Research Article

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

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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 rate, stomatal conductance, CO₂ 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, CO₂ 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).

A considerable 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

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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 tolerance 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/m³ 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 performed based on minimum significant difference test (LSD) at 5% probability level. Statistical analysis of data of all traits was performed 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 Maehl 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%.
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Table 1. The analysis of pollutants and their concentration

<table>
<thead>
<tr>
<th>Gas contents</th>
<th>NO (ppm)</th>
<th>NO₂ (ppm)</th>
<th>NOₓ (ppm)</th>
<th>SO₂ (ppm)</th>
<th>CO₂ (%)</th>
<th>CH₄ (mg/m³)</th>
<th>CO (ppm)</th>
<th>O₂ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>22±3</td>
<td>0/7</td>
<td>7</td>
<td>105±10</td>
<td>20</td>
</tr>
</tbody>
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Figure 1. The effect of gases 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 CO₂ 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, CO₂ 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 gases 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 pollutant 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.
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Figure 4. The effect of gasses and time of exposure on photosynthesis rate (a), stomatal conductance (b) and CO₂ 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 lebbeck* 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...
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 SO₂ and NOₓ. Pollutants such as SO₂, NO₂ and O₃ damage the membranes and related molecules, including chlorophyll pigments (Ramakrishnaiah and Somasekhar, 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 SO₂ 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, NO₂, SO₂ 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. Pourkhhabbaz (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 previouse 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 CO₂ 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 CO₂ 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 NO₂ 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 SO₂, 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 molecule activity of the catalase enzyme in the contaminated site can be attributed to the presence of environmental pollutants which strongly have produced H₂O₂ in these species under oxidative stress (Faromibi et al., 2007).

**Ascorbate peroxidase:** The ascorbate peroxidase enzyme acts as a scavenger 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 (SO₂, NO₂, and O₃) 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 (Emerson 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 Quisquilia 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 stoma'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 coccinina 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 (Majermik 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 (Majermik 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 Ahtuilion 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 CO2 in photosynthesis process and the presence of nitrogen and sulfur in NOx and SO2 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


