSTRACT

Visual symptoms of phytotoxicity of heavy metals were observed on Lettuce (*Lactuca sativa* L. cv. Romaine) plants grown at industrial and urban areas, where the concentrations of metals was about 36 times higher than in other sites. Moreover, dramatic changes in enzymatic activities as well as net photosynthetic rates (A), variable to maximum chlorophyll fluorescence (F_v/F_m) and stomatal conductance (g_s). The decrease in chlorophyll reached 70 and 64% in plants cultivated in the industrial and urban regions, while lengths of shoots reduced by 50 and 41% in plants collected from the same locations, respectively. Antioxidant enzymes were significantly altered during exposure. On the other hand, and lipid peroxidation was enhanced by exposure to airborne heavy metals. The reduction in chlorophyll and other physiological and biochemical parameters were correlated with the concentrations of airborne pollutants measured in the atmosphere of the locations examined. Moreover, lettuce plants cultivated in the industrial region accumulated heavy metals, which can pass into the human food chain. The biochemical and physiological parameters measured in the present study clearly showed that they could form the basis of a plant biomarkers battery for monitoring and predicting early effects of exposure to airborne heavy metals.

To Cite This Article: Ibrahim A. Hassan, Jalal M. Basahi, Iqbal M. Ismail, Asharf A. Zahran., Effects of airborne heavy metal pollution on physiological and biochemical processes in lettuce (Lactuca sativa L.Romaine) Plants. ***Adv. Environ. Biol., 7(14),*** *4741-4748, 2013*

INTRODUCTION

Increasing industrialization, urbanization and vehicular traffic metropolitan cities could increase levels of heavy metals in air and soil [1 – 4]which lead to a high pollution pressure on the biota and eventually, would pose a threat to food safety and human health [5,6]. Presence of airborne heavy metals in vegetable crops above the permissible limit may lead to severe health hazards to the people consuming it [7]. So the estimation of their levels in contaminated food is very important for the safety of human health [8- 11].

Leaves of plants are especially useful as biological indicators to assess air pollution for metal pollution because metal pollutants found as superficial contaminants on them [12 -16] Although it was reported that mosses and lichens are good monitors of heavy metal pollution, higher plants can be used as biomonitors in areas that do not have these species [17 – 19].

Photosynthesis (A) is inhibited by air pollution and other environmental stresses [20 – 28]. Maize and wheat plants exposed to Ni stress had lower photosynthetic rates (A), lower stomatal conductance (g_s), reduced photochemistry, and lower chlorophyll concentration [29 -32]. Moreover, they found a decline of fluorescence induction kinetics as well as of carotenoids concentrations in Ni-stressed plants. However, the main mechanism primarily affecting photosynthesis in response to heavy metals is not clear [23 – 34]. Heavy metals have detrimental effects on the enzymatic capacity and g_s of the photosynthetic apparatus [12].

In Saudi Arabia, air pollution due to the heavy metals arises from road traffic that uses fossil fuel, industry, agriculture, sewage sludge, and waste incineration as well as from the dust storms [34 – 35]. However, Studies regarding the contamination of heavy metals in the vegetable crops are scanty. Therefore, it is important to study the heavy metals contamination in plants that could presumably be used as a biological indicator of heavy metal pollution so as to decide if it is safe or not for human consumption [2,14, 20, 34, 35].

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It was aimed in the present investigation to evaluate impact of airborne heavy metals on some physiological and biochemical processes in lettuce (*Lactuca sativa* L. cv Romaine) plants and to use them as a biomonitor of airborne heavy metals.

MATERIALS AND METHODS

Plant material, growth conditions and experimental design:

Seeds of Lettuce (*Lactuca sativa* L. cv Romaine) plants were washed with tap water to break dormancy and to remove excess pesticides or herbicides. Experimental design and growth conditions were discussed elsewhere [2]. Shoot length was measured on all plants grown at different urbanization gradients.

Gas exchange and fluorescence measurements:

Gas exchange parameters and chlorophyll fluorescence yield were measured simultaneously, with a portable photosynthesis system LI 6000 (Li- Cor, USA) and Fluorescence Monitoring System (FMS, Hansatech Instruments, U.K.), respectively. For each measurement, the first top fully developed leaves from the main stems of six plants were used on weekly basis [2, 24 – 26, 33].

Pigment concentration:

Chlorophylls (*a* & *b*) and carotenoids were extracted, measured on a UV Spectrophotometer and their concentrations were calculated [19].

Antioxidant enzymes:

Ten leaves (5 young and 5 expanded ones) were homogenized and dialyzed [36]. The dialyzed samples were used for enzymatic and protein content determinations. Activities of CAT, APX, and SOD were determined (Hassan, 2006). One unit of CAT and APX is defined as the number of mmoles of H_2O_2 consumed per minute, and one unit of SOD as the enzyme content which gives 50% inhibition of cytochrome *c* reduction.

Lipid peroxidation:

Ten leaves were homogenized in 0.1% trichloro acetic acid, centrifuged (20,000g, 15min) and the supernatants were collected. To 1 ml aliquots of supernatant, 4 ml of a solution of 20% trichloroacetic acid and 0.5% thiobarbituric acid was added; the mixture was heated (95 1C; 30min), quickly cooled, and then centrifuged (10,000g,10min). Supernatants were used to determine Malondialdehyde (MDA) content at 532 nm [23, 39].

Statistical analysis:

Data were subjected to one way ANOVA, using the SATATGRAPHICS statistical software package. Least Significant Difference (LSD) Test was applied to assess the significant differences among the mean values of different attributes. The values are means of ten replications. Data were log transformed prior to analysis to ensure normality and equality of variance. The relationships between sites and different parameters were assessed using correlation analysis. There were 6 replicates

RESULTS AND DISCUSSION

Leaves developed visible injury symptoms in the form of chlorotic and brown accentuated necrotic lesions especially in older leaves collected from industrial and urban areas which exhibited (Fig. 1).

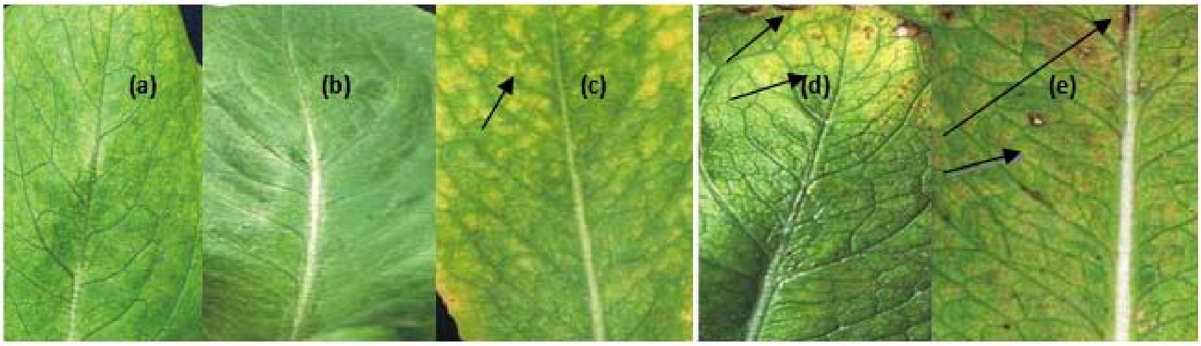

Fig. 1: Leaves collected from different sites. (a) Control, (b) Rural, (c) Residential and suburban (d) Urban and (e) industrial. Small chlorotic stippling on the old leaves of the plant. Arrows indicate Chlorotic and necrotic lesions on leaves collected from urban, suburban and industrial sites.

The growth of lettuce shoots was significantly reduced in industrial, urban and residential areas ($p \leq 0.05$) when compared to the control. Length of shoot was decreased by 51, 41, 25, 30 and 18% in plants collected from industrial, urban, suburban, residential and rural sites, respectively (Fig 2).

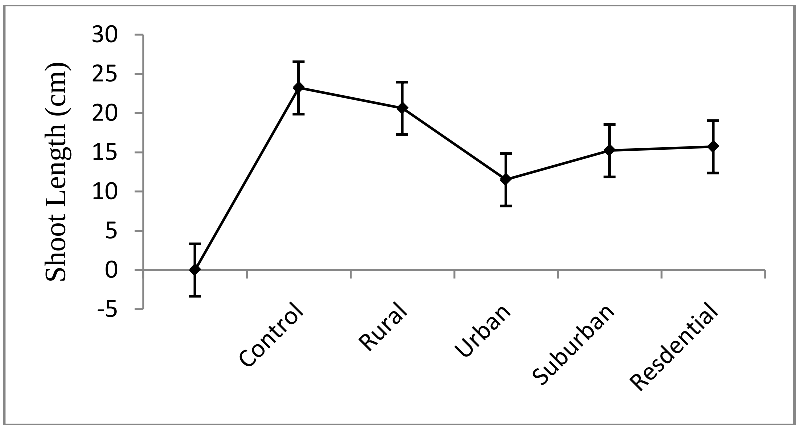

Fig. 2: Shoot lengths of plants collected from different sites. (Each figure is a mean value of 10 replicates ± SE)

Net Photosynthetic rates (A) were decreased by 53, 51, 37, 36 and 14% in plants collected from industrial, urban, suburban, residential and rural sites, respectively. Stomatal conductance (g_s) was also decreased by 46, 49, 29, 33 and 12% in plants collected from the same sites, respectively (Figure 3).

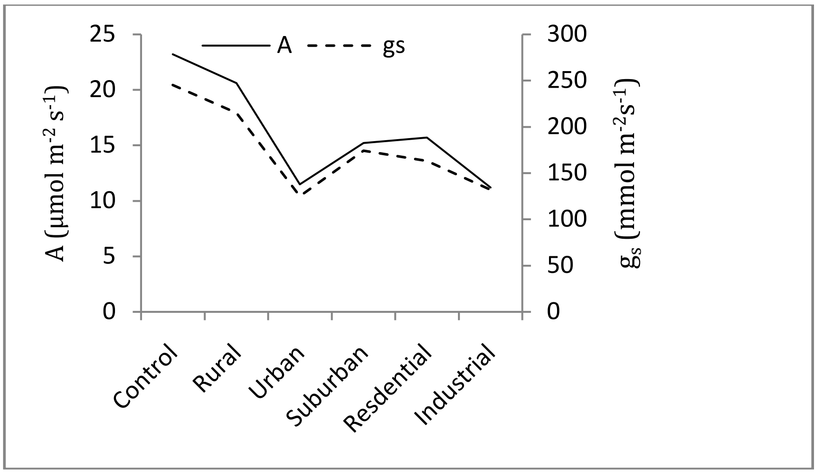

Fig. 3: Net Photosynthetic rates (*A*) (µmol m^{-2} s^{-1}), (g_s) (mmol $m^{-2}s^{-1}$) of lettuce (*Lactuca sativa* L) plants collected from different sites along urbanization gradient. Legends as Fig 2.

Chl a , b and Chl a/b ratio were decreased by 70, 59 and 27% in plants collected from industrial area, and by 64, 57 and 16 in plants collected from Urban areas, respectively. These parameters were decreased at other sites but at relatively lower extents (Figure 4).

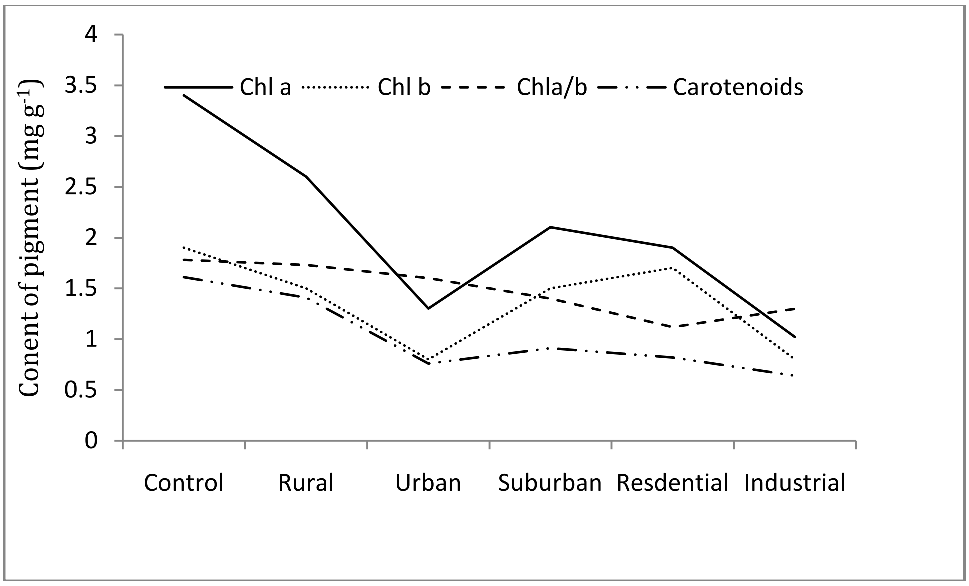

Fig. 4: Chl *a* and *b* contents(mg g^{-1}), Carotenoids (mg g^{-1}) of lettuce (*Lactuca sativa* L) plants collected from different sites along urbanization gradient. Legends as Fig.2.

Table 1 shows that the maximum quantum yield of PSII (F_v/F_m) was decreased significantly ($P \leq 0.05$) at industrial and urban sites by 13 and 10%, respectively, while the reductions were insignificant ($P \geq 0.05$) in other sites (Table 1). Leaves collected from different sites had a higher basal fluorescence (F_0) level ($p \leq 0.01$), and a significant decrease in both maximal fluorescence induction (F_m) and variable fluorescence (F_v) value ($p \leq 0.05$) when compared to control.

Table 1: fluorescence parameters) of lettuce (*Lactuca sativa* L) plants collected from different sites along urbanization gradient. (Each figure is a mean value of 10 replicates ± SE)

Parameter	Control	Rural	Urban	Suburban	Residential	Industrial
F_0	613±34.0^{a}	713±29.5^{a}	901±27.2^{e}	769±22.9^{c}	726±30.6^{b}	854±33.7^{d}
F_m	3037±397.2	2829±75.2	2206±68.9	2511±65.4	2699±89.7	2304±71.6
F_v	2423±15.0^{d}	2116±67.8^{c}	1580±88.9^{a}	1881+91.7^{b}	1980±54.8^{b}	1589±78.5^{a}
F_v/F_m	0.794±0.01^{f}	0.747±0.009^{e}	0.716±0.04^{b}	0.749±0.03^{d}	0.733±0.07^{c}	0.689±0.004^{a}

SOD was increased by 38, 40, 18, 32 and 31% in leaves collected from industrial, urban, suburban, residential and rural areas, respectively (Table 2). On the other hand, CAT activities were reduced by 41, 38, 18, 22 and 14% in the same site respectively (Table 2). Moreover, APX was reduced by 30, 28, 11 in leaves collected from industrial, urban, suburban sites, respectively, while there was no significant ($P > 0.05$) effect on leaves collected from residential or urban areas (Tab.2).

Table 2: Response of Antioxidant Enzymes (U/mg protein) and lipid peroxidation (nmol g^{-1} FW) due to accumulation of heavy metals

Enzyme	Control	Rural	Urban	Suburban	Residential	Industrial	LSD
SOD	300^{a}	310^{a}	421^{b}	354^{c}	395^{d}	413^{e}	19
CAT	3.67^{c}	3.16^{b}	2.27^{a}	3.01^{b}	2.87^{a}	2.16^{a}	0.71
APX	36.2^{bc}	31.9^{b}	26.1^{ab}	32.3^{b}	33.6^{b}	25.4^{a}	6.45
MDA	0.154^{a}	0.163^{a}	0.201^{b}	0.187^{b}	0.190^{b}	0.235^{c}	0.02

Means not followed by the same letter(s) are significantly different from each other at $P < 0.05$

MDA content in lettuce leaves increased significantly ($p \leq 0.05$) in plants collected from industrial, urban, suburban and residential areas by 52, 30, 21 and 23%, respectively (Table 2). Rural area had no significant ($P > 0.05$) effect on MDA (Table 2).

A least-squares linear regression analysis was obtained for all sites and different physiological and biochemical markers (Table 3). The results show that the correlation coefficients (r) were significant at $p < 0.001$ for gas exchange measurements (A, g_s. F_v/F_m, Chl contents, SOD, CAT, APX and MDA (Table 3).

Table 3: Correlation matrix of different physiological and biochemical parameters (*p< 0.01, **p< 0.001). n= 20

	Site	*A*	g_s	Chl *a*	Chl *b*	Chl *a/b*	F_v/F_m	SOD	CAT	POX	MDA
site	--	-0.75^{*}	-0.69^{*}	-0.60^{*}	-0.55^{**}	-0.26^{*}	-0.11^{*}	0.38^{**}	-0.52^{**}	-0.37^{*}	0.417^{**}
A		--	0.528^{**}	0.764^{**}	0.36^{**}	0.209^{*}	0.87^{**}	-0.41^{**}	0.01	0.073	-0.163^{*}
g_s			--	0.017	0.01	0.002	0.11	-0.021	0.003	0.107	-0.261^{*}
Chl *a*				--	-0.32^{*}	0.43^{**}	0.52^{**}	0.028	0.017	0.017	-0.192^{*}
Chl *b*					--	-0.4^{**}	0.20^{*}	0.005	0.011	0.001	0.031
Chl *a/b*						--	0.23^{*}	0.021	0.021	0.003	-0.105
F_v/F_m							--	0.002	0.002	0.012	-0.219^{*}
SOD								--	-0.11^{*}	-0.12^{*}	0.205^{*}
CAT									--	0.016	-0.201^{*}
POX										--	-0.015
MDA											--

Urban atmospheres, particularly those of megacities tend to have higher concentrations of heavy metals and other pollutants than rural (agricultural) ones, reflecting varying contents of contaminants from industrial and vehicular emissions as well as ash and soot coal fires [40, 41]. Nevertheless, here are limited studies on environmental pollution by heavy metals in Saudi Arabia.

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity [3,42 -45]. Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals [43]. Heavy metals inhibit metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition [46 -49]. The percentage reduction in Chl. Contents reported in our study is higher than those recorded in other urban areas in Turkey [19, 28] and Nigeria [11]. This higher percentage of reduction in Chl content of lettuce in the present study is an indicator of disturbances of the pigment synthesis mechanism and inhibition of degradation due to heavy metal effects. Such reductions in Chl content would lead to reduction in photosynthetic rates and eventually growth. Both chlorophyll and A showed a strong negatively correlation with urban and industrial sites, which are characterized by high heavy metal contents in their soils. The chlorophyll ratio, which is used as a stress indicator, decreased significantly with increasing metal concentrations. Such alteration indicates a change in the PSII/PSI ratio in stressed leaves [50]. The superficial observations were consistent with the chlorophyll contents and the fluorescence parameters.

The reduction in growth recorded in the present study is in agreement with the results reported in literature about effects of heavy meal pollution on growth and yield of lettuce (*L. sativa* L.), bean (*phaseolus vulgaris* L.) and *Lupinus albus* L. plants [48-50].

Plants have evolved a complex antioxidant system to mitigate oxidative stress caused by heavy metals and by other biotic and abiotic stresses. These antioxidants play an important role in the cellular defense strategy. Metals are known to cause molecular damage to plant cells either directly or indirectly through the burst of Reactive Oxygen Species (ROS), which can react with fatty acids leading to the peroxidation of lipids, destroying biological membranes [44].

Antioxidants like POX, SOD and CAT are ubiquitous and they play an important role in detoxification of toxic metal ions [18, 33, 50]. They play a crucial role in plant growth and development. Moreover, they are a potential indicator for metal toxicity 23, 51, 52].

Our results demonstrated that SOD increased linearly with urbanization and contents of heavy metals in soils. Excess of heavy metals can persuade oxidative stress in plants, which can escort formation of ROS. Antioxidant enzymes may alter the H_2O_2 to the H_2O in the plant cells and counteract the toxicity effect of H_2O_2 [53 -54]. Hence to shield cells against oxidative stress, antioxidant enzymes augmented proportionally, which is also consistent with our results. On the other hand, activities of CAT and APX were decreased linearly with increasing concentrations of heavy metals. Both increases and decreases were detected in APX and CAT [50, 55, 56]. Exposure to high concentrations of heavy metals resulted in a decreased antioxidant capacity [57]. CAT and APX were inhibited with extended exposure to heavy metals at different sites, in exposed leaves. This is in agreement with other studies bean (*Phaseolus vulgaris* L.) [37], pea (*Pisum sativum* L.) [38], and in lettuce (*Lactuca sativa* L.) plants [46].

MDA is a cytotoxic product of lipid peroxidation and its formation is routinely used as a general indicator of the extent of lipid peroxidation resulting from oxidative stress [26, 46]. The elevated MDA content obtained in lettuce leaves in the present study suggests that heavy metals, induced oxidative damage in lettuce as evidenced by increased lipid peroxidation through either indirect production of ROS or through inhibition of oxidative stress enzymes ([46]. Furthermore, MDA content was increasd in leaves of a mangrove plant (*Bruguiera gymnorrhiza*) when exposed to multiple metals [57]. Therefore lipid peroxidation is recommended as a biomarker of heavy metal stress for pollution monitoring purposes.

Reductions in photosynthetic efficiency and antioxidant capacities of lettuce, as well as MDA production are good indicators of leaf senescence due airborne heavy metal pollution. These alterations were correlated to shoot growth, at the end of exposure. Therefore these biomarkers could form the basis for monitoring and be predictive of early effects of this pollutant before they give rise to significant changes in natural community structures.

Conclusions:

Lettuce plants can be used as bioindicator of airborne heavy metals through the use of biomarkers. However, a better understanding of the overall process of metal-induced senescence, describing the cascade of their effects in plants is needed for a selection of relevant biomarkers of heavy metal stress.

AKNOWLLEDGMNTS

This work is supported with a grant from CEES. We would like to thank Samah K. Shata for her assistance and help as well as Prof Samir Khalil (Alexandria University) for help in analysis.

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