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Gas Exchange, Chlorophyll Fluorescence and Antioxidants as Bioindicators of Airborne Heavy Metal Pollution in Jeddah, Saudi Arabia

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ABSTRACT

Lettuce (*Lactuca sativa* L. cv. Romaine) plants were exposed to different levels of urbanization in Jeddah city, Saudi Arabia. They showed different degrees of visible injury symptoms and dramatic changes in enzymatic activities as well as net photosynthetic rates (P_N), variable to maximum chlorophyll fluorescence (F_v/F_m) and stomatal conductance (g_s). Visual symptoms of phytotoxicity of heavy metals were observed on plants grown at industrial and urban areas, where the concentrations of metals was about 36 times higher than in other sites. 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. 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 more heavy metals than others, which can pass into the human food chain. Photosynthetic efficiency was significantly decreased and lipid peroxidation was enhanced. Antioxidant enzymes were significantly altered during exposure. 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.

Key words: P_N – net photosynthetic rate; g_s - stomatal conductance;
F_v/F_m - maximum quantum efficiency of PSII photochemistry; biomonitoring.

INTRODUCTION

The rapid increasing population in urban areas led to anthropogenic activities and fossil fuel combustion. Emissions from road traffic that uses fossil fuel, industry, agriculture, sewage sludge, and waste incineration are the chief sources of air pollution[1, 2]. Air pollutants especially heavy metals are hazardous and toxic to human beings depending on their concentrations in the food stuff[3-4]. Presence of airborne heavy metals in vegetable crops above the permissible limit may lead to severe health hazards to the people consuming it[5]. So the estimation of their levels in contaminated food is very important for the safety of human health[3, 6].

Increasing industrialization, urbanization and vehicular traffic in Jeddah city could increase levels of heavy metals in air and soil [2] which lead to a high pollution pressure on the biota and eventually, would pose a threat to food safety and human health[7,8].

Metal pollutants found as superficial contaminants on leaves thereby, they are especially useful as biological indicators to assess air pollution indicator for metal pollution[9 - 17]. Because of the different characteristics of foliar uptake, accumulation and translocation of atmospheric heavy metals by leaves, plant leaves are used as bioindicators and/or biomonitors of heavy metal pollution in the terrestrial environment[18-20]. 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[21- 23].

Photosynthesis (P_N) is inhibited by air pollution and other environmental stresses[24-28]. Ouzounidou *et al.*[29] found reductions in rate of photosynthesis stomatal conductance (g_s), the maximum quantum yield of primary photochemistry, variable fluorescence (Fv) and chlorophyll concentration in Ni-stressed wheat. Recently, a marked toxicity of heavy metal pollution to photosynthetic apparatus in maize plants was reported[30]. They found a decline of fluorescence induction kinetics as well as of chlorophyll and carotenoid concentrations in Ni-stressed plants of maize. However, the main mechanism primarily affecting photosynthesis in response to heavy metals is not clear[17]. Heavy metals have detrimental effects on the enzymatic capacity and g_s of the photosynthetic apparatus[31].

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[32-34]. 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 m 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, 34].

Airborne heavy metals are hazardous and toxic to human beings depending on their concentrations in the food stuff[4]. During the past few decades, there has been an increase in the use of levels of higher plant as biomonitors of heavy metal pollution in the arid and semi-arid environments such as Saudi Arabia[9, 32 - 36].

The aim of present study was aimed at evaluating lettuce (*Lactuca sativa* L. cv Romaine) leaves as a biomonitor of airborne heavy metals in order to assess whether the vegetable crops were safe for human consumption.

MATERIALS AND METHODS

Plant material, growth conditions and experimental design

Seeds of Lettuce (*Lactuca sativa* L. cv Romaine) plants were washed with distilled water to remove excess pesticides or herbicides and to break dormancy. Experimental design and growth conditions were discussed elsewhere[2].

Gas exchange and fluorescence measurements

The photosynthetic gas-exchange measurements were done by a portable photosynthesis system LI 6000 (Li- Cor, USA). The pots were located in a climatic box, where plants were adapted for 1 h at a photon flux density (PFD) of 450 mmol m^{-2} s^{-1} (PAR). The leaf gas-exchange was determined under the following conditions: PFD of 900 mmol m^{-2} s^{-1}, leaf temperature of 31.5^{0}C, ambient CO_2 concentration of ca 400 mmol mol^{-1} and relative air humidity of about 65%. For each measurement, the first top fully developed leaves from the main stems of six plants were used on weekly basis[16].

Chlorophyll fluorescence was measured by a Fluorescence Monitoring System (FMS, Hansatech Instruments, U.K.). Measurements were made in ambient [CO_2] (Ca, 450 mmol mol^{-1}) on individual leaves enclosed into a leaf cuvette under a rate of 0.44 L min^{-1} air flow, relative humidity within the cuvette at 50-55%, a leaf temperature of 40^{0}C and 900 mmol m^{-2} s^{-1} of light intensity[31]. The maximum quantum yield of PSII in dark adapted leaves was estimated by the ratio between variable and maximal fluorescence, $F_v/F_m = (F_m - F_0)/F_m$. The efficiency of water-splitting apparatus was estimated by ratio between basal and variable fluorescence, F_0 / F_v [37]. Oxygen concentration was lowered to 1.5% when testing leaf gas exchange under non-photorespiratory conditions[17].

Gas exchange parameters and chlorophyll fluorescence yield were measured simultaneously.

Pigment concentration

Chlorophylls (*a* & *b*) were extracted in 85% acetone and measured on a UV-1800 Spectrophotometer (SHIMADZU) and their concentrations were calculated[21]. Leaves of the same age as those in the gas-exchange analyses were used.

Antioxidant enzymes

Tissue samples of 5 young and 5 expanded leaves were homogenized and dialyzed[38]. The dialyzed samples were used for enzymatic and protein content determinations. Activities of CAT, POX, and SOD were determined[39]. One unit of CAT and POX 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

Lipid peroxidation of lettuce leaves (n = 10) was determined by measuring malondialdehyde (MDA) production[40]. Tissues samples 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 MDA content at 532 nm[41].

Elemental analysis

The elemental analysis was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using IRIS Intrepid II XSP instrument[2]. Six point calibration procedure was applied with multi-element calibration solution (Merck ICP multi-element standard solution IV)[42].

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

Toxicity symptoms and plant growth

Lettuce plants developed visible injury symptoms, especially in older leaves collected from industrial and urban areas which exhibited chlorotic and brown necrotic lesions (Fig. 1). Furthermore,

Table 1: Physiological parameters (Net Photosynthetic rates (P_N), Stomatal conductance (g_s), Chlorophyll *a* and *b* contents, and 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
P_N(µmol m $^{-2}$ s^{-1})	23.47±3.2	20.19±2.8	11.45±2.1^a	14.72±1.9	15.04±2.5	10.93±1.5^a
g_s(mmol m^{-2}s^{-1})	245±25.2	215±19.6	125±15.3^a	174±11.8	163±14.7^b	132±20.1^a
Chl *a*(mg g^{-1})	3.42+0.05	2.59+0.04	1.24+0.009^a	2.07+0.01	1.94+0.03^b	1.02+0.17^a
Chl *b*(mg g^{-1})	1.86+0.008	1.47+0.009	0.80+0.03^a	1.51+0.03^b	1.72+0.05	0.76+0.04^a
Chl *a*/Chl *b*	1.84+0.005^d	1.76+0.007^d	1.55+0.09^c	1.37+0.006^a	1.13+0.002^b	1.34+0.04^a
Cartenoids (mg g^{-1})	1.56+0.007^d	1.39+0.008^c	0.75+0.007^b	0.91+0.008^c	0.82+0.008^b	0.64+0.006^a
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±153.0^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±0.001^f	0.747±0.009^e	0.716±0.004^b	0.749±0.003^d	0.733±0.007^c	0.689±0.004^a

accentuated necrosis and leaf fall were observed in the oldest plant leaves collected from the same areas during the third week of exposure.

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).

Figure 3 shows that soils collected from industrial and suburban sites have the highest concentrations of heavy metals.

Gas exchange, chlorophyll fluorescence and pigments

Net Photosynthetic rates (P_N) were decreased by 53, 51, 37, 36 and 14% in plants collected from industrial, urban, suburban, residential and rural sites, respectively (Table 1). Stomatal conductance (g_s) was also decreased by 46, 49, 29, 33 and 12% in plants collected from the same sites, respectively (Table 1).

Table 1 also 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.

The superficial observations were consistent with the chlorophyll contents and the fluorescence parameters (Table 1). 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

Table 2: Response of Antioxidant Enzymes (U/mg protein) and lipid peroxidation (nmol/g fresh wt.) to accumulation of heavy metals

Parameter	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
POX	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 $P < 0.05$

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

	Site	P_N	g_s	Chl *a*	Chl *b*	Chl *a/b*	F_v/F_m	SOD	CAT	POX	MDA
site	—	-0.745^{**}	-0.639^{**}	-0.602^{**}	-0.549^{**}	-0.261^{*}	-0.109^{*}	0.374^{**}	-0.521^{**}	-0.370^{*}	0.417^{**}
P_N		—	0.528^{**}	0.764^{**}	0.361^{**}	0.209^{*}	0.872^{**}	-0.402^{**}	0.011	0.073	-0.163^{*}
g_s			—	0.017	0.010	0.002	0.106	-0.021	0.003	0.107	-0.261^{*}
Chl *a*				—	-0.318^{*}	0.428^{**}	0.519^{**}	0.028	0.017	0.017	-0.192^{*}
Chl *b*					—	-0.371^{**}	0.198^{*}	0.005	0.011	0.001	0.031
Chl *a/b*						—	0.231^{*}	0.021	0.021	0.003	-0.105
F_v/F_m							—	0.002	0.002	0.012	-0.219^{*}
SOD								—	-0.109^{*}	-0.121^{*}	0.205^{*}
CAT									—	0.0162	-0.201^{*}
POX										—	-0.015
MDA											—

and 16 in plants collected from Urban areas, respectively. These parameters were decreased at other sites but at relatively lower extents (Table 1).

Antioxidant enzymes and Lipid peroxidation

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, POX 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 (Table 2).

Lipid peroxidation as measured by 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 ($P_{N,}$ g_s. F_v/F_m), Chl contents, SOD, CAT, POX and MDA (Table 3).

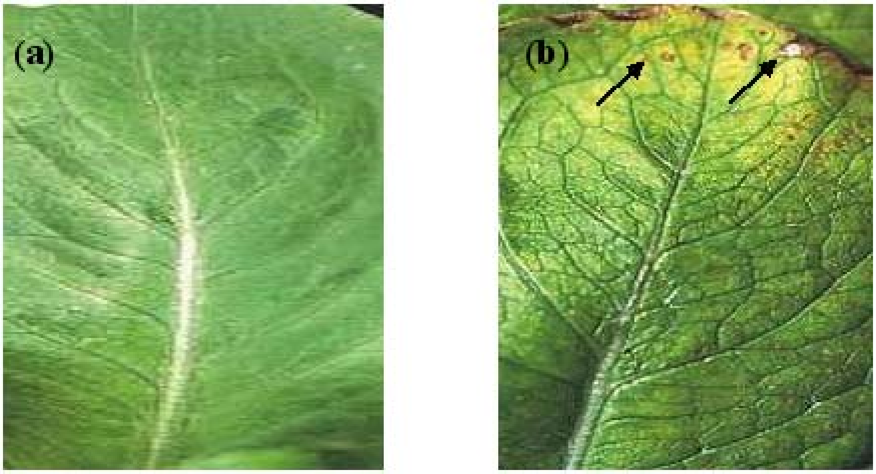

Fig. 1: Small chlorotic stippling on the old leaves of the plant. (a) Control plants, (b) plants collected from urban and industrial areas. Arrows indicate Chlorotic and necrotic lesions on leaves

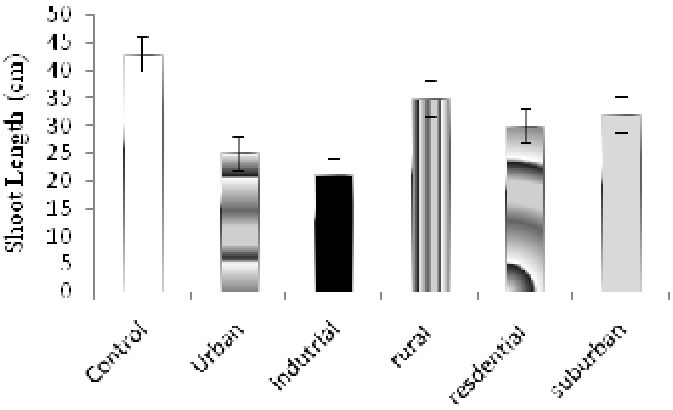

Fig. 2: Shoot lengths of plants collected from different sites. Results are expressed as mean ± 1 SE of ten replicates

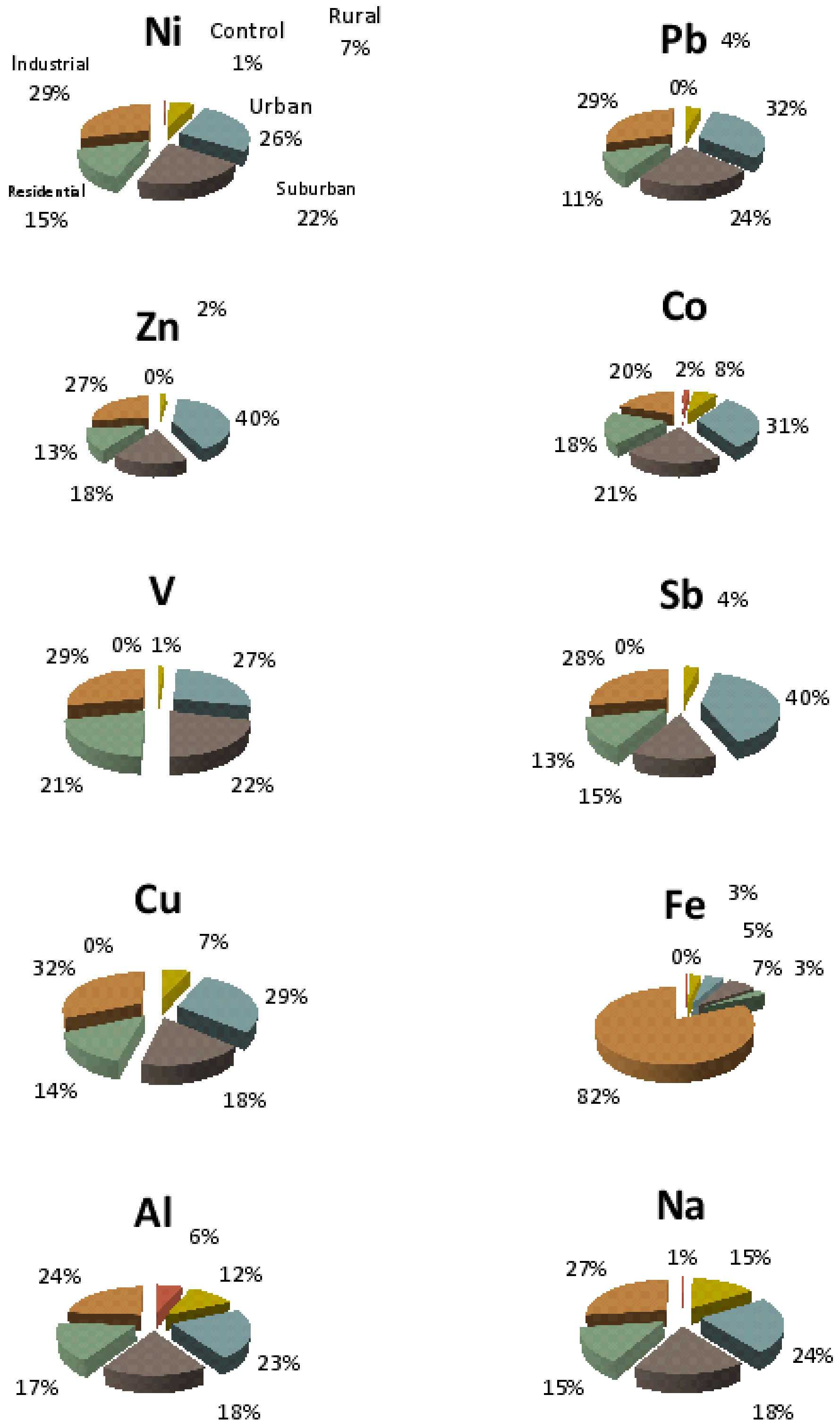

Fig. 3: Percentage of different heavy metals collected from different areas

DISCUSSION

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[5,7,14,43, 44]. Nevertheless, here are limited studies on environmental pollution by heavy metals in Saudi Arabia.

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[40, 42 - 48].

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[46-49]. Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals[21]. Heavy metals inhibit metabolic processes by inhibiting the action of enzymes, and this may be the most important cause of inhibition[21, 47, 50, 51]. The percentage reduction in Chl. Contents reported in our study is higher than those recorded in other urban areas in Turkey[21, 51] and Nigeria[48]. 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[47].

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[40].

Antioxidants like POX, SOD and CAT are ubiquitous and they play an important role in detoxification of toxic metal ions[47, 53]. They play a crucial role in plant growth and development. Moreover, they are a potential indicator for metal toxicity[21, 51, 54].

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 [54 -55]. 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 POX were decreased linearly with increasing concentrations of heavy metals. Both increases and decreases were detected in POX and CAT[21, 51,54]. Exposure to high concentrations of heavy metals resulted in a decreased antioxidant capacity[56-57]. In our study, CAT and POX were inhibited with extended exposure to heavy metals at different sites, in exposed leaves. This is in a agreement with other studies bean, (*Phaseolus vulgaris* L.)[58 - 59], pea, (*Pisum sativum* L.),[60], and in lettuce (*Lactuca sativa* L.) plants[40].

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[40,61]. 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[40]. Furthermore, MDA content was increased in leaves of a mangrove plant (*Bruguiera gymnorrhiza*) when exposed to multiple metals[62]. Therefore lipid peroxidation is recommended as a biomarker of heavy metal stress for pollution monitoring purposes.

In general, airborne heavy metal pollution induced senescence in lettuce in the present study, as measured in general as photosynthetic efficiency reduction, decrease in the overall antioxidant capacities of lettuce plants and a MDA production. These alterations were accompanied by an inhibition in the classical endpoint, shoot growth, at the end of exposure. These biomarkers could be used in integrative approaches with classical endpoints in ecotoxicological tests; especially this study was conducted real field conditions. Therefore they 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

Laboratory and field studies have provided encouraging insights into the capacity of lettuce plants to act as biomonitors of air pollution 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. Lettuce plants proved to be suitable as usage in environmental studies as a bioindicator.

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