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Some morphological and biochemical responses due to industrial air pollution in *Prosopis juliflora* (Swartz) DC plant

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The current study was done to examine the impact of ambient air pollution on some biological factors in *Prosopis juliflora* plants using two sites; control and polluted (around one of the oil fields in south west of Iran). Various morphological and biochemical characteristics of the plants were studied in both sites and compared with each other. Plants exposed to pollution showed lower leaf area, petiole length, chlorophylls, carotenoids and soluble carbohydrate contents as compared to plants growing in control site. Proline levels in polluted leaves significantly increased ($p < 0.01$), suggesting the activation of protective mechanism in these plants under air pollution stress, and also the observed responses are regarded as adaptive and compensative to the adverse effects of air pollution.

Key words: Air pollution, chlorophyll, sugare, proline, *Prosopis juliflora*.

INTRODUCTION

In recent past, air pollutants, responsible for vegetation injury and crop yield losses, are causing increased concern (Joshi and Swami, 2007). Urban air pollution is a serious problem in both developing and developed countries (Li, 2003). The increasing number of industries and automobile vehicles are continuously adding toxic gases and other substances to the environment (Jahan and Iqbal, 1992). All combustion release gases and particles into the air. These can include sulphur and nitrogen oxides, carbon monoxide and soot particles, as well as smaller quantities or toxic metals, organic molecules and radioactive isotope (Agbaire and Esiefarienrhe, 2009). Environmental stress, such as air pollution, is among the factors most limiting plan productivity and survivorship (Woo et al., 2007). Pollutants could be classified as either primary or secondary. Pollutants that are pumped into the atmosphere and directly pollute the air are called primary pollutants while those that are formed in the air when primary pollutants react or interact are known as secondary pollutants (Agbaire, 2009). It has been observed that plants particularly growing in the urban areas affected greatly due to varieties of pollutants [oxides of nitrogen and sulphur, hydrocarbon, ozone, particulate matters, hydrogen fluoride, peroxyacyl nitrates (PAN) etc.] (Jahan and Iqbal, 1992). Sulphur dioxide (SO_2^-), nitrogen oxides (NO_x) and CO_2 as well as suspended particulate matter. These pollutants when absorbed by the leaves may cause a reduction in the concentration of photosynthetic pigments viz., chlorophyll and carotenoids, which directly affected to the plant productivity (Joshi and Swami, 2009). Industrialization and the automobiles are responsible for maximum amount of air pollutants and the crop plants are very sensitive to gaseous and particulate pollutions and these can be used as indicators of air pollution (Joshi et al.,

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2009). In urban environments, trees play an important role in improving air quality by taking up gases and particles (Woo and Je, 2006). Plants provide an enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollutant level in the air environment, with various extent for different species (Liu and Ding, 2008). They act as the scavengers for many air borne particulates in the atmosphere (Joshi and Swami, 2009).

The genus *Prosopis* is in the family Leguminosae (Fabaceae), sub-family Mimosoideae. The native range of *Prosopis* species can be approximately divided into five regions, simply defined as Asia, Africa, North America, Central America and South America. The genera *Prosopis* contain some of the most widespread and important tree species in the arid and semiarid zones of the tropical and sub-tropical world. The genus *Prosopis* contains 44 species and a large number of varieties. The genetic variation of *P. juliflora* is high for all characters tested in the species (Kumar et al., 1998).

Prosopis is able to improve the soil in which it is growing by means of biological nitrogen fixation, leaf litter accumulation, nutrient pumping from deeper soil layers, loosening of a hard soil structure, stabilizing of loose sands, and an increase of the fauna above and below the ground (Kaushik and Kumar, 2003).

Adverse effects of air pollution on biota and ecosystems have been demonstrated worldwide. Much experimental work has been conducted on the analysis of air pollutant effects on crops and vegetation at various levels ranging from biochemical to ecosystem levels (Tiwari et al., 2006).

In order to understand the effects and alterations due to air pollutants in plants, present study examines the impact of air pollution on *P. juliflora* around one of the oil factory in Iran.

MATERIALS AND METHODS

Area of study

This study was conducted between October 2009 and December 2009. The area of study is situated in industrial region around one of the oil fields in south west of Iran. This is designated as polluted site (main contaminants in the polluted area were SO_2 and H_2S). A site with similar ecological conditions was selected as the control (unpolluted) site. The leaf samples (three replicates of the three plant samples) in two regions were taken and immediately taken to the laboratory for analysis. The plants studied in two regions were 10 to 15 years old. The climate in this land is warm and humid.

Morphological measurements

1. Leaf area (mm^2): Leaf area was measured by means of leaf area meter.
2. Petiole length (cm): It was done for 20 leaves from each replicates.
3. Dry weight (g): 20 g of fresh leaves (from each replicates) were dried over night in an oven at 40°C and reweight to obtain the dry weight.

Biochemical measurements

The chlorophyll a, b, total chlorophyll and carotenoids in the leaves of selected plant were determined by use of acetone 80% according to the method described by Lichtenthaler (1987). Analysis of soluble sugar was done after making hydro alcoholic extract of leaf powder. Total soluble carbohydrate concentration was determined by phenol sulphuric acid method of Helle-bust and Graigie (1978).

To determine proline, fresh leaves, ninhydrin and acetic acid were used according to the Bates et al. (1975) procedure.

The results were analyzed statistically by using the statistical software MSTATC in order to determine the significant differences between polluted and control stands. Analysis of variance was done by Completely Randomized Design.

RESULTS

Morphological changes

The study revealed that leaves of *P. juliflora* collected from polluted site showed a decrease (34.85%) in their surfaces (Figure 1). The average leaf area of the plant in control site as compared to polluted site decreased from 18150.808 mm^2 to 11825.613 mm^2. The average of petiole length from leaves in polluted site (0.447 cm) was lower than control (0.480 cm). This decrease was 6.87% (Figure 2). An increase of 29.65% in dry weight was exhibited at polluted leaves. The average dry weight of leaves increased from 6.790 g in control site to 8.803 g in polluted site (Figure 2). Neither of morphological changes in this study was significant.

Biochemical changes

At polluted site, *P. juliflora* leaves exhibited 7.28, 7.82, 7.50 and 8.61% decrease in chlorophyll a, b, total chlorophyll and carotenoid contents, respectively (Figure 3).

A decrease 2.17% in soluble carbohydrate concentration was observed in leaves collected from polluted site (Figure 4). In this study the changes in chlorophylls, carotenoid and sugar contents were not significant while proline in leaves subjected to air pollution significantly ($p < 0.01$) increased (121.48%) from 17.074 in control to 37.815 in polluted site (Figure 5).

DISCUSSION

Air pollution is one of the severe problems facing the world today. It deteriorates ecological condition and can be defined as the fluctuation in any atmospheric constituent from the value that would have existed without human activity (Tripathi and Gautam, 2007). The instrumental and bio-monitoring of air pollution carried out at suburban sites have shown that often the major phytotoxic agents are present at levels above the

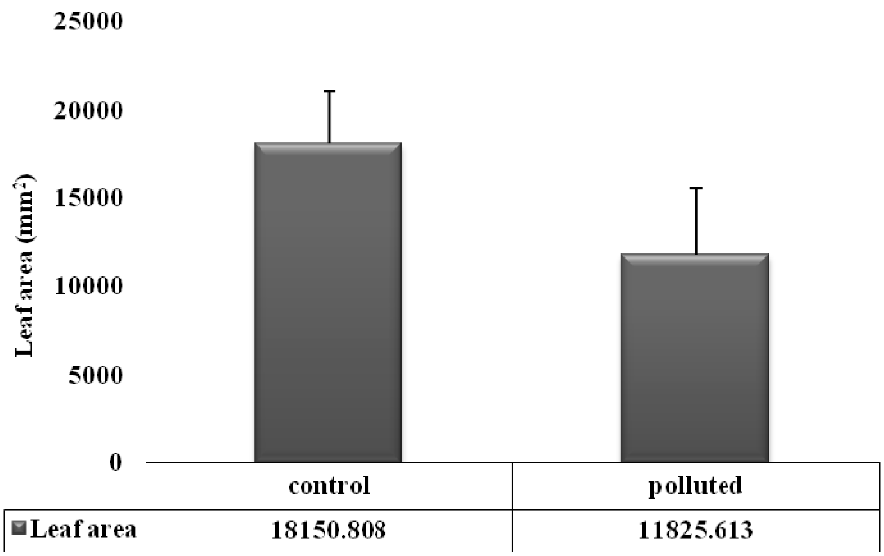

Figure 1. Leaf area of *P. juliflora* grown in control and polluted sites. Bars represent standard deviation. * = $p < 0.05$; ** = $p < 0.01$.

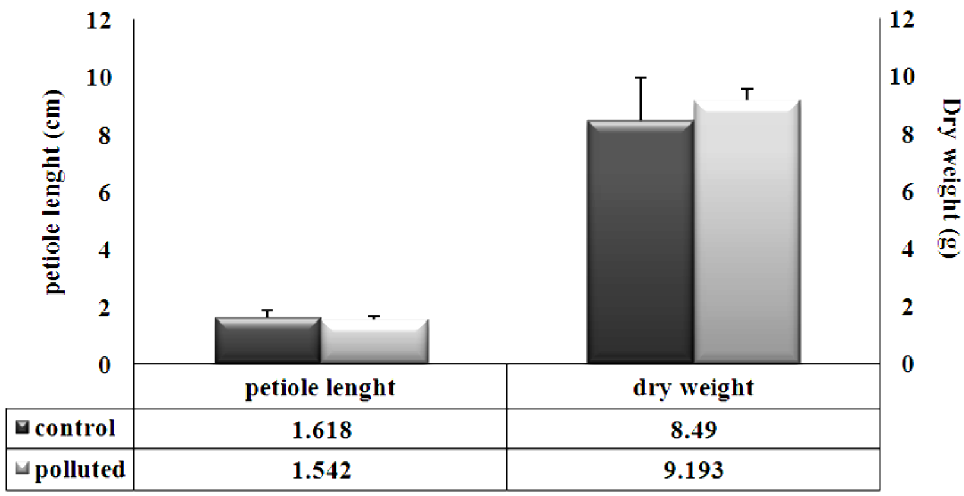

Figure 2. Leaf dry weight and petiole lenght of *P. juliflora* grown in control and polluted sites. Bars represent standard deviation. * = $p < 0.05$; ** = $p < 0.01$.

threshold of plant damage. It has been observed that ozone concentrations are higher in suburban and rural areas as compared to the urban areas, whereas SO_2 and NO_2 concentrations are higher at urban sites (Tiwari et al., 2006). The atmospheric SO_2 adversely affects various morphological and physiological characteristics of plants. High soil moisture and high relative humidity aggravated SO_2 injury in plants (Tankha and Gupta, 1992). Vegetation is an effective indicator of the overall impact of air pollution, and the effect observed is a time-averaged result that is more reliable than the one obtained from direct determination of the pollutant in air over a short period. Although a large number of trees and shrubs have been identified and used as dust filters to check the rising urban dust pollution level (Rai et al., 2009). Air pollution can directly affect plants via leaves or indirectly via soil acidification. When exposed to airborne pollutants, most plants experienced physiological changes before exhibiting visible damage to leaves (Liu and Ding, 2008). Air pollution stress leads to stomatal closure, which reduces CO_2 availability in leaves and inhibits carbon fixation. Net photosynthetic rate is a commonly used indicator of impact of increased air pollutants on tree growth (Woo et al., 2007). Plants that are constantly exposed to environmental pollutants absorb, accumulate and integrate these pollutants into

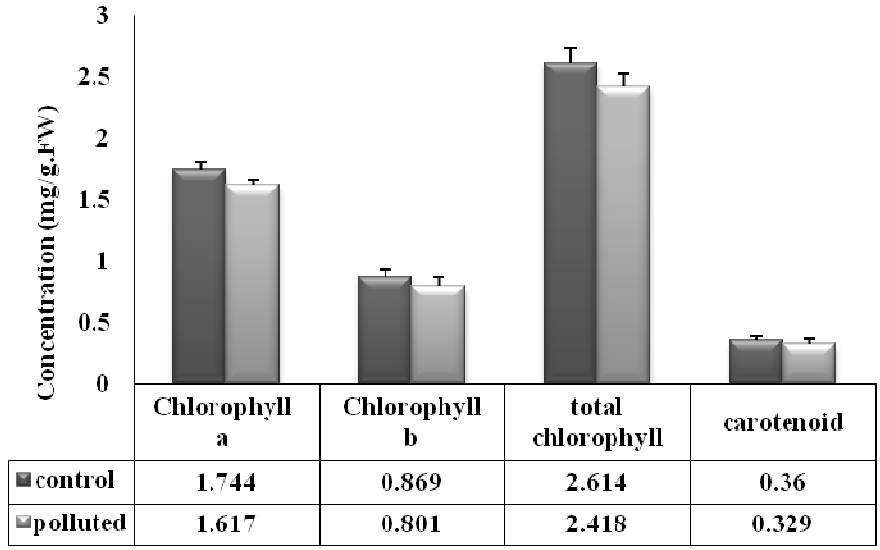

Figure 3. Clorophyll a, b, total and carotenoid contents of *P. juliflora* grown in control and polluted sites. Bars represent standard deviation. * = $p < 0.05$; ** = $p < 0.01$.

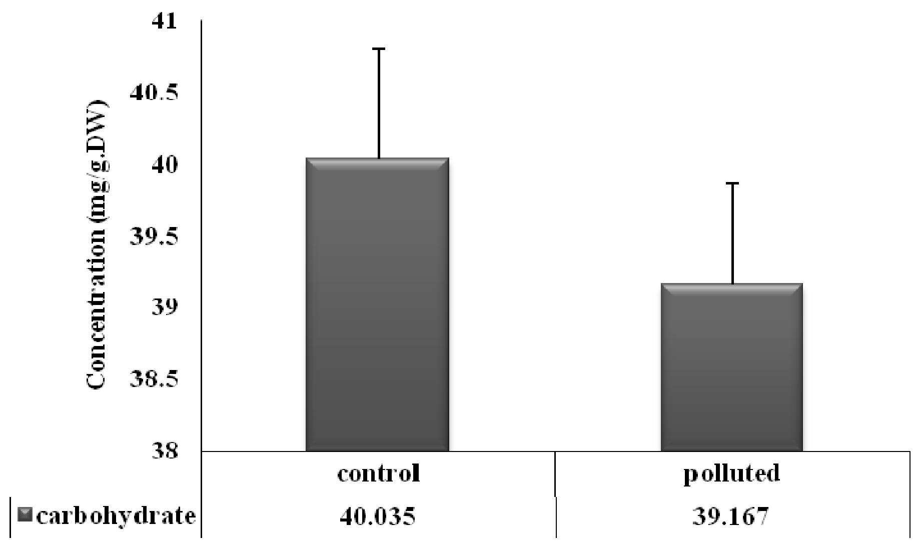

Figure 4. Soluble carbohydrate content of *P. juliflora* grown in control and polluted sites. Bars represent standard deviation. * = $p < 0.05$; ** = $p < 0.01$.

their systems. It reported that depending on their sensitivity level, plants show visible changes which would include alteration in the biochemical processes or accumulation of certain metabolites (Agbaire and Esiefarienrhe, 2009).

Pollutants can cause leaf injury, stomatal damage, premature senescence, decrease photosynthetic activity, disturb membrane permeability and reduce growth and yield in sensitive plant species (Tiwari et al., 2006). Reductions in leaf area and leaf number may be due to decreased leaf production rate and enhanced senescence. The reduced leaf area result in reduced absorbed radiations and subsequently in reduced photosynthetic rate (Tiwari et al., 2006). In this study, plants subjected to air pollution showed reductions in leaf area and petiole length. These results are in agreement with those of Dineva (2004), Tiwari et al. (2006) and Jahan and Iqbal (1992) that also recorded reduction of leaf area and petiole length under pollution stress conditions (Dineva, 2004; Tiwari et al., 2006; Jahan and Iqbal, 1992). Plant adaptation to changing environmental factors involves both short-term physiological responses and long-term physiological, structural and morphological modifications. These changes help plants minimize stress and maximize use of internal and external resources (Dineva, 2004).

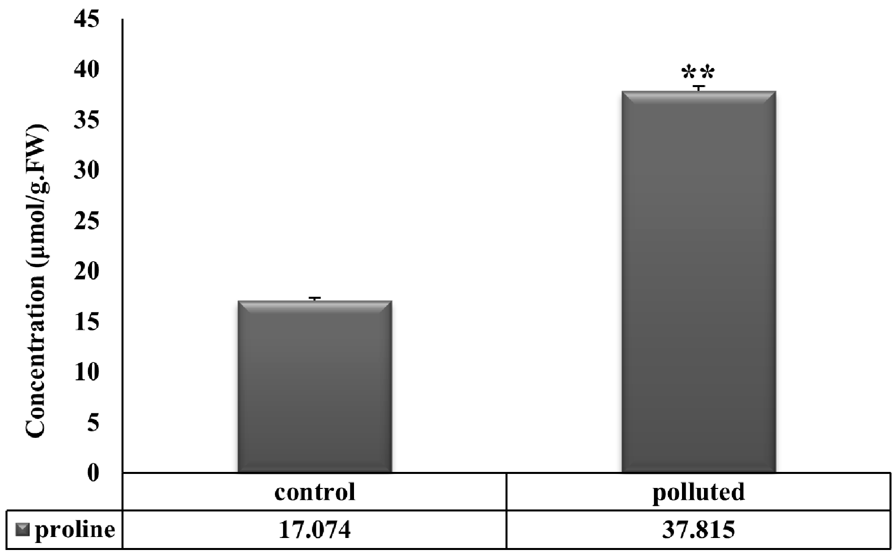

Figure 5. Proline content of *P. juliflora* grown in control and polluted sites. Bars represent standard deviation. * = $p < 0.05$; ** = $p < 0.01$.

A relationship between traffic density and photosynthetic activity, stomatal conductance, total chlorophyll content and leaf senescence has been reported (Honour et al., 2009). One of the most common impacts of air pollution is the gradual disappearance of chlorophyll and concomitant yellowing of leaves, which may be associated with a consequent decrease in the capacity for photosynthesis (Joshi and Swami, 2007). Chlorophyll is found in the chloroplasts of green plants and is called a photoreceptor (Joshi et al., 2009). Chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process in which carbon dioxide is "fixed" to yield carbohydrates and oxygen. When plants are exposed to the environmental pollution above the normal physiologically acceptable range, photosynthesis gats inactivated. The distribution of plant diversity is highly dependent on presence of air pollutants in the ambient air and sensitivity of the plants. Chlorophyll measurement is an important tool to evaluate the effects of air pollutants on plants as it plays an important role in plant metabolism and any reduction in chlorophyll content corresponds directly to plant growth (Joshi and Swami, 2009). Chlorophyll is an index of productivity of plant. Whereas certain pollutants increase the total chlorophyll content, others decrease it (Agbaire and Esiefarienrhe, 2009). In present study it was exhibited that chlorophyll content from *P. juliflora* leaves exposed to pollution decreased as compared with control. The shading effects due to deposition of suspended particulate matter on the leaf surface might be responsible for this decrease in the concentration of chlorophyll in polluted area. It might clog the stomata thus interfering with the gaseous exchange, which leads to increase in leaf temperature which may consequently retard chlorophyll synthesis. Dusted or encrusted leaf surface is responsible for reduced photosynthesis and thereby causing reduction in chlorophyll content (Joshi and Swami, 2009). A considerable loss in total chlorophyll, in the leaves of plants exposed to pollution supports the argument that the chloroplast is the primary site of attack by air pollutants such as SO_2 and NO_x. Air pollutants make their entrance into the tissues through the stomata and cause partial denaturation of the chloroplast and decreases pigment contents in the cells of polluted leaves. High amount of gaseous SO_2 causes destruction of chlorophyll (Tripathi and Gautam, 2007). Several researches have recorded reduction in chlorophyll content under air pollution (Tiwari et al., 2006; Tripathi and Gautam, 2007; Joshi and Swami, 2007; Joshi and Swami, 2009; Joshi et al., 2009).

Carotenoids protect photosynthetic organisms against potentially harmful photoxidative processes and are essential structural components of the photosynthetic antenna and reaction center (Joshi and Swami, 2009). Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They act as accessory pigments in higher plants. They are tougher than chlorophyll but much less efficient in light gathering; help the valuable but much fragile chlorophyll and protect chlorophyll from photoxidative destruction (Joshi et al., 2009). In Present investigation, carotenoid content of polluted leaves was decreased. Reduction in carotenoid content under air pollution stress has been recorded by many researchers (Tiwari et al., 2006; Tripathi and Gautam, 2007; Joshi and Swami, 2007; Joshi and Swami, 2009; Joshi et al., 2009).

Soluble sugar is an important constituent and source of energy for all living organisms. Plants manufacture this organic substance during photosynthesis and breakdown during respiration (Tripathi and Gautam, 2007). In this examine soluble sugar content were reduced under air pollution stress conditions. Tripathi and Gautam (2007), in their study revealed significant loss of soluble sugar in all tested species at all polluted sites. The concentration of soluble sugars is indicative of the physiological activity of a plant and it determines the sensitivity of plants to air pollution. Reduction in soluble sugar content in polluted stations can be attributed to increased respiration and decreased CO_2 fixation because of chlorophyll deterioration. It has been mentioned that pollutants like SO_2, NO_2 and H_2S under hardening conditions can cause more depletion of soluble sugars in the leaves of plants grown in polluted area. The reaction of sulfite with aldehydes and ketones of carbohydrates can also cause reduction in carbohydrate content (Tripathi and Gautam, 2007). Typical environmental stress (high and low temperature, drought, air and soil pollution) can cause excess reactive oxygen species (ROS) in plant cells, which are extremely reactive and cytotoxic to all organisms (Pukacka and Pukacki, 2000). High exposure to air pollutants forces chloroplasts into an excessive excitation energy level, which in turn increases the generation of ROS and induces oxidative stress (Woo et al., 2007). The deleterious effects of the pollutants are caused by the production of reactive oxygen species (ROS) in plants, which cause peroxidative destruction of cellular constituents (Tiwari et al., 2006). It has been reported that proline act as a free radical scavenger to protect plants away from damage by oxidative stress. Although the scavenging reaction of ROS with other amino acids, such as tryptophan, tyrosine, histidine, etc. are more effective compared with proline, proline is of special interest because of its extensive accumulation in plants during environmental stress (Wang et al., 2009). The effects of pollutants on plants include pigment destruction, depletion of cellular lipids and peroxidation of polyunsaturated fatty acid (Tiwari et al., 2006). There appears to be a relationship between lipid peroxidation and proline accumulation in plants subjected to diverse kinds of stress (Wang et al., 2009). If such a relationship exists, proline accumulation might play an important role in inhibiting air pollution-induced lipid peroxidation. Proline accumulation often occurs in a variety of plants in the present of different stresses. For example, proline accumulation in leaves of plants exposed to SO_2 fumigation (Tankha and Gupta, 1992), heavy metals (Wang et al., 2009) and salt (Woodward and Bennett, 2005) stress has been reported (Tankha and Gupta, 1992; Wang et al., 2009; Woodward and Bennett, 2005). This study also exhibited that in response to air pollution conditions, proline level of polluted leaves significantly increased ($p < 0.01$). The mechanism of the influence of different pollutants on plant organism is associated with oxidative damage at cellular level. Changes in important physiological processes such as respiration, photosynthesis, carbohydrate transport are results of damages at cellular level. The sensitivity to different pollutants differs between plants and even between clones of the same species (Pukacka and Pukacki, 2000).

Several factors can alter the results of such studies. For example, determination being tolerance or sensitive for one tested plant species could be changed during different seasons (Liu and Ding, 2008). It is suggested for some species that the biggest effects of pollution treatment are associated with the early stages of the life cycle (Honour et al., 2009). The long term, low-concentration exposures of air pollution produces harmful impacts on plant leaves without visible injury (Joshi et al., 2009). Species differed in the magnitude of response to pollutant exposure, although differences were not consistent within taxonomic or functional groups (Honour et al., 2009). The present knowledge of effects of air pollution on plants is mostly based on experiments where plants have been exposed to high concentrations of air pollutants for short periods under experimental conditions. However, less is known about responses of plants to air pollutants at environmentally relevant concentrations and for long durations in field conditions (Li, 2003). The present study was intended to increase the understanding of the adaptation and survival mechanisms of plants under air pollution. In summary, many changes in plant physiology and growth, such as those caused by air pollution, are biological compensatory responses to environmental stress. The main stress compensatory strategy in plants is to minimize damage from stress (Woo et al., 2007).

In this study, the leaves of *P. juliflora* samples tolerated pollution stress by decreasing most of the parameters (morphological and biochemical). As well as, the increased proline level in this plant, minimized damages from stress.

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