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Physiological and biochemical response of traffic-generated particulate matter captured by some vegetables

Dalia F. Slima[1], Hadeer M. Nageeb[1*], Mohamed A. Zayed[1] and Yasser H. Ibrahium[2]
1- Botany and Microbiology Department, Faculty of Science, Menoufia Univ., Egypt.
2- Environment Division, National Research Centre, Dokki, Cairo, Egypt.
* Corresponding author E-mail: hadeer_nageeb@yahoo.com

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ABSTRACT

Amplified traffic is one of the main factors that increase particulate matter (PM) in the air. Green plants play an important role in reducing PM from the air. The present study aimed to investigate the impact of traffic pollution on three vegetable crops (*Lactuca sativa* L., *Eruca sativa* L. Cav., and *Brassica oleracea* var. *capitata* f. *alba*) cultivated on roadside at El-Bajour city, Menoufia governorate Egypt. Some physiological and biochemical parameters such as chlorophyll, total carbohydrates, protein, phenolics, flavonoids, reducing power, lipid peroxidation some antioxidant enzymes and heavy metals were determined in these plants. Results showed a significant difference (according to the studied biochemical parameters) between the samples affected by traffic pollution compared to control. The variation in the studied parameters in the leaves and root were found to be pollution load dependent and can be used as indicators of air pollution stress or as a marker for physiological damage to crops prior to the onset of visible injury symptoms.

Keywords: Traffic pollution, roadside plants, photosynthetic pigments, antioxidant enzymes.

INTRODUCTION

Air pollution is considered one of the most important abiotic stresses for the agricultural crops. It has direct and indirect impacts on the agricultural crops so it represents a serious threat to the agricultural fields. Air pollution occurs when gases, dust particles, fumes (or smoke) or odour are introduced into the atmosphere in a way that makes it harmful to humans, animals and plant. Air pollution threatens the health of humans and other living beings in our planet. It creates smog and acid rain, causes cancer and respiratory diseases, reduces the ozone layer atmosphere and contributes to global warming[1]

Traffic-generated particulate matter (PM) that is responsible for a significant portion of urban particulate matter pollution. Road traffic in Europe is responsible for more than 50% of PM10 and globally, 25% of PM2.5 (particulates with 10µm and 2.5µm aerodynamic diameter, respectively)[2]**.** Motor vehicles are responsible for 60-70% of the pollution found in an urban environment. The combustion of fuel in engines of motor gives rise to sulphur dioxide (SO_2), nitrogen oxides (NO_x) and CO as well as suspended particulate matter. These pollutants when absorbed by the plant leaves causing a reduction in the concentration of photosynthetic pigments e.g. chlorophyll and carotenoids[3]. In addition, motor vehicles also emit carbon dioxide (CO_2), carbon monoxide (CO), methane (CH_4), polyaromatic hydrocarbons (PAHs), particulate matter

(PM), heavy metals (HM), volatile organic chemicals (VOC) and contribute to the formation of the secondary pollutant ozone (O_3)[4].

Roads can also change other environmental factors as the basic compounds applied to roads can influence pH and salt concentrations locally[5]. Road surfaces themselves are decayed and also impede drainage[2,6]. Higher levels of physical disturbance (wind turbulence) are associated with increasing rates of traffic flow[7].

The European Environmental Agency has identified dust as the most dangerous air pollutant contributing to the premature death of 4,200,000 people worldwide, especially in undeveloped and intermediate regions[8].

Deposition of airborne PMs cause interference in light absorption at the leaf surface and plugging of stomata, leading to increase of leaf temperature and several physiological responses observed as impaired growth, photosynthesis, transpiration and stomatal conductance[9,10]. PMs affect different biochemical and metabolic pathways including decrease in chlorophyll and carotenoid content, soluble sugar and protein contents, increased production of ROS and free radical scavengers, increased or decreased activity of antioxidative enzymes and altered amino acid metabolism[11–13].

Generally, concern is raising due to increasing levels of the toxic substances and highly reactive heavy metal enriched particles (metal-PMs) in the atmosphere, uptake of metal-PMs by crop plants, their transfer to the food chain, and ultimate impact on human and animal health[14,15]. On the other hand, PMs may have beneficial effect on plants as they may contain sulfates, nitrates, ammonium, several inorganic ions such as Ca^{+2}, K^{+}, Na^{+}, Mg^{+2}, and carbon[16].

Plants growing in polluted habitats have shown a wide variety of symptoms which can even be used in the overall assessment of habitat quality. Leaf functional traits are reliable markers of environmental stress; these traits as physiological and morphological traits, leaf water status, leaf ultrastructure, non-enzymatic and enzymatic antioxidants, photosynthetic pigments, nutritional status and resource utilization[17].

Considering previously mentioned studies, the present study deals with the quantification of ambient traffic air pollutants, and assesses the impacts of these pollutants with special reference to PM on physiological, biochemical and enzymatic activities of three common crops; *Lactuca sativa* (L.) (Lettuce), *Eruca sativa* (L.) Cav. (Rocket), and *Brassica oleracea* var. *capitata* f. *alba* (white Cabbage)] growing in fields at different distances from roadside at El-Bajour city, Menoufia governorate Egypt.

MATERIALS AND METHODS

Crop samples:

Three different agricultural crops {(*Lactuca sativa* (L.) (Lettuce), *Eruca sativa*(L.) Cav.(Rocket), and *Brassica oleracea* var. *capitata* f. *alba* (Cabbage)} were collected at different distances from the traffic rood of El-Bagour city, Menoufia governorate Egypt (30˚26\394\\ N, 31˚02\103\\E). The samples were collected from fields at the roadside (0m) then after 70 and 140m from the road. Control samples were collected from fields away from road. Plant samples were rinsed with tap and distilled water to remove debris and separated into (roots and leaves) then left to be dried for 24h in an oven at (40-60ºC). Later, these samples were grinded separately into fine powder using electrical grind. The powdered samples were kept in well-sealed bags for further analysis.

Plant analysis:

1.Photosynthetic pigments:

Fresh leaf extract was used for determination of the main photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) following the spectrophotometric method recommended by Metzner *et al.*[18]. Acetone (85%) was used for homogenizing 5g fresh leaves for 5 minutes and centrifuged. The volume of supernatant was completed to 100mL with 85 % acetone. All samples were measured at three different wavelengths of 452.5, 644 and 663 nm. Plant pigments were expressed as μg/mL using the following equations:

Chlorophyll a = 10.3 E663 - 0.918 E644,

Chlorophyll b = 19.7 E644 -3.87 E663

Carotenoids = 4.2 E452.5 - (0.264 Chlorophyll a + 0.426 Chlorophyll b).

Where, E is the absorbance at the definite wavelength (nm).

2. Enzyme extraction and assays:

Extraction of enzymes was done by homogenizing 1g fresh leaves with 10mL 50mM sodium phosphate buffer (pH 7.0) in an ice-cold mortar. Then centrifugation was done at 20,000xg for 20min and the supernatant was used for determination of catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) activities.

Catalase (CAT) activity was estimated according to Aebi[19] by monitoring the decomposition of H_2O_2. 50 µL enzyme extract and 10mM H_2O_2 were added to 1mL of reaction mixture containing potassium phosphate buffer (pH 7.0) to initiate the reaction. The reaction was measured at 240 nm for 5 min using spectrophotometer (spectroUVS-2700/uvs-2800) and H_2O_2 consumption was calculated using extinction coefficient, 43.6 M^{-1} cm^{-1}.

Peroxidase (POD) activity was determined using the guaiacol oxidation method described by Chance and Maehly[20]. The reaction was initiated by adding 10 mM H_2O_2 to 3mL reaction mixture containing 10mM potassium phosphate buffer (pH 7.0), 8 mM guaiacol, and 50 µL enzyme extract. Within 5 min, due to the formation of tetraguaiacol there was increase in absorbance recorded at 470 nm. A unit of peroxidase activity was expressed as the change in absorbance per minute and specific activity as enzymes units per mg soluble protein (extinction coefficient 6.39mM^{-1} cm^{-1}).

Polyphenol oxidase (PPO) activity was determined by adding 0.2mL supernatant to 2.8mL of catechol solution (0.1mol/L). The absorbance at 410nm in three minutes was recorded at 25°C using UV-spectrophotometer (spectroUVS-2700/uvs-2800). One unit of PPO activity (U) was defined as the amount of enzyme that led to a change of 0.001 in absorbance per minute. PPO activity was calculated using the following equation according to Yang *et al.* [21].

$$PPO\ (U\ g^{-1}\ FW\ min^{-1}) = (\Delta A \times V_t) / (W \times V_s \times 0.01 \times T)$$

Where, ΔA: change in absorbance at 410nm; T: reaction time; V_t: total volume of enzyme extract; W: weight of sample; and V_s: amount of enzyme solution employed in the reaction.

3. Determination of heavy metals (Cd^{+2} andPb^{+2}**):**

Samples were prepared by wet digestion method[22] by adding 5mL HNO_3 to 1g dry plant powder and stirred well. Then, 4mL 33% H_2O_2 were added in a hood and stirred slowly. The flasks were heated on a hot plate for 7-8min after a strong effervescence was produced. Then washed the filtrate with 5mL HCI: H_2O (1:1) and diluted up to 25mL with distilled H_2O. The contents of Cd^{+2} andPb^{+2}were measured using atomic absorption spectroscopy (model Perkin Elmer, 2380A Analyst 100).

4. Statistical analysis:

The dissimilarities among plant variables were done, besides one way analysis variance (ANOVA-1) was used to evaluate the significant variations among different locations. Post-Hoc analysis (Tukey LSD test) was performed. Statistical tests were carried out through SPSS program (SPSS 2006).

RESULTS

1. Chlorophyll contents:

In the present study, the results for chlorophyll a and b illustrated in figure 1. All plants away from roadside were significantly different at P<0.05 and P<0.01 for chlorophyll a, b and carotenoids. All the results for Chlorophyll a, b and total contents increased by increasing the distance from rood side. For the leaf samples of Lettuce,there was a decrease in the chlorophyll content recorded for polluted sites (rood side) by 37.67% and 82.78% in chlorophyll a and b, respectively in comparison to control site.

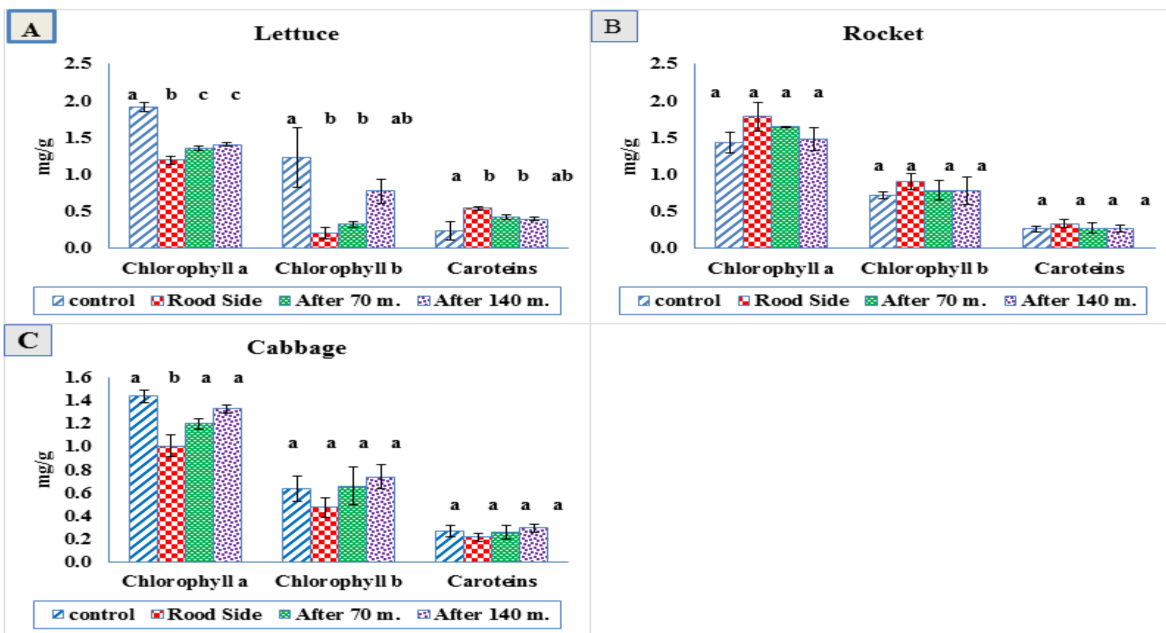

Fig. 1:Chlorophyll a, b and carotenoids (mg/g fresh weight) in leaves of studied plant samples collected at different distances from rood side (Mean ±SD). Different lowercase letters are significantly differing at (P < 0.05).

While carotenoids content behaves different pattern as it increased in roadside (0.54mg g^{-1}) compared to control samples (0.24mg g^{-1}).For the leaf samples of Rocket there was an increase in chlorophyll a and b contents for samples collected from rood sides by 25.21% and 25.56% respectively, compared to control. While carotenoid contents were increased by 27.55% in rood side samples compared to control. There was no significant difference recorded between the control and polluted sites in all the studied pigments for Rocket. For the leaf samples of Cabbage, Chlorophyll a and b contents decreased by 29.98% and 25.87%, respectively at polluted site in comparison to control site. While carotenoid contents decrease by 18.87% in comparison to control.

2. Enzymes :

The activity of CAT, POD and PPO has been reported to be changed under the air pollution. The present study showed that, the CAT activity of lettuce leaves recorded its highest value in samples collected from roadside (117.62U g^{-1} FW) which increased by 58.9 % in comparison to control.

Whereas, for rocket the highest value was 190.83U g^{-1} FW, while the cabbage leaf samples collected after 70 m from roadside recorded the highest value for CAT activity that increased by 38.89 % in comparison to samples collected from roadside (figure 2A).

The POD activity represented in figure 2B showed that lettuce leaves recorded the highest value 20.89U g^{-1} FW for samples collected from control compared to roadside samples. For rocket leaves, the highest value 45.31U g^{-1} FW was recorded for samples collected after 70 m from roadside revealing an increase by 193.64 % in comparison to control. Moreover, the cabbage leaves recorded the highest value 57.64U g^{-1} FW for samples collected after 140 m from roadside.

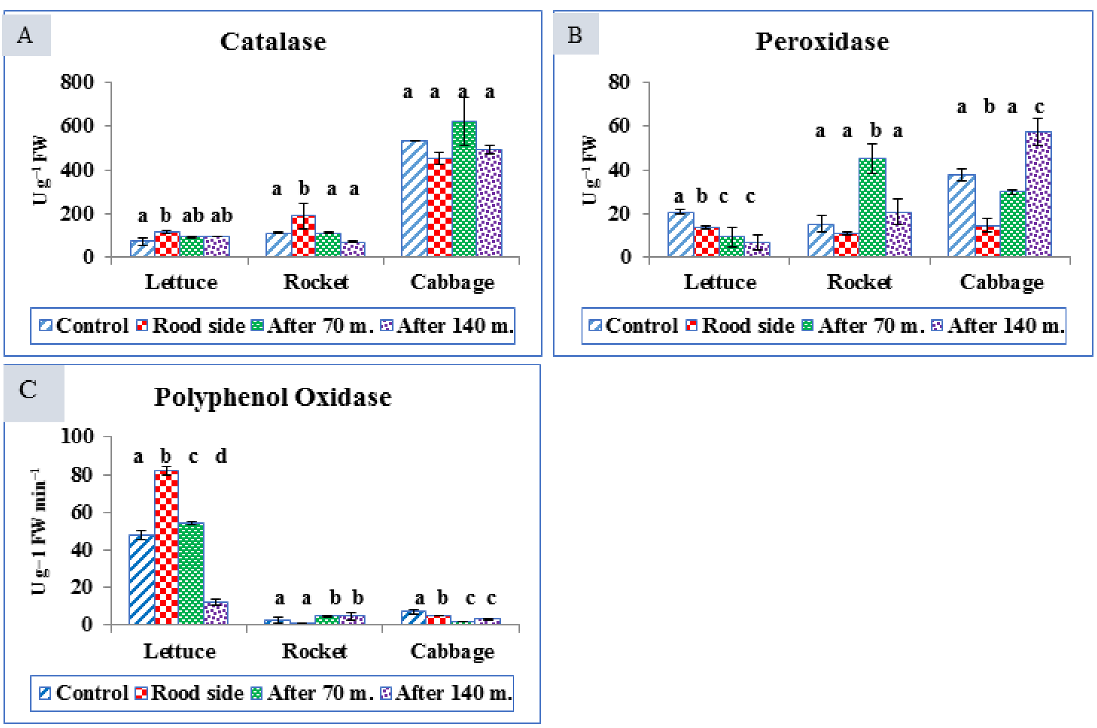

Fig. 2: Activity of some antioxidant enzymes {catalase (CAT), peroxidase (POD) and polyphenol oxidase (POD)} of leaf extracts for plant samples collected at different distances from roadside. Different lowercase letters are significantly differing at (P < 0.05).

The activity of PPO enzyme of lettuce leaf samples recorded the highest value $82Ug^{-1}$ FW min^{-1} for samples collected from roadside, contrarily rocket leaves recorded the lowest value at roadside samples 0.62U g^{-1} FW min^{-1} in comparison to control. While for cabbage leaves, the highest value was recorded in samples collected from control site revealing an increase by 29.9 % compared to roadside (Fig. 2C).

3. Heavy metals:

The concentration of studied heavy metals (Pb and Cd) represented in Figure (3). The results showed that lettuce roots collected from roadside recorded 300.1 and 23.49 (ppb) of Pb and Cd, respectively, and these were about 10 and 8 fold of the concentration recorded for control site. While for leaf samples the highest value 734.5 (ppb) was recorded for samples collected after 140m from roadside. In rocket, the highest concentration of Pb (227.5 ppb) was recorded in roots for samples collected from roadside. On the other hand, the highest concentration in leaf (351.5ppb) was recorded in samples collected after 140 m. from roadside. In cabbage roots collected from roadside the concentration of Pb and Cd was 153.1and 5.59 (ppb), respectively. While for leaf samples, the highest value was 80.4 and 5.49 (ppb) for Pb and Cd, respectively in samples collected from roadside.

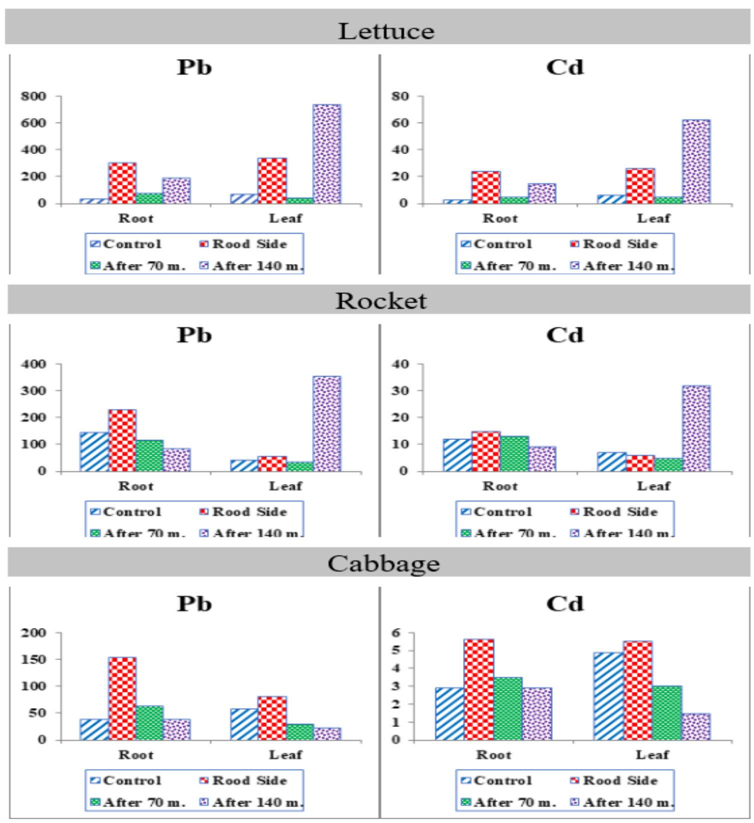

Fig. 3: Concentration of lead (Pb) and cadmium (Cd) expressed as ppb in root and leaf samples of studied plants collected at different distances from roadside.

DISCUSSION

Chlorophyll:

Plants Chlorophyll content is an indication of the photosynthetic activity and plant growth. In the present study, all of chlorophyll a, b and total chlorophyll contents were lower in polluted site (roadside) samples; and these contents were significantly increased by increasing the distance from roadside for Lettuce and Cabbage. These results agreed with Giri *et al* [23], theystudied the effect of air pollutants generated from the exhaust of industries and automobiles on the chrorophyll content of *Azadirachta indica*, *Nerium oleander*, *Mangifera indica* and *Dalbergiasissoo*, and found that chlorophyll a, b and carotenoids decreased in higher polluted sites as compared to non or less polluted ones. Similarly, studies of Mir *et al*.[24] who improved that high levels of automobile pollution decreases chlorophyll content in higher plants near roadsides. The possible explanation could be that, chlorophyll b content converted into chlorophyllide. Because of the high concentration of SO_2 in the air (released by vehicular emission) that eliminates the phytol group of chlorophyll b and also the denaturation of chlorophyll structure into the pheophytin occurs by substituting Mg^{2+} of chlorophyll molecule by the two H atoms in presences of high concentration of SO_2. Also, the destruction of thylakoid membrane structure might cause a decline in chlorophyll[25,26].

The increased concentration of air pollutants may exhibit an increase in chlorophyllase enzyme activity which could be a reason for reduction in chlorophyll content[26,27]. The plant species having low total chlorophyll content may be due to the alkaline condition created by the dissolution of chemicals present in the dust particulate in the cell sap[28]. On the other hand, Rocket leaves showed an increased content of all studied pigments in polluted site (roadside) compared to the control. This result agreed with that of Verma and Singh[29], who recorded reductions by 14–49% in Chl a content at higher pollution load in *F. religiosa*. In addition, the current results agreed with that of Kamble *et al*.[30] , who found higher chlorophyll content in *Delonix regia* collected from polluted site compared to non-polluted site. The possible explanation behind that could be that some plants are resistant to high air pollution levels.

Carotenoids in lettuce and Rocket showed an increase at samples collected from roadside compared to control. While carotenoids content in cabbage revealing a reduction in carotenoids compared to control; this results agreed with Abhishek *et al*.[31] who reported significant reduction in carotenoid contents in *Shorea robusta* and *Mallotus phillipinensis*, due to roadside automobile emission. Similarly, Samina *et al*.[32] also reported a reduction in carotenoids content in *Albizzia lebbek Benthdue* exposed to coal smoke, and Joshi and Swami[33] also reported Carotenoids contents of Mango (*Mangifera indica*) recorded at control and polluted sites were 2.97±0.41 and 2.42±0.60 mg gm^{-1} respectively. A decrease of 18.51% was recorded in the caratenoids amount in the polluted site as compared to control. Carotenoids had the primary function of shielding chlorophyll from photo-oxidant degradation[34]. Moreover, the difference in pigment content in the three studied plants as a response to traffic air pollution is related to the species of the plant. Some researcher has concluded that chlorophyll activity depends on the plant species[26,35].

Generally, the atmospheric PM reduces the total chlorophyll content but not at all times. The deposition of PM on the leaf surface can prevent the absorbed light thus decreasing the effective photosynthetic activity of plants, also stomatal clogging can decrease photosynthesis. The impact of PM accumulation on the leaf can primarily contribute to a reduction in total chlorophyll content of needle-leaved plants[36]. Moreover, Arkadiusz *et al.*[37] found that PM has a negative correlation with the chlorophyll content but that association is unique to each plant species. However, with the same environment condition, the level of decrease in chlorophyll is depended on the plant species[38].

Enzymes:

Stress such as air pollution enhances ROS formation in plant cells, resulting in oxidative stress[39]. The plant cells have several antioxidative enzymes to protect plants against these oxidative stressors such as (superoxide dismutase, glutathione reductase, catalase, peroxidase and polyphenol oxidase) and low molecular antioxidants such as ascorbic acid, glutathione, a tocopherol, flavonoids, and carotenoids[40]. Primary protection mechanism is offered by CAT which scavenges the product of oxidative stress and thus helps in amelioration of the adverse effect of oxidative damage[41]. The enzyme POD is an important enzyme, which governs the growth of plants. Pollution load increases CAT and POD activity which can be inferred to be due to ROS generated under stress caused by air pollution stress[42]. The scavenging properties of enzymes like CAT and POD[43] seem to be enhanced under pollution stress. In addition Rai[44] suggested that increased POD activity is a specific indication of SO_2 and NO_X pollution. Therefore, an increase in CAT and POD is considered to be linked with resistance to stress and self-defense as it shows variation with the plant being studied and also the level of pollutants[41].

In this study, an increase in catalase activity was recorded for lettuce and rocket leaf samples, which could be attributed to the dust deposited on the leaf or the content of heavy metals in such particles. This result is in agree with Rai[42] who found a significant and positive correlation of enzyme catalase and peroxidase with dust deposition of plant species. Pollution load increases catalase and peroxidase content and may be a function of ROS production in response to air pollution stress.

The activity of PPO enzyme of lettuce leaf samples showed an increase in samples collected from roadside. Contrarily the lowest activity of PPO found in rocket and cabbage leaves collected from roadside samples. This difference in enzyme activity may be based on plant internal physiological mechanisms, degree of plant tolerance and the amount of pollutants it perceive. In high polluted sites the enzymatic activity of CAT and POD was high and that pollution load leads to increased production of ROS, which in turn increases CAT activity. In addition, this is considered to be a physiological adaptation for reducing the level of H_2O_2 to sub toxic levels during metabolism[42].

Heavy metals:

Airborne heavy metals when deposited on soil are taken up by the plants through their root system and translocated to other parts of the plant through an active uptake mechanism. Therefore, plants growing along the roadside may work as phytoremediators of airborne metals released from vehicles and street dust[41].

The present study showed that lettuce roots collected from roadside had concentration of Pb and Cd 10 and 8 fold, respectively compared to control site, and for rocket root collected from roadside the concentration of Pb recorded 1.5 fold compared to control. While, for cabbage roots the concentration of Pb and Cd for samples collected from roadside was 4 and 2 fold, respectively compared to control. Moreover, the highest concentration in leaf was about 9 fold of the control concentration in samples collected after 140 m. from roadside. In addition, Wang *et al.*[45] indicated that Zn, Pb and Cu declined logarithmically with distance from the roadside but concentrations were unrelated to traffic density. Generally, elevated Pb at the roadside may represent a legacy of the use of leaded petrol, deposition from brake wear or diesel exhaust emissions. While, cadmium being a metal generally associated to oil or fossil fuel combustion.

It is known that, smaller particles have higher loading of heavy metals and PAH (poly aromatic hydrocarbons). Therefore smaller particles will generate higher toxicity in cells and induce higher antioxidative response compared to larger particles[17], which may explain the higher concentration of Pb and Cd in leaves of lettuce and rocket samples collected after 140m from roadside that the smaller particles are more lighter and could be carried to greater distance from rood side.

Heavy metals in foliar dust can enter the plant leaf tissue and eventually accumulate in the plant body through foliar uptake[46]. The main way for heavy metals in foliar dust to enter the leaves include adsorption and internalization through the cuticle and penetration via stomatal pores.

Plants of different species have different ability to bioaccumulate and transport heavy metals. Plants of different species have different morphological characteristics, such as the density and size of leaf stomata, the density and length of trichomes, the roughness of leaf surface, and the surface wax, significantly affecting the foliar uptake of heavy metals[15]. Therefore, whether the metal elements of plant leaves are transported through root uptake or directly absorbed from foliar dust through foliar-uptake. The species identity plays a major role in the whole process. The foliar dust, rather than soil, explained more variation of elemental composition of plant leaves. By exposing lettuce and rye grass to either a contaminated atmosphere or contaminated soil, Li *et al.*[47] found that Pb accumulation via foliar uptake was higher than that via root uptake. Using isotope tracing, He and Gao[48] confirmed that atmospheric particulate matter was the major source of Pb in Chinese cabbage leaves. The present study further supported the important role of foliar dust in shaping the elemental composition of plant leaves through a field experiment involving multiple sample plots, multiple plant species, and multiple detected elements in urban soil-plant systems. Future research needs to be carried out to reveal the effects of atmospheric particulate matter and foliar dust on food crops, especially leafy and fruiting vegetables, in agricultural ecosystems, which is relevant to food security and biological health.

Conclusions

A considerable amount of damage is caused to plants as a result of PM or dust deposition, even though plants have some stress-tolerant mechanisms that may cause inhibition of photo-synthetic activities and antioxidant enzymes. Each of the investigated plants showed specific response to the traffic pollution owing to stress-tolerant mechanisms of each plant species.

Moreover, the physiological, and biochemical responses of plants to PM pollution can be viewed as potentially adaptive changes. Evaluation of physiological and biochemical changes in plants on exposure to PM pollution is an important step to monitor the tolerant plants to pollution.

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الإستجابة الفسيولوجية والبيوكيميائية للجسيمات الناتجة عن الحركة المرورية فى بعض الخضروات

داليا فهمي سليمه١ هدير محمد نجيب*١ محمد احمد زايد١ ياسر حسن ابراهيم٢
١- قسم النبات والميكروبيولوجي ، كلية العلوم ، جامعة المنوفية ، مصر.
٢- قسم البيئة ، المركز القومى للبحوث، الدقى، مصر.
*البريد الالكترونى للباحث الرئيسى: hadeer_nageeb@yahoo.com

المستخلص

زياده الكثافه المروريه تعد واحده من أهم العوامل التي تعمل علي زياده الجسميات في الهواء وتلعب النباتات الخضراء دور رئيسي في تقليل تلك الجسيمات من الهواء. تهدف هذه الدراسه إلي معرفه تاثير التلوث الناتج عن المركبات علي بعض النباتات المجاوره للطريق محل الدراسه وذلك عن طريق إختيار ثلاثه نباتات مزروعه وهي الخس والجرجير والكرنب البلدي. حيث تم جمع هذه النبات علي مسافات مختلفه من الطريق المروري و بدراسه بعض العوامل الفسيولوجيه والبيوكميائيه متمثلة في محتوي الصبغات وبعض الإنزيمات المضاده للأكسده وكذلك بعض العناصر الثقيله، أظهرت النتائج اختلافات ذات أهمية بين العينات النباتيه التي تم جمعها من المسافات المختلفه من الطريق بالمقارنه مع الكنترول. وأيضا أشارت النتائج أن الإختلافات في العوامل التي تم دراستها في كل من الجذور و الاوراق للنباتات محل الدراسه كانت معتمدة علي كميه الملوثات والتي تعتبر مؤشرا علي تلوث الهواء او مؤشرا علي التدمير الفسيولوجي للنباتات قبل ظهور أعراض مظهريه علي النباتات.