Journal of Plant Process and Function, Vol. 9, No. 40, Year 2021

Research Article

Effect of air pollution resulting from exhaust emission on the morphological, physiologic and biochemical responses of lettuce (*Lactuca sativa* var. longifolia)

Amin Kohan[1], Maryam Haghighi*[1], Nourollah Mirghaffari[2] and Mohammad Hossein Ehtemam[3]

[1] Department of Horticulture- College of Agriculture- Isfahan University of Technology, Isfahan, Iran
[2] Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran
[3] Department of Agronomy and Plant Breeding, Collage of Agriculture, Isfahan University of Technology, Isfahan, Iran
(Received: 06/04/2019 Accepted: 07/07/2020)

Abstract

Most of leafy vegetables grow near cities and exhaust emission affects their morphological, physiologic and biochemical attributes of them. The experiment was conducted in a factorial experiment based on a completely randomized design with three replications in the greenhouse of the Isfahan University of Technology of Iran. Lettuce seedlings were exposed to exhaust emissions or pollution in three different exposure times (10, 20 and 30 days). Then, their morphological, physiological and biochemical traits were measured and compared with the control plants. Results showed that in plants that were exposed to pollution for 30 days, the catalase, ascorbate peroxidase, guaiacol peroxidase activity, proline content, electrolyte leakage, relative water content and the number of stomata significantly increased. It was observed that the photosynthetic factors, leaf area, plant height, shoot fresh and dry weight and length of stomata in lettuce plants decreased after 30 days, compared to the controls. The photosynthetic rate, stomatal conductance, CO_2 intracellular concentration and leaf area of lettuce seedlings increased when exposed to emissions for 10 days. Generally, the results showed that the exposure of lettuce plants to pollution, especially after 30-days, caused more morphological (leaf area, plant height), physiological (catalase, ascorbate peroxidase, guaiacol peroxidase activity, proline content, electrolyte leakage, relative water content, photosynthetic rate, stomatal conductance, CO_2 intracellular concentration) and anatomical (stomata density) changes due to contaminants.

Keywords: Guaiacol peroxidase, City pollution, Leafy vegetable

Introduction

With the industrialization of communities in many developing countries, the concentration of primary and secondary pollutants in the atmosphere has increased (Mage *et al*., 1996). Vehicles increase air pollution levels in urban centers more than 75% of the overall pollution (Breusgem *et al*., 2001). Airborne gaseous pollutants, especially sulfur dioxide, nitrogen dioxide and, secondary photochemical oxide and ozone, can have many adverse effects on the livelihoods and welfare of producers and consumers (Rai *et al*., 2011). The response of plants to air pollutants may be very different, and the differences depend on the concentrations of contaminants and time distribution, genetic origins and physiological activity of plants, meteorological factors, plant nutrition status and other environmental factors (Atri *et al*., 2005). Exposure to a single pollutant, without the interference of other pollutants, rarely occurs under the conditions of the real environment, while the combination of several pollutants, either sequentially or simultaneously, is by far the most real (Kostka-Rick and Mannin, 1993). Estimating the effects of air pollutants is difficult because organisms are exposed to a wide range of uncontrolled variables (parasites, climatic conditions, blend of pollutants) (Kostka-Rick and Mannin, 1993).

Aconsiderable amount of cultivated lettuce, are in outdoors and on the side of the roads or contaminated urban areas but little research has been done on the effect of vehicle pollutants on lettuce. The effects of some vehicle exhaust gases on physiological and morphological characteristics of lettuce were investigated in this study. Researches have shown that many pollutants in exhaust gases in high concentrations can be destructive for plants (Adrees *et al*., 2016). Pollutants can cause leaf damage, stomatal closer, premature aging, photosynthetic effects, membrane permeability impairment, and loss of growth and yield in sensitive plant species (Verma and Singh, 2006; Tiwari *et al*., 2006). Iqbal *et al*. (2010) showed that the number of leaves per plant in the contaminated area was slightly increased, which could be a compensatory mechanism adapted to the plants under stress, but its

*Corresponding Author, Email: mhaghighi@cc.iut.ac.ir

leaf size decreased that the leaf area compared to the control area significantly decreased. Leaf dry weight was significantly reduced under the stress of contamination (Iqbal *et al*., 2010). Air pollutants can affect stomatal conductance (Verma and Singh, 2006). The air pollution stress causes the formation of active oxygen species in plant cells, which in turn causes oxidative stress and affects the biochemical processes of the plants and decreases plant tolerate to other stress as well (Miller *et al*., 2010; Rai *et al*., 2011). Pollution reduced the levels of chlorophyll a, chlorophyll b, carotenoids, ascorbic acid, relative leaf water content, and contamination tolerance index (Chukwu and Adams, 2016). The number of stomata and epidermal cells in the plants grown along the busy roads of Khartoum, Sudan, increased due to the impact of vehicle emissions (Achille *et al*., 2015). Mandal (2006) showed that roadside plants including *Nerium indicum* Mill., *Boerhaavia diffusa* L., *Amaranthus spinosus* L., *Cephalandra indica* Naud, and *Tabernaemontana divaricata* L. were able to avoid the effects of air pollution by changing their physiological pathways associated with photosynthesis and respiration (Mandal, 2006). Plant leaves of *Picea pungens* were exposed to automobile exhaust fumes for 30 days and anatomical structures, such as epidermis, cuticle, ladder parenchyma and spongy parenchyma, were altered when exposed to high concentrations of 0.4 mg/m^3 of vehicle exhaust (Qin *et al*., 2014).

Material and methods: The experiment was conducted in the factorial plan based on a completely randomized design with three replications in the greenhouse of Isfahan University of Technology (Longitude 18 ´ 7 ° 23 ° North, latitude 2 ´53 ° 51 east). For treatment management, 12 small chambers (length: 100 cm, width 70 cm, and height: 70 cm) and an exhaust generator with commercial lead-free gasoline for transferring the contaminants to the chambers were constructed.

The analysis of pollutants and their concentration was presented in table 1, using theVARIO PLUS MRU device to analyze the output gases and to determine the concentration of contaminants from burning gasoline.

The seeds of *Lactuca sativa* var. *longifolia* were cultured in plastic pots (20 cm diameter, 18 cm height) which were filled with sand and soil mixtures of 1:2 ratio. The soil texture in this study sandy-loamy contained 68.05% sand 17% clay, 14.95% silt with pH of 6.32 and an EC of 671 µs/m was used .Lettuce seedlings transferred to the chambers in 4-5 leafy stage, and exposed to the gases listed in the table above for three hours once in the morning and once in the afternoon (based on peak weather hours of urban air pollution) in three different exposure times (10, 20 and 30 days) separately. After these three hours, the greenhouse plastic was removed to ventilate. At the final of the experiment in every gas exposure time, their anatomical, physiological and morphological traits of plants were measured and compared with the control plants that were not exposed to gases. The relative water content was measured by the method of Ritchie *et al*. (1990). The relative water content was determined by using 7 mm leaf discs. The leaf discs of each treatment were weighed (FW). Then, they were hydrated until saturation (constant weight) for 48 h at 5°C in darkness (TW). The leaf discs were dried in an oven at 105°C for 24h (DW). RWC was calculated according to the following formula:

$$RWC\% = (FW - DW)/ (TW - DW) \times 100$$

Electrolyte leakage was measured by Lutts *et al.* (1995). Samples were cut into equal-sized pieces (0.3 g per treatment) and placed in 25 mm culture vessels containing 15 ml of distilled water and shaken at 100 rpm on an orbital shaker for 24 h at room temperature. The initial conductance of the bathing solution was measured using a conductivity meter. Then, the tubes were autoclaved at 115°C for 10 min and final readings were taken following autoclaving and additional 24 h incubation at room temperature. EL (%) was calculated as initial measurements/final measurements 100.

The total chlorophyll content and carotenoid content of leaves were extracted by 100% acetone solvent method (Biddington and Dearman, 1985). The absorbance of light at 661.6 and 168.8 for total chlorophyll and 470 nm for carotenoids were read (Lichtenhaler, 1987). The chlorophyll fluorescence concentration was measured by a chlorophyll meter machine (OS-30P model manufactured by Opti-science, UK) and the Fv/Fm ratio (photochemical effect of photosystem II) was reported. Factors related to leaf gas exchange (photosynthesis rate, stomatal conductance and CO_2 intracellular concentration) were measured by a portable photosynthesis measurement (LCi model manufactured by ADC Bioscientific Ltd, UK). The amount of proline was measured using the Bates method (1973). Catalase activity was measured using the modified Aebi method (1984) at 240 nm wavelength (Aebi, 1984). Ascorbate peroxidase activity was estimated by modified Nakano and Asada (1981) by absorbance reduction at 290 nm. The activity of the guaiacol peroxidase enzyme was determined by the Chance and Maehli method (1955) with a slight change. Anatomical traits were observed by optical microscope and measured through the Edn-2 software. Statistical analysis of data of all traits was performed using statistical software Statistix 8 and comparison of meanings performed based on minimum significant difference test (LSD) at 5% probability level.

Results

The effect of exhaust gases on morphological characteristics of lettuce: Height, fresh and dry weight of the aerial part and leaf area of lettuce plants, decreased when exposed to exhaust emissions after 30 days, around 11.05%, 12.66%, 36.84% and 5.81%, respectively. The dry weight of the aerial part and leaf area of plants exposed to contaminants for 10 days increased significantly by 16.5% and 7.26%,

Table 1. The analysis of pollutants and their concentration

NO (ppm)	NO_2 (ppm)	NOx (ppm)	SO_2 (ppm)	CO_2 (%)	CH_4 (mg/m3)	CO (ppm)	O_2 (%)	Gas contents
0	1	1	22±3	0/7	7	105±10	20	Concentration

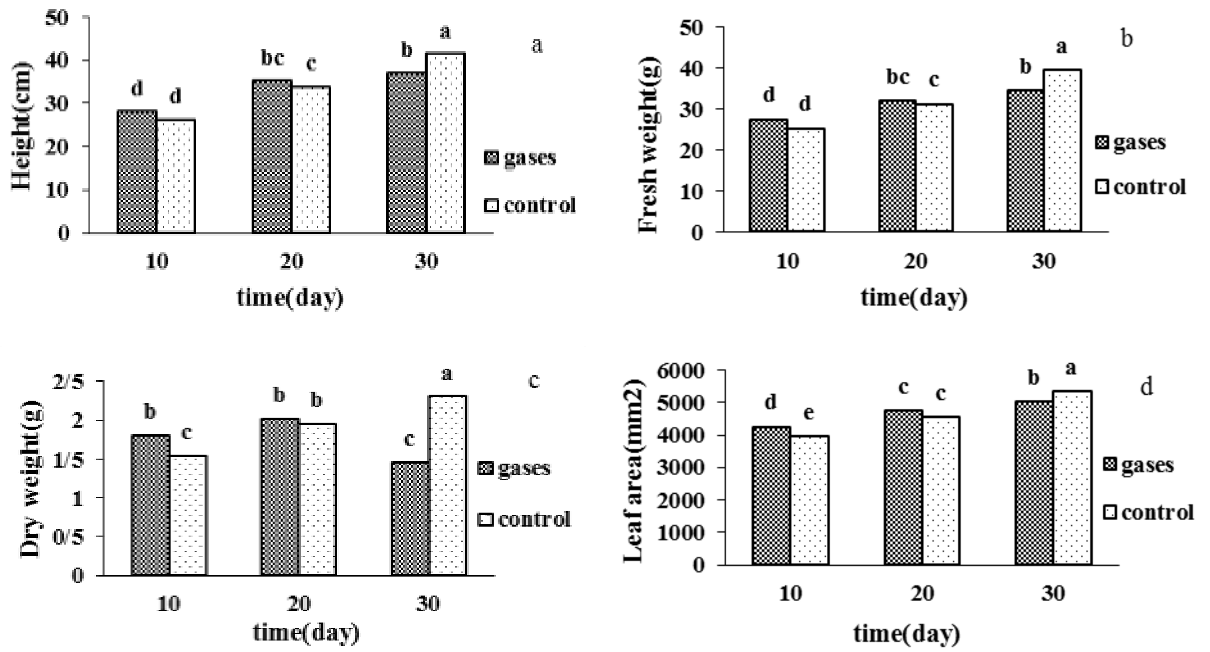


Figure 1. The effect of gasses and time of exposure on height (a), fresh weight (b), dry weight (c) and leaf area (d) of lettuce

respectively, compared to the control plants (Figure 1 a,b).

The effect of exhaust gases on physiological characteristics of lettuce: The amount of relative water content and ion leakage of leaves of plants exposed to exhaust gases for 30 days increased by 10.15% and 24.44%, respectively, compared to the control plants (Figure 2 a,b).

Significant decrease in total chlorophyll content of plants exposed to exhaust emissions for 20 and 30 days was observed in 11.51% and 29.17% compared to the control plants, respectively. There was no significant difference in the carotenoid content of plants in all three exposure times compared to the control plants. Significant decrease in chlorophyll fluorescence amount in lettuce plants exposed to emissions for 20 and 30 days was observed in comparison with the control plants by 2.43% and 2.74% respectively (Figure 3 a,b,c).

The net photosynthetic rate, stomatal conductance and CO_2 intracellular concentration of plants exposed to exhaust gases for 30 days decreased significantly by 35.66%, 36.68% and 9.61%, respectively, compared to the control plants. The net photosynthetic rate and the stomatal conductance of plants exposed to contaminants for 10 days increased significantly by 16.07% and 28.98%, respectively, compared to the control plants. Also, CO_2 intracellular concentration of plants exposed to exhaust gases for 10 and 20 days significantly increased by 6.30% and 6.81%, respectively, compared to the control plants (Figure 4 a,b,c).

Proline content of lettuce leaves exposed to gasses for 10 and 20 days was not significantly different from that of the control plants, but a significant increase of 54.34% in proline accumulation of the plants that had 30 days' exposure to emissions was shown in comparison with the control plants. The activity of catalase, ascorbate peroxidase and guaiacol peroxidase enzymes in plants exposed to exhaust gases for 20 and 30 days showed a significant increase compared to the control plants. In the 10-day exposure, plants showed a significant increase in the activity of the ascorbate peroxidase and the guaiacol peroxidase enzyme compared to the control plants (Figure 5a, b, c, d).

The effect of exhaust gases on anatomical characteristics of lettuce: The number of stomata of lettuce that was exposed to emissions for 10 days did not change significantly compared to the control plants, but a significant increase in the number of stomata of plants for 20 days and 30 days were exposed to the contaminants, were 14.56% and 71.04%, respectively, compared to the control plants. The length of stomata of the plants exposed to the gases for 20 and 30 days significantly decreased than the control plants (Figure 6 a,b).

The stomata in the control plants and the plants which had the least exposure time to the pollutants were completely open. But the stomata in the plants which had the most exposure to the contaminants showed high degrees of closure (Figure 7 a,b,c). According to the microscopic images, it was observed that the size of

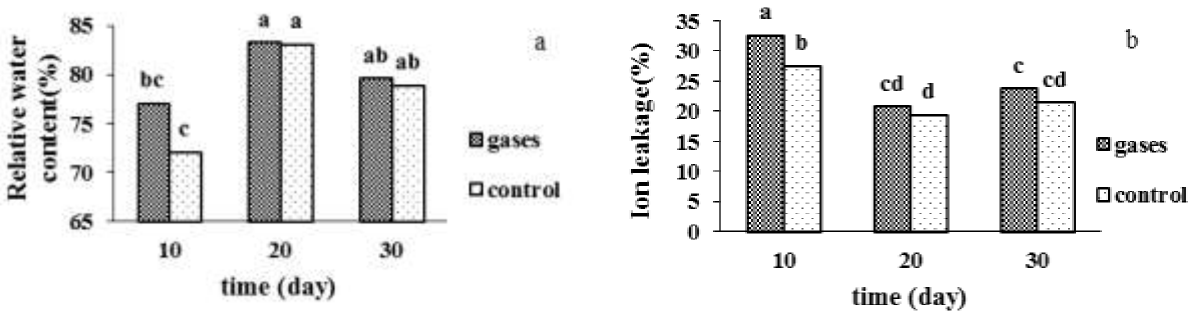


Figure 2. The effect of gasses and time of exposure on relative water content (a) and ion leakage (b) of lettuce

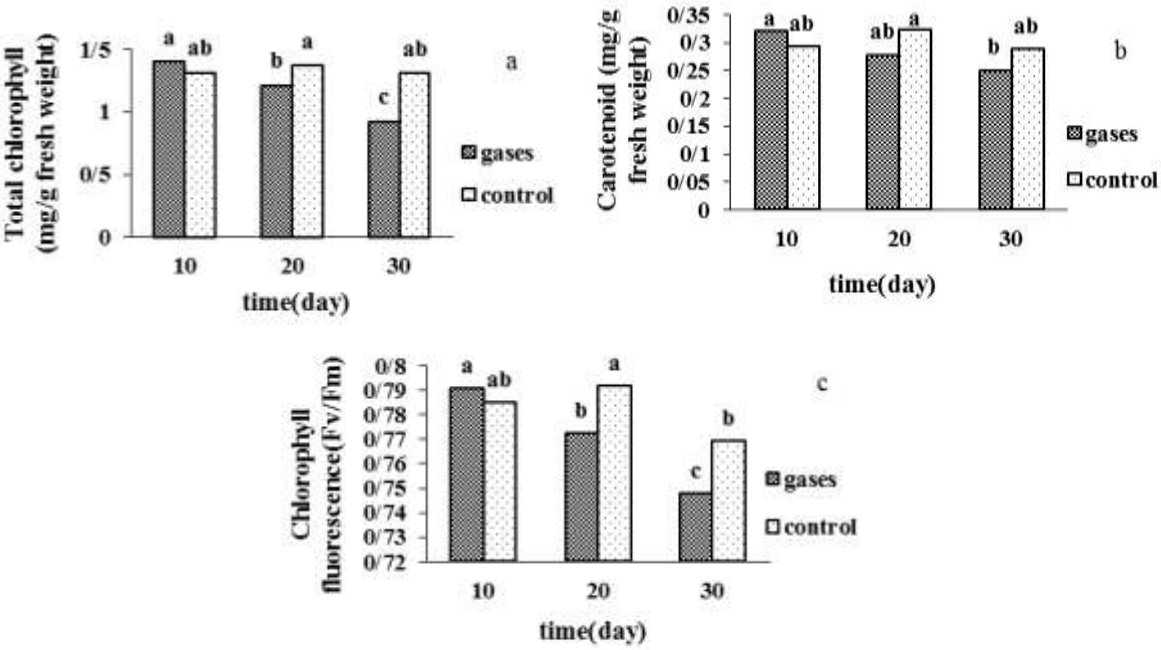


Figure 3. The effect of gasses and time of exposure on total chlorophyll (a), carotenoid (b) and chlorophyll fluorescence(c) of lettuce

parenchymal cells and the diameter of xylem opening in plants that had the highest exposure time to the emissions increased compared to the controls and 10 days of exposure plants. In contrast, the number of xylem in plants that were exposed to emissions for 30 days decreased compared to the 10 days exposure plants and the control plants (Figure 8 a, b,c).

Discussion

Height: John *et al*. (2006) showed that toxic compounds in exhaust pollutants reduced plant growth. They showed that the lowest plant height of tomatoes was related to plants near to the source of contamination. They expressed that pollutants inhibited plant growth (Chukwu and Adams, 2016). The height of rice and mustard plants under the stress of air pollution was significantly less than the control plants (Chauhan and Joshi, 2010). A decrease in the growth of plants exposed to urban pollutants compared with the control plants has been reported and it has been reported that this decline in plant growth is because of polutant that are present in vehicle pollutants (Leghari and Zaidi, 2013).

The fresh and dry weight of the aerial part: Reduction of stem fresh weight may be due to the prevention of chlorophyll formation and leaf tissue damage (Chauhan, 2010). It has been shown that toxic substances in smoke decreased reduced the growth rate and thus reduced the weight of tomato plants exposed to the source of smoke production (Chukwu and Adams, 2016). Reduction in the leaf surface leads to a decrease in the amount of absorption of radiation, followed by a decrease in the amount of photosynthesis and loss of leaf dry weight (Tiwari, 2006). Khan and Khan (1993) stated that decrease in the amount of photosynthesis and loss of leaf dry weight (Tiwari, 2006). Khan and Khan (1993) stated that a decrease in photosynthetic activity and poor plant growth apparently reduced dry matter and yield performance. Chukwu and Adams (2016) showed that the dry weight of tomato plants that had the closest distance to the pollutant source showed a significant decrease compared to the control plants.

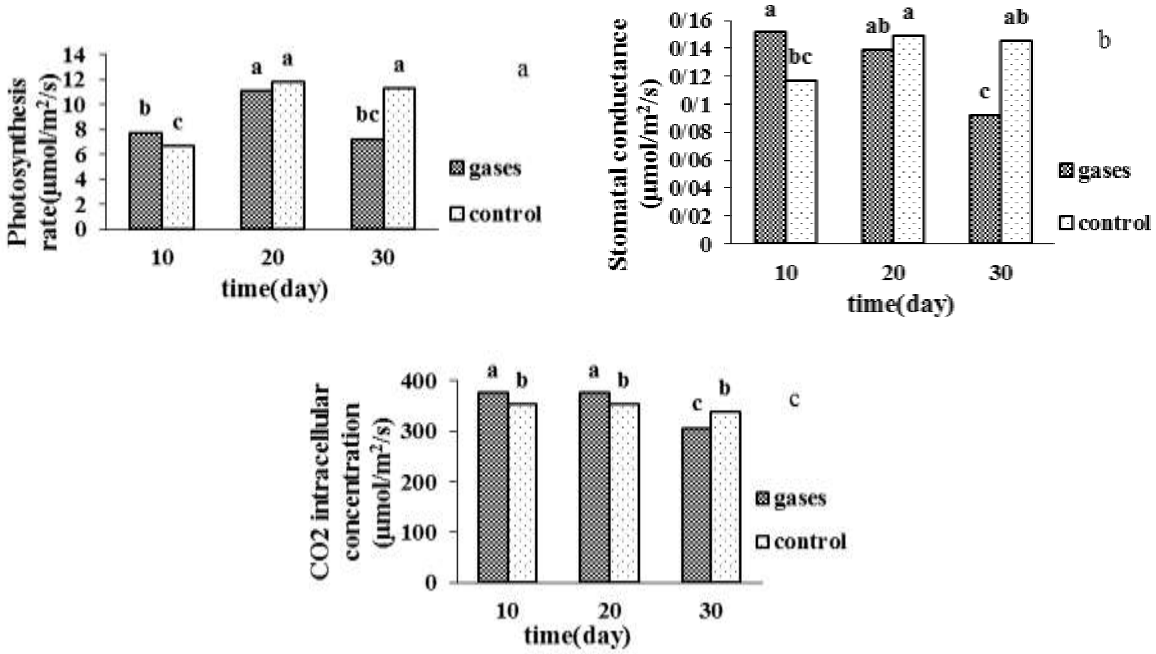


Figure 4. The effect of gasses and time of exposure on photosynthesis rate (a), stomatal conductance (b) and CO_2 intracellular concentration (c) of lettuce

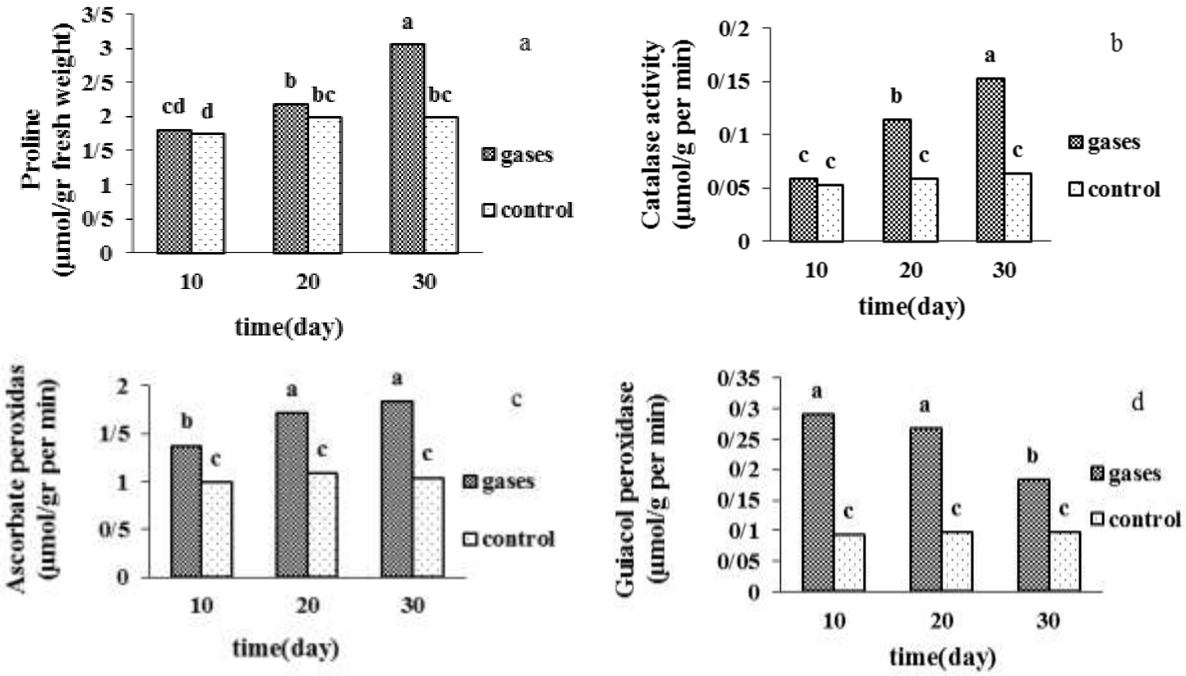


Figure 5. The effect of gasses and time of exposure on proline (a), catalase activity (b), ascorbate peroxidase (c) and guaiacol peroxidase (d) of lettuce

Leaf area: Reduction in leaf area due to air pollution can affect the plant's ability to photosynthesis and finally its ability to adapt to the stress of air pollution (Seyyednejad and Koochak, 2011). The plant may reduce its leaf area as a defense mechanism to limit the level of exposure to airborne contamination (Jochner *et al*., 2015). The reduction of leaf area in leaves of *Albizia lebbek* was reported under the stress of air pollution (Seyyednejad *et al*., 2009). It has been shown that the leaf area of plants that had the furthest distance from the source of contamination was higher than those which were close to the source of contamination (Chukwu and Adams, 2016). Areington *et al*. (2015) showed that the leaf area of plants that had the closest distances to pollutants had a significant decrease compared to plants located at distant distances.

The leaf relative water content: The plants exposed to pollution. Significantly increased the water

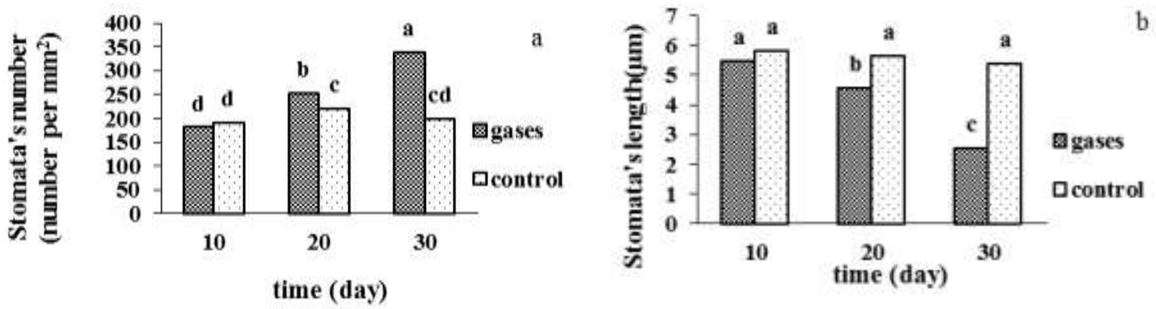
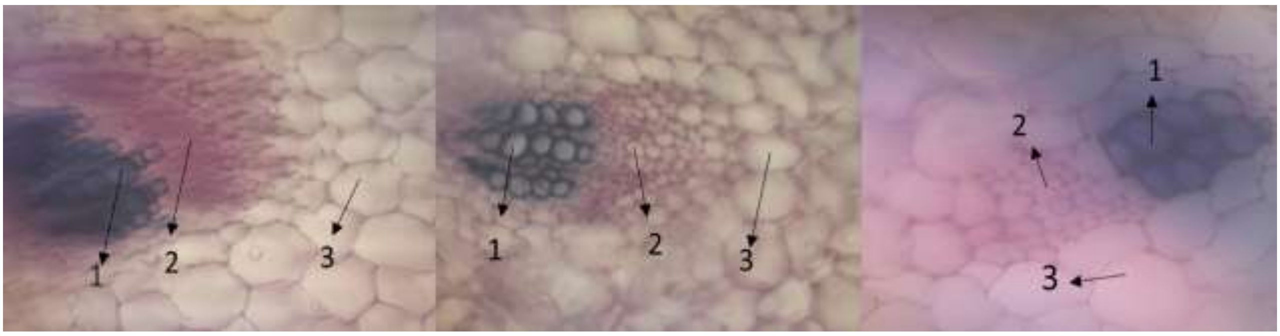


Figure 6. The effect of gasses and time of exposure on stomata's number (a) and stomata's length(b) of lettuce

a b c

Figure 7. Microscopic image of stomata in control plants at 40 × magnification (a), stomata exposed to the gases for 10 days with the magnification of 40 × (b), stomata exposed to the contaminants for 30 days with the magnification of 40 × (c).



a b c

Figure 8. The image of the xylem (1), phloem (2) and parenchyma (3) of control plants with the magnification of 40 × (a), 10-days exposure plants (b) and 3٠-days exposure plants (c)

content of the root, stem and leaf components (Kammerbauer and Dick, 2000). High water content in the plant will help maintain a physiological balance of the plant under stress conditions such as exposure to air pollution when transpiration is usually high and may lead to drying (Singh *et al*., 1995).

Electrolyte leakage: Electrolyte leakage is used as an indicator of damage to the membrane by Reactive oxygen species (ROS) (Valavanidis *et al*., 2006). Probably prolonged exposure of lettuce plants (30 days) subject to contaminants, has produced ROS and caused oxidative damage in them, and damaged their cell membranes. Therefore, more electrolyte was removed from the membrane. Pearson and Henriksson (1981), in a laboratory study, showed that ion leakage levels were higher in plants exposed to contamination than the control plants. Areington et al. (2015) also showed that the ion leakage rate in plant leaves of *Brachylaena discolor*, which had the closest distance to contaminated industrial areas, was more than control plants.

Photosynthetic pigments: Air pollutants enter the tissues through the stomata, resulting in malformed chloroplasts and reduce the content of pigments in infected leaf cells (Seyyednejad and Koochak, 2013). Free radicals produced during contamination could contribute to the decomposition of photosynthetic pigments and thus the reduction of pigmentation (Sairam *et al.,* 1998). Chlorophyll a, b and carotenoid levels in leaf samples collected from infected areas were significantly lower than samples collected from the control areas (Giri *et al*., 2013; Stevovic *et al*., 2010). The significant loss of total chlorophyll in contaminated plants indicates that chloroplasts are the main source of attack by air pollutants such as SO_2 and NOx. Pollutants such as SO_2, NO_2 and O_3 damage the membranes and related molecules, including chlorophyll pigments (Ramakrishnaiah and Somashekar, 2003). Carotenoids act as auxiliary pigments in plants. Carotenoid helps chlorophyll and protects it from damage caused by photooxidation (Joshi and Swami, 2009). The amount of carotenoid of lettuce plants exposed to contaminants for 10, 20 and 30 days did not show any significant difference in comparison with the control plants (figure 3b), which was consistent with the results of Giri *et al*. (2013), which showed that the amount of carotenoid of plants *Azadirachta indica*, *Nerium oleander Mangifera indica* and *Dalbergia sissoo* that was exposed to vehicle pollutants did not show any significant change. Besides, Saquib *et al*. (2010) showed that the destroy of carotenoids to air pollution was 4.5 times lower than chlorophyll a.

Chlorophyll fluorescence: Glaz *et al*. (2004) stated that as the result of the closure of the stomata due to contaminants, the carbon dioxide capture is limited and the photosynthesis, and Fv/Fm ratio was decreased. Muneer *et al*. (2014) reported a decrease in Fv/Fm ratio in strawberry plants exposed to CO, NOx, and SO_2 compared to the control plants. They stated that the reduction of Fv / Fm under the stress of dangerous gases may be due to the inactivation of photosystems reaction centers that receive the initial amount of light energy and cannot be exploited due to the presence of oxidative stress. Researchers showed that chlorophyll fluorescence levels of lichen plants in areas with higher contamination levels were lower than those with lower levels of contamination (Muneer *et al*., 2014).

Leaf gas exchanges: The decrease in factors related to the gas exchange of leaf, maybe due to physical damage to the stomata due to the presence of high CO, NO_2, SO_2, and PM10 in ambient air (Aebi, 1984). Saquib *et al.* (2010) reported a significant decrease in photosynthesis rate and stomatal conductance in *Croton bonplandianum* BAILL. in contaminated areas compared to the control area. Pourkhabbaz (2010) showed that the urban environment contributed to the structural characteristics of the plane leaves and reduced the photosynthesis by reducing the leaf area, reducing the density and width of the stomatal pores. The previuse study showed that in *Kandelia candel*, reduction of stomatal conductance under stress conditions could be related to decreasing the number and density of leaf stomata of the plant (Qiu *et al*., 2007). Also, a study on some plants has shown that lower CO_2 intracellular concentration should be accompanied by lower stomatal conductance (Rivelli *et al*., 2002). Reduction of stomatal conductance is the main way of reducing the water loss of leaves in exposure to stress, which is associated with lowering the entrance of carbon dioxide into the leaves and subsequently reducing photosynthesis. Air pollution stress leads to the closure of stomata, which in turn reduces the availability of CO_2 in leaves and prevents carbon stabilization (Glaz *et al*., 2004).

The gas exchanges of lettuce plants increased with the lowest exposure time of pollution compared to the control plants. Kammerbauer and Dick (2000) showed that the presence of exhaust gases produced a 15% increase in the production of photosynthetic active pigments. The increase of chlorophyll may be due to NO_2 absorption of nitrogen precursors. Besides, sulfur, which is part of the compounds in the pollutants, is a structural component of amino acids, proteins, vitamins and chlorophylls (Maugh, 1979; Yang *et al*., 2006). According to the results of current research, it seems that the pollution composition in the gases compounds in the gases are likely to increase the number of photosynthetic pigments in lettuce plants in the first 10 days, and thus the photosynthesis rate is also decreased.

Proline: Seyyednejad and koochak (2013) have shown that the increase of proline levels in *Prosopis juliflora* increased its resistance to pollution stress and reduced its damages due to pollution. Proline has been reported to act as a free radical cleaner and protects plant proteins and membranes against damage from oxidative stress (Wang *et al*., 2009). Wang (2009) has shown that the presence of SO_2, carbon monoxide and other pollutants in the content of vehicle exhaust gases increases the proline content of the plants. Agbaire and Akporhonor (2014) reported an increase of proline content in the plants near the contaminated areas compared to the control areas. Patidar *et al*. (2016) showed that the proline content of plants exposed to urban pollutants was significantly increased compared with the control plants.

Catalase activity: Rai (2016) showed that the catalase activity of roadway plant species increased compared to the non-contaminated areas. He has stated that this increase was due to the production of ROS due to the presence of pollutants. In a study by Xue *et al*. (2013), it was shown that catalase activity in *Buxus Sinica* plants adjacent to vehicle contaminants significantly increased compared to control plants. The more activity of the catalase enzyme in the contaminated site can be attributed to the presence of environmental pollutants which strongly have produced H_2O_2 in these species under oxidative stress (Farombi *et al*., 2007).

Ascorbate peroxidase: The ascorbate peroxidase enzyme acts as a sweeper of active oxygen species, and it seems to be produced to protect plants against the active forms of oxygen (hydrogen peroxide) when exposed to plants. (Miyake *et al*., 1993). Mehlhorn *et al*. (1987) has shown that ascorbate peroxidase activity in *Pisum sativum* var. *Waverex* in a mixture of pollutants (SO_2, NO_2, and O_3) was significantly increased compared to the control plants. Atri *et al*. (2005) showed a significant increase in the activity of the ascorbate peroxidase enzyme *in Lavandula vera* and *Rosmarinus officinalis* L. plants grown in contaminated areas compared to the control plants.

Guaiacol Peroxidase: Increased activity of peroxidase in response to vehicle exhaust emissions has been reported by researchers (Emberson *et al*., 2003). Rai (2016) showed that the peroxidase activity of roadway plant species increased compared to the non-polluted areas. He has said this increase could be due to the production of ROS by contaminants. A study on the effect of air pollution in Tehran on acacia showed that acacia with its physiological mechanisms such as anthocyanin increase and increase activity of peroxidase enzyme could show a suitable response to air pollution (Maddah *et al*., 2015).

The number of stomata: Stomatal compression fluctuation is a response to environmental stresses and an important way to control the absorption of pollutants (Verma *et al*., 2006). Increasing the number of stomatal cells on the surface of the leaves the in population's leaves can be a physiological development by plant species in order to remain healthy in the unpleasant environmental conditions created by the high rate of pollution in the area (Sukumaran, 2014). Sukumaran (2014) showed that the number of stomata significantly increased in plants in contaminated areas, which may be an adaptive feature to reduce the damage caused by air pollutants. The results of the study of Rai *et al*. (2004), also showed that the stomata in the plants of *Terminalia arjuna and Quisquali indica* increased in contaminated areas. They stated that a number of plants are resisting to air pollutants by increasing their stomata's number.

Stoma's pore length: Sharma and Butler (1973) according to microscopic studies, have reported a minor reduction in pore size of the stoma in plants grown in contaminated areas. According to their research, the decrease in the stomata's pore size of plants in the contaminated areas can be considered as a suitable adaptation, which may help to reduce the absorption of gas contaminants. It has been shown that stoma's pore length in *Muntingia calabura* and *Ixora coccinia* plants in urban contaminated areas was reduced compared with control plants (Thara *et al*., 2015).

Stomata's shape: The importance of the stomata in protecting plants against the air pollutants has been investigated, which indicates that the closure of the stomata helps the plants to protect themselves against the air damage (Majernik and Mansfield, 1970). When the stomata are closed, other cells are protected against contaminants inside the leaf. However, if the stomata's closure is permanent (for example, if the guard cells are damaged and unable to rejuvenate again) the effect is likely to be serious and the subsequent growth of the plants will be affected, since the main route for absorption carbon dioxide will be blocked for photosynthesis (Majernik and Mansfield, 1970). In a study, the simple closure of the stomata in *Boerhaavia*, *Amaranthus* and *Cephalandra* and stomata's obstruction in *Nerium* plant and *Tabernaemonatana* leaves were evident under the stress of pollution (Mandal, 2006). It has been shown that in the contaminated area, the number of stomata and closed stomata were more than control, while the number of stomata closed in the control area was much less reported (Amulya *et al*., 2015).

Xylem, phloem, and parenchyma: Reduction of vascular bundles, resulting in a reduction of transfers of pollutants to the plant (Lorestani *et al*., 2014). Sukumaran (2014) has reported that a significant increase in fiber's length and width of the vessel in the infected area than the natural site, in plants like *Abutilon indicum* G.Don., *Croton sparsiflorus* Morong. and *Cassia occidentalis* L. occurred. This may be the adaptation of plants to survive pollution and tension. The vessel's length of *Tamarindus indica* L. which was exposed to contaminants was significantly reduced (Maajitha Begam and Muhammad Ilyas, 2016). The number of xylem in plants that were exposed to emissions for 30 days decreased compared to 10 days of exposure plants and the control plants. Wahlmann *et al*. (1986) stated that the decrease in photosynthetic activity delayed the cambium activity and as a result, the production of xylem and phloem. The size of parenchymal cells of lettuce, which had the highest exposure to contaminants, increased, and its number decreased. The leaf anatomy of *Lotus corniculatus*, *Trifolium montanum*, *Trifolium pretense* and *Trifolium repens* showed a decrease in palisade parenchyma cells and spongy parenchyma cells in infected leaves compared to the non-infected leaves collected from non-infected areas (Irina, 2009).

Conclusion

In lettuce plants, which had the highest exposure time (30 days exposure to gases), it has been observed that antioxidant enzymes (catalase, ascorbate peroxidase, and guaiacol peroxidase), proline, ion leakage, relative water content, number of stomata per unit area significantly increased compared to the control plants. But significant decrease in chlorophyll a, b and total chlorophyll, leaf gas exchanges (photosynthesis rate, stomatal conductance and carbon dioxide intracellular concentration), chlorophyll fluorescence, leaf area, height, fresh weight and dry weight and the stomata's length was observed in lettuce plants with the highest exposure time (30 days), compared to the control plants. The results showed that the lettuce plants that had the greatest exposure to pollutants showed damages and signs of damage to the pollutants sooner than apparent and morphological symptoms. Exposure to contaminants for 10 days, significantly increased total chlorophyll content and leaf gas-exchange parameters compared to the controls that are most likely because of the contents in contaminants and also the role of CO_2 in photosynthesis process and the presence of nitrogen and sulfur in NO_2 and SO_2 gases. The results showed that the exposure of lettuce plants to pollutants, especially 30-days exposure, caused morphological, physiological and anatomical reactions due to contaminants that could be considered these reactions as the adaptation of these plants to the stress of contamination, in order to adapt or to deal with stress conditions and thus to survive the plant under pollution stress conditions.

References

Achille, K. N., Maxime, A. D., Sabas, B. Y. S. and Kouame, D. B. (2015) Detoxifying hydrogen peroxide enzymes activity in two plant species exposed to air pollution in Abidjan city (Cote dIvoire). International Journal of Plant, Animal and Environmental Sciences 5: 140-145.

Adrees, M., Ibrahim, M., Shah, A. M., Abbas, F., Saleem, F., Rizwan, M., Hina, S., Jabeen, F. and Ali, S. (2016) Gaseous pollutants from brick kiln industry decreased the growth. photosynthesis, and yield of wheat (*Triticum aestivum* L.). Environmental Monitoring *and* Assessment 188: 1-11.

Aebi, H. (1984) Catalase in vitro. Methods in Enzymology 105: 121-126.

Agbaire, P. O. and Akporhonor, E. E. (2014) The effects of air pollution on plants around the vicinity of the Delta Steel Company, Ovwian-Aladja, Delta State, Nigeria. IOSR Journal of Environmental Science, Toxicology and Food Technology 8: 61-65.

Amulya, L., Hemanth Kumarand, N. K. and Jagannath, S. (2015) Air pollution impact on micromorphological and biochemical response of *Tabernaemontana divaricata* L. (Gentianales: Apocynaceae) and *Hamelia patens*. (Gentianales: Rubiaceae). Brazilian Journal of Biological Sciences 2: 287-294.

Areington, C. A., Varghese, B. and Ramdhani, S. (2015) An assessment of morphological, physiological and biochemical biomarkers of industrial air pollution in the leaves of *Brachylaena discolor*. Water, Air and Soil Pollution 226: 1-14.

Atri, S., Ghorbanli, M. L. and Motesadi, Z. S. (2005) The effect of atmospheric pollutants on the activity of antioxidant enzymes (ascorbate peroxidase and catalase) and soluble carbohydrates of *lavandula vera* dc and *rosmarinus officinalis* L. Journal of Environmental Science and Technology 25: 42-50.

Bates, L. S., Waldern, R. P. and Tear, I. D. (1973) Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207.

Breusgem, F.V., Vranova, E., Dat, J. F. and Inze, D. (2001) The role of active oxygen species in plant signal transduction. Plant Science 161: 405-414.

Biddington, N. L. and Dearman, A. S. (1985) The effect of mechanically induced stress on the growth of cauliflower, lettuce and celery seed-lings. Annals of Botany 55: 109-119.

Chance, B. and Maehly, C. (1955) Assay of catalase and peroxidases. Methods in Enzymology 11: 764-775.

Chauhan, A. and Joshi, P. C. (2010) Effect of ambient air pollutants on wheat and mustard crops growing in the vicinity of urban and industrial areas. New York Science 3: 52-60.

Chauhan, A. (2010) Photosynthetic pigment changes in some selected trees induced by automobile exhaust in Dehradun, Uttarakhand. New York Science 3: 45-51.

Chukwu, M. N. and Adams, E. A. (2016) Effect of generator (exhaust) fumes on the growth and development of *Lycopersicum esculentus* (tomato). Journal of Applied Sciences and Environmental Management 20: 335-340.

Emberson, L. D., Ashmore, M. R. and Murray, F. (2003) Air Pollution Impacts on Crops and Forests: A Global Assessment. Imperial College Press, London.

Farombi, E. O., Adelowo, O. A. and Ajimoko, Y. R. (2007) Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. International Journal of Environmental Research and Public Health 4: 158-165.

Giri, S., Shrivastava, D., Deshmukh, K. and Dubey, P. (2013) Effect of air pollution on chlorophyll content of leaves. Current Agriculture Research 1: 93-98.

Glaz, B., Dolen, R. M. and Samira, H. D. (2004) Sugarcane photosynthesis, transpiration and stomatal conductance due to flooding and water table. Crop Science 44: 1633-1641.

Iqbal, M., Mahmooduzzafar, Nighat, F. and Khan, P. R. (2010) Photosynthetic, metabolic and growth responses of *Triumfetta rhomboidea* to coal-smoke pollution at different stages of plant ontogeny. Journal of Plant Interactions 5: 11-19.

Irina, N. G. (2009) Air pollution effects on the leaf structure of some Fabaceae species. Notulae botanicae Horti Agrobotanici Cluj 37: 57-63.

Jochner, S., Markevych, I., Beck, I., Traidl-Hoffmann, C., Heinrich, J. and Menzel, A. (2015) The effects of short-and long-term air pollutants on plant phenology and leaf characteristics. Environmental Pollution 206: 382-389.

John, W., Wargo, L., Alderman, N. and Brown, D. (2006) The harmful effects of vehicle exhaust. A case for policy change. Environment and Human Health, Inc.

Joshi, P. C. and Swami, A. (2009) Air pollution induced changes in the photosynthetic pigments of selected plant species. Journal of Environmental Biology 30: 295-298.

Kammerbauer, J. and Dick, T. (2000) Monitoring of urban traffic emissions using some physiological indicators in *Ricinus communis* L. Plants. Archives of Environmental Contamination and Toxicology 39: 161-166.

Khan, M. R. and Khan, M. W. (1993) The interaction of SO_2 and root-knot nematode on tomato. Environmental Pollution 81: 91-102.

Kostka-Rick, R. and Mannin, W. J. (1993) Radish (*Raphanus sativus* L.): A model for studying plant responses to air pollutants and other environmental stresses. Environmental Pollution 82: 107-138.

Leghari, S. K. and Zaidi, M. A. (2013) Effect of air pollution on the leaf morphology of common plant species of Quetta city. Pakistan Journal of Botany 45: 447-454.

Lichtenhaler, H. K. (1987) Chlorophylls and carotenoids. Pigments of photosynthetic biomembranes. In: Methods in Enzymology (eds. Douce, R. and Packer, L.) Pp. 350-382. Academic Press Inc, New York.

Lorestani, B., Kolahchi, N., Ghasemi, M. and Cheraghi, M. (2014) Changes germination, growth and anatomy *Vicia ervilia* in response to light crude oil stress. Journal of Chemical Health Risks 4: 45-52.

Lutts, S., Kinet, J. M. and Bouharmont, J. (1995) Changes in plant response to Nacl during development of rice varieties differing in salinity resistance. Journal of Experimental Botany 46: 1843-32.

Maajitha Begam, A. and Muhammad Ilyas, M. H. (2016) Pharmacognostic studies leaves and wood of *Tamarindus indica* Linn. Subjected to Cement Dust Pollution. International Journal of Advanced Research 4: 1946-1954.

Maddah, S. M., Moraghebi, F., Kashani, Q., Farhangiyan, S. and Afdideh, F. (2015) Physical, physiological responses and resistance of acacia tree (*Robinia pseudoacacia* L.) under the influence of air pollution in Tehran. Journal of Plant Physiology 38: 56-48.

Mage, D., Ozolins, G., Peterson, P., Webster, A., Orthofer, R., Vandeweerd, V. and Gwynne, M. (1996) Urban air pollution in mega-cities of the world. Atmospheric Environment 30: 681-686.

Majernik, O. and Mansfield, T. A. (1970) Direct effects of SO_2 pollution on the degree of opening of stomata. Nature 227: 377-378.

Mandal, M. (2006) Physiological changes in certain test plants under automobile exhaust pollution. Journal of Environmental Biology 27: 43-47.

Mansfield, T. A. and Majernik, O. (1970) Can stomata play a part in protecting plants against air pollutants? Environmental Pollution 1: 149-154.

Maugh, T. H. (1979) SO_2 pollution may be good for plants. Science 205: 383-383.

Mehlhorn, H., Cottam, D. A., Lucas, P. W. and Wellburn, A. R. (1987) Induction of ascorbate peroxidase and glutathione reductase activities by interactions of mixtures of air pollutants. Free Radical Research communications 3: 193-197.

Miller, G. A. D., Suzuki, N. and Mittler, R. O. N. (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell and Environment 33: 453-467.

Miyake, C., Cao, W. H. and Asada, K. (1993) Purification and molecular properties of the thylakoid-bound ascorbate peroxidase in spinach chloroplasts. Plant, Cell and Environment Physiology 34: 881-889.

Muneer, S., Kim, T. H., Choi, B. C., Lee, B. S. and Lee, J. H. (2014) Effect of CO, NOx and SO_2 on ROS production, photosynthesis and ascorbate–glutathione pathway to induce *Fragaria× annasa* as a hyperaccumulator. Redox Biology 2: 91-98.

Nakano, Y. and Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant, Cell and Environment Physiology 22: 867-880.

Patidar, S., Bafna, A., Batham, A. R. and Panwar, K. (2016) Impact of urban air pollution on photosynthetic pigment and proline content of plants growing along the AB road indore city, India. International Journal of Current Microbiology and Applied Sciences 5: 107-113.

Pearson, L. C. and Henriksson, E. (1981) Air pollution damage to cell membranes in lichens. II. Laboratory experiments. Bryologist 515-520.

Pourkhabbaz, A. R., Rastin, N., Olbrich, A., Langenfeld Heiser, R. and Polle, A. (2010) Influence of environmental pollution on leaf properties of urban plane trees *Platanus orientalis* L. The Bulletin of Environmental Contamination and Toxicology 85: 251-255.

Qin, X. M., Sun, N., Ma, L., Chang, Y. and Mu, L. (2014) Anatomical and physiological responses of Colorado blue spruce to vehicle exhausts. Environmental Science and Pollution Research 21: 11094-11098.

Qiu, D. L., Lin, P. and Guo, S. Z. (2007) Effects of salinity on leaf characteristics and CO_2/H_2O exchange of *Kandelia candel* (L.) druce seedlings. Journal of Forest Science 53: 13-19.

Rai, A. and Kulshreshtha, K. (2004) Effect of particulates generated from automobile emission on some common plants. Journal of Food Agriculture and Environment 4: 253-259.

Rai, P. K. (2016) Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: implications for urban ecosystem restoration. The Journal of Asia-Pacific Biodiversity 9: 47-55.

Rai, R., Rajput, M., Agrawal, M. and Agrawal, S. B. (2011) Gaseous air pollutants: A review on current and future trends of emissions and impact on agriculture. Journal of Scientific Research 55: 77-102.

Ramakrishnaiah, H. and Somashekar, R. K. (2003) Higher plants as biomonitors of automobile pollution. Ecology, Environment and Conservation 9: 337-343.

Ritchie, S. W., Nguyen, H. T. and Halody, A. S. (1990) Leaf water content and gas exchange parameters of two wheat genotypes differing in drought resistance. Crop Science 30: 105-111.

Rivelli, A. R., Lovelli, S. and Perniola, M. (2002) Effects of salinity on gas exchange, water relations and growth of sunflower (*Helianthus annuus*). Plant Biology 29: 1405-1415.

Sairam, R. K., Deshmukh, P. S. and Saxena, D. C. (1998) Role of antioxidant systems in wheat genotype tolerance to water stress. Plant Biology 41: 387-394.

Saquib, M., Ahmad, A. and Ansari, K. (2010) Morphological and physiological responses of *Croton bonplandianum Baill*. to air pollution. International Journal of Environmental Science and Technology 17: 35-41.

Seyyednejad, S. M. and Koochak, H. (2011) A study on air pollution effects on *Eucalyptus camaldulensis*. In: Proceedings of the International Conference on Environmental Biomedical and Biotechnology.16:98-101.

Seyyednejad, S. M. and Koochak, H. (2013) Some morphological and biochemical responses due to industrial air pollution in *Prosopis juliflora* (Swartz) DC plant. African Journal of Agricultural Research 8: 1968-1974.

Seyyednejad, S. M., Niknejad, M. and Yusefi, M. (2009) Study of air pollution effects on some physiology and morphology factors of *Albizia lebbeck* in high temperature condition in Khuzestan. Journal of Plant Sciences 4: 122-126.

Sharma, G. K. and Butler, J. (1973) Leaf cuticular variations in *Trifolium repens* L. as indicators of environmental pollution. Environmental Pollution 5: 287-293.

Singh, N., Yunus, M., Srivastava, K., Singh, S. N., Pandey, V., Misra, T. and Ahmad, K. J. (1995) Monitoring of auto-exhaust pollution by roadside plants. Journal Environmental Monitoring and Assessment 34: 13-25.

Stevovic, S., Mikovilovic, V. S. and Calic-Dragosavac, D. (2010) Environmental impact on morphological and anatomical structure of Tansy. The African Journal of Biotechnology 9: 2413-2421.

Sukumaran, D. (2014) Effect of air pollution on the anatomy some tropical plants. Applied Journal of Environmental Science 2: 32-36.

Thara, S. B., Kumar, N. K. H. and Jagannath, S. (2015) Micro-morphological and biochemical response of *Muntingia calabura* L. and *Ixora coccinia* L. to air pollution. Research Plant Biology 5: 11-17.

Tiwari, S., Agrawal, M. and Marshall, F. M. (2006) Evaluation of ambient air pollution impact on carrot plants at a suburban site using open top chambers. Environmental Monitoring and Assessment 119: 15-30.

Valavanidis, A., Vlahogianni, T., Dassenakis, M. and Scoullos, M. (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicology and Environmental Safety 64: 178-189.

Verma, A. and Singh, S. (2006) Biochemical and ultrastructural changes in plant foliage exposed to auto-pollution. Environmental Monitoring and Assessment 120: 585-602.

Verma, R. B., Mahmooduzzafar, T., Siddiqui, O. and Iqbal, M. (2006) Foliar response of Ipomea pes-tigridis L. to coal-smoke pollution. The Turkish Journal of Botany 30: 413-417.

Wahlmann, B., Braun, E. and Lemark, S. (1986) Radial increment in different tree heights in beech stands affected by air pollution. IAWA Bulletin 7: 285-288.

Wang, F., Zeng, B., Sun, Z. and Zhu, C. (2009) Relationship between proline and Hg^{2+} induced oxidative stress in a tolerant rice mutant. Ecotoxicology and Environmental Safety 56: 723-731.

Xue, M., Dong, X. L., Ma, C., Zhang, X. and Ma, H. C. (2013) Response of urban roadside greening plant exposed to automobile exhaust pollution. Advanced Materials Research 610: 3398-3401.

Yang, L., Stulen, I. and De Kok, L. J. (2006) Sulfur dioxide: Relevance of toxic and nutritional effects for Chinese cabbage. Environmental and Experimental Botany 57: 236-245.