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Physiological Evaluation of Apricot (*Prunus armeniaca* L.) Leaves to Air Pollution for Biomonitoring of Atmospheric Quality

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ABSTRACT: Industrialization releases significant amounts of various air pollutants such as F, Cd, Pb, particulate matter, etc., which can in turn have a deleterious effect on a variety of biochemical and physiological processes as well as the structural organization within the cells. Responses from plants species to air pollutants is varied with certain species being very sensitive to such pollutants, ending up with well visible and measurable symptoms. Morphological damage is generally visible through lesions on the aerial parts, while biochemical and physiological changes which are invisible can be measured and quantified. This study has been designed to investigate the biochemical and physiological biomarkers of apricot (*Prunus armeniaca* L.) exposed to air pollution. It has been observed that, in comparison to unpolluted sites, lipid peroxidation level has increased in the leaves of apricot trees, grown in polluted areas, whereas photosynthetic capacity (Net photosynthesis, stomatal conductance, transpiration rate, total chlorophyll, and carotenoids) along with osmotic regulator (proline and soluble sugars) levels have declined. In *P. armeniaca* leaves, these symptoms can be used as indicators of air pollution stress for its early diagnosis, making them a reliable marker for a particular physiological disorder.

Keywords: *Prunus armeniaca*, biomonitoring, lipid peroxidation, gas exchange, photosynthetic pigments, proline

INTRODUCTION

Air pollution is one of the severe problems facing the world today, due to increasing levels of some gaseous and dust particles in the environment that result from anthropogenic activities like road transportation and industries. The release of such pollutants not only disturb the air quality, but cause adverse health effects in humans, animals, and plants living in immediate vicinity of such sources (Niu et al., 2014).

The response from plant species to air pollutants varies, making it vital to classify them into sensitive and tolerant groups since the members of the former class can serve as biological indicators, while those of the latter could be regarded as sinks for air pollutants. Moreover, tolerant species could preserve a green landscape in any area, threatened by important anthropogenic activities (Domingos et al., 2015). Fluoride and heavy metals are considered strong phytotoxic pollutants, which have adverse impacts on

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physiological comportment of plants, like altering their nutritional status, inhibiting their photosynthetic capacity (Niu et al., 2014; Sadati et al., 2016; Raeesi et al., 2016), or water relations (Sen et al., 2017). In suboptimal conditions, these pollutants become phytotoxic, resulting in the production of reactive oxygen species (ROS) like superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet -}$), and hydrogen peroxide (H_2O_2). The ROS react very rapidly with lipids membrane, resulting in lipid peroxidation induction (Zouari et al., 2016a). Plants respond to environmental stresses, improving their persistence to such conditions in various ways, which encompass changes at biological, physiological, and biochemical functions. Lipid peroxidation (TBARS), photosynthetic capacity (gas exchange and photosynthetic pigments), and osmotic regulator (such as proline and soluble sugars) are important indicators for plant physiological functions. In fact, TBARS is a common cytotoxic product of lipid peroxidation, considered to be a sensitive diagnostic index for oxidative injury under environmental stress (Aria et al., 2016; Zouari et al., 2016b). Photosynthesis (Pn), the most important assimilation process in higher plants, has been proven to be sensitive to pollutants. Proline and soluble sugars that are considered cellular osmotic regulators and free radical scavengers, have been found to get accumulated significantly in plant leaves under heavy metal stress (Zouari et al., 2016c). Hence, lipid peroxidation, photosynthetic capacity, and osmotic regulators in leaf tissue can reflect the plant physiological response under air pollution stress.

The Sfax Region, located in the central eastern part of Tunisia (270 km from the capital), harbors one of the most important industrial parks of the country, including the phosphate fertilizer plant (known as SIAPE) in the southern suburbs of the city. This factory releases several particles (both solid and liquid) and gaseous pollutants, principally fluoride compounds and heavy metals, to the atmosphere. Over the years, biomonitoring has become an important complement to the traditional techniques for air quality measurements. Bioindicator organisms are able to reveal alterations in air quality, before they severely affect human health or the biotope. According to Hodkinson and Jackson (2005), bioindicator means a species that is able to generate information about the environment quality. Such organisms are characterized by low ecological tolerance to certain chemicals. Indeed, they can present a physiological, morphological, or behavioral alteration, when they are exposed to certain pollutants. Considering the role of plants in pollution biomonotiring, the present study has been conducted to clarify the physiological and biochemical responses of apricot (*Prunus armeniaca* L.) leaves, including TBARS, gas exchange, photosynthetic pigments, proline, and soluble sugars to air pollution.

MATERIALS AND METHODS

The studied apricot plants (*Prunus armeniaca* L.) were taken from two experimental stations, located along the Sfax coast: the polluted site (PS), 1 km from the lead smelter and phosphate fertilizer industries (34°70'N, 10°72'E); and the control site (CS), 30 km west of the industries (34°54'N, 10°58'E). The main air pollutants, emitted by these factories, included F, Cd, and Pb (Ben Abdallah et al., 1990; Mezghani et al., 1999; Elloumi et al., 2003, 2017). Contradictory, there was no pollution source present in the CS, commonly used as an unpolluted site. The apricot plants from the two sites (PS and CS) were similar in age. They were planted on a loamy sand soil. Both polluted and control sites were submitted to an arid climate, being presented with very similar geochemical, ecological, and climatic conditions. The mean annual precipitation was 220 mm (with the minimum mean

precipitation (1 mm) being in July, and the maximum (300 mm) in December). Also, the mean annual temperature was 19.0°C (with the minimum mean temperature (6.5±2°C) being in January, and the maximum (32.5±2.5°C) in July).

Three plants were selected for each site with 50–70 leaves collected from several branches on all sides of each plant. The sampling took place in May, 2011.

The lipid peroxidation level in leaf and root tissues was estimated by determining the content of Thiobarbituric Acid Reactive Substances (TBARS), expressed as nmol of malondialdehyde equivalents (MDAeq), themselves a product of lipoperoxidation. TBARS content was determined by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). The absorbance of the homogenized mixture was read at 532 nm, and corrected at 600 nm for non-specific absorbance and the TBARS content was calculated, using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Net photosynthesis (Pn), stomatal conductance (Gs), and transpiration rate (E) were determined on expanded leaves from 10:00 to 12:00 A.M., using a portable gas exchange system (Li-Cor model 6200, Lincoln, USA). The measurements were done on a sunny day with 1252 μmol m^{-2} s^{-1} photosynthetic active radiations, 26 °C of air temperature, and at 50% relative air humidity.

For total chlorophyll and carotenoids analyses, 0.2 g of fresh leaves were ground in 5 ml of 80% acetone solution, using pestle and mortar. After filtration, the extracts were adjusted to 15 ml with 80% acetone solution and the pigment contents were determined with UV/vis spectrophotometer (Perkin Elmer, Norwalk, USA), in accordance with the method of Lichtenthaler and Wellburn (1983).

The proline contents of the apricot leaves were assayed based on the method, described by Bates et al. (1973) with absorbance being measured at 520 nm with UV/vis spectrophotometer (Perkin Elmer, Norwalk, USA). Toluene was used as a blank and proline content was calculated, using L-proline for the standard curve.

The soluble sugar contents of the apricot leaves were assayed, based on the method described by Robyt and White (1987) and the absorbance was measured at 640 nm with UV/vis spectrophotometer (Perkin Elmer, Norwalk, USA). Soluble sugar content was calculated, using glucose solutions to develop a standard curve.

A one way analysis of variance (SPSS software, 19.0) was carried out, using Duncan test ($p \leq 0.05$) to compare the average rates of all measured parameters with at least three replicates being performed for each measurement.

RESULTS AND DISCUSSION

Biomarkers can be defined as molecular, biochemical, or physiological changes in the cells, tissues, or organs that are indicative of organism exposure to xenobiotics. The current study analyzed different physiological and biochemical markers of apricot (*Prunus armeniaca* L.) leaves, grown in an industrial zone of Sfax-Tunisia.

Thiobarbituric acid reactive substances (TBARS) is a major reactive aldehyde, resulting from lipid-membrane peroxidation by ROS. Results showed that TBARS ascended by 141% in the leaves of apricot trees, grown in polluted site, as compared to the values belonging to the control plants (Fig. 1), which reflected the oxidative stress, induced by air pollution. Several studies have reported the increase in TBARS content under pollution stress, which was more important in susceptible plants than in tolerant ones (Domingos et al., 2015; Elloumi et al., 2017). Niu et al (2014) reported an increase in TBARS content in maize (*Zea mays* L.) leaves, parallel with the increase of mercury concentration applied to the plant. Elloumi et al. (2017) reported significant positive

correlations between F concentration and TBARS content in the leaves of loquat plants (*Eriobotrya japonica* L). The significant increase ($p \leq 0.05$) in TBARS content in apricot leaves under air pollution stress could be regarded an effective biomarker of this plant's sensibility to adverse environmental conditions, as suggested by Domingos et al. (2015).

Figure 2 demonstrates the impact of air pollution stress on photosynthetic pigments' contents of apricot plants under the studied conditions. Results indicated that air pollution stress decreased chlorophyll a and b as well as total chlorophyll contents. These parameters declined by 43%, 38%, and 41% in polluted leaves.

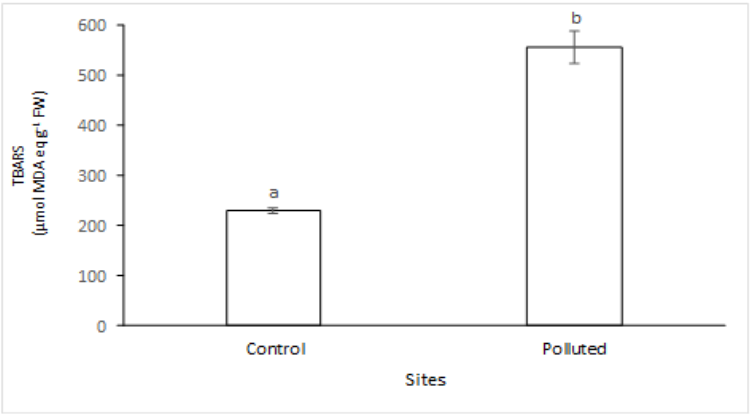

Fig. 1. Thiobarbituric acid reactive substances (TBARS) content in the leaves of *Prunus armeniaca* grown in both control and polluted sites. Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences among the treatments ($p \leq 0.05$, Ducan test).

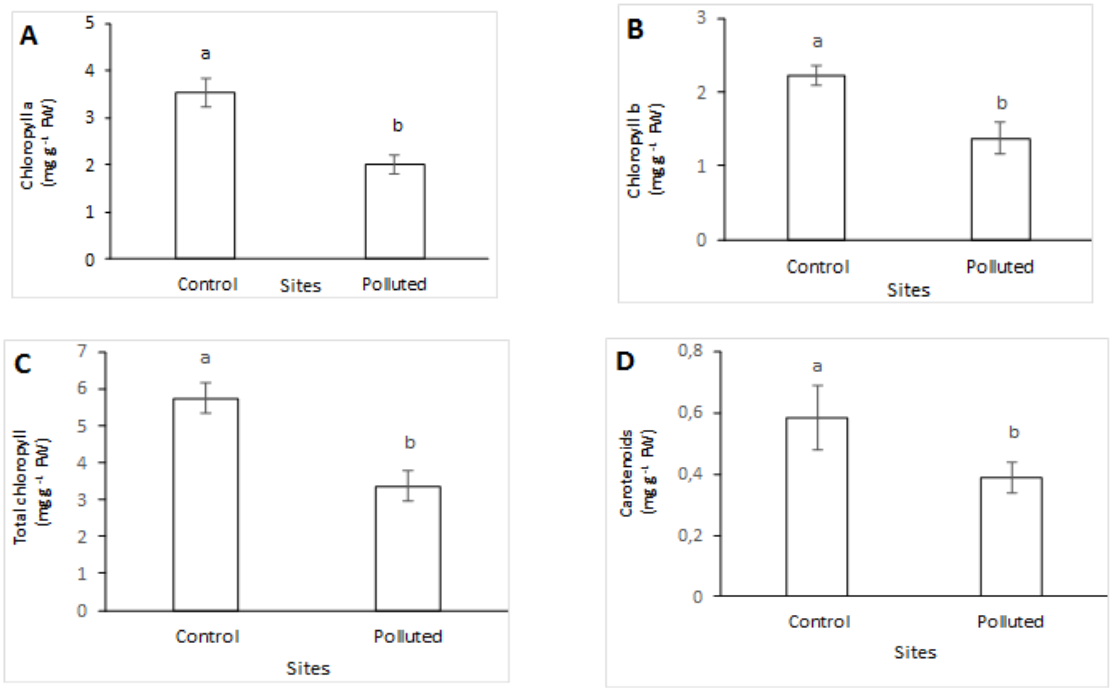

Fig. 2. Photosynthetic pigments' content in the leaves of *Prunus armeniaca* grown in control and polluted sites. Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences among the treatments ($p \leq 0.05$, Ducan test).

Depending on the species and the stress level, itself, chlorophyll content can be affected by several stresses, such as high temperature, drought, salt stress, and air or soil pollution (Sen et al., 2017). Reduction in chlorophyll content, as observed in the present study, might be due to (i) reduction of essential enzymes or mineral ions, required for chlorophyll synthesis; and (ii) enhanced chlorophyll degradation due to chloroplast damage (Elloumi et al., 2017). Results also showed a reduction in carotenoid content of stressed apricot plants (Fig. 2), which could have severe consequences on chlorophyll pigments, since carotenoids act as photoprotective agents of chlorophyll from photooxidative alteration inside the chloroplasts. Under pollution-stressed environments, their normal protective role may be hampered, consequently leading to cellular destruction including pigment degradation (Park and Jung, 2017). In this study, lower photosynthetic pigments, measured in apricot leaves, were in line with their sensitivity to contaminated environments.

Figure 3 illustrates that air pollution stress caused a significant ($p \leq 0.05$) decrease in gas exchange parameters (net photosynthesis Pn, transpiration rate E, and stomatal conductance Gs) in apricot plants, grown in polluted site, in comparison to the control ones. The reduction rates of Pn, E, and Gs were 50%, 44%, and 46%, respectively.

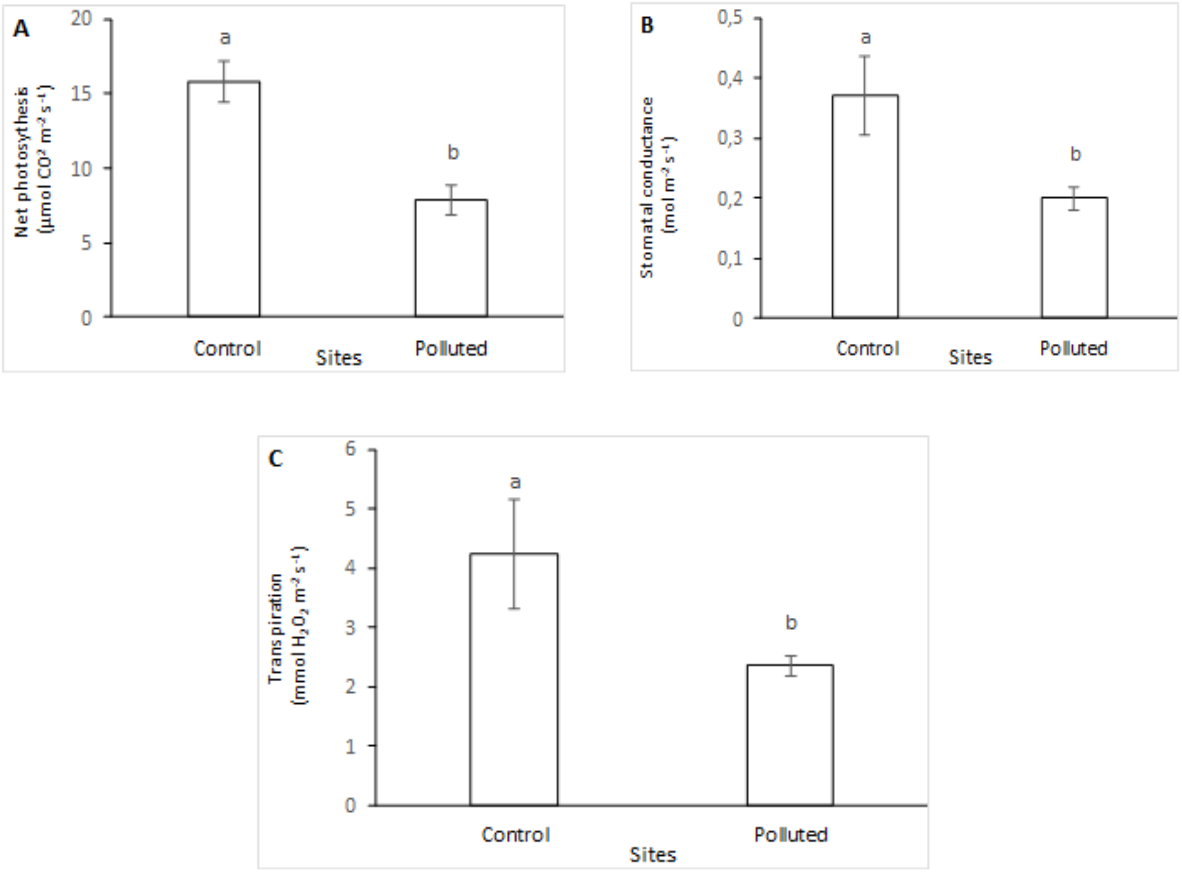

Fig. 3. The impact of air pollution on gas exchange parameters (Net photosynthesis, stomatal conductance, and transpiration rate) of *Prunus armeniaca.* Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences between treatments ($p \leq 0.05$, Ducan test).

In this study, dust accumulation on apricot leaf surfaces could act as a physical barrier that declined (i) the leaves' capability of absorbing photosynthetically-active radiation along with (ii) transpiration rate. As a result, leaf surface temperature was increased. In the hot dry summer period, the dust deposit on the leaves contributed to plant overheat, consequently affecting the normal course of photosynthesis and other biosynthetic processes (Sen et al., 2017). The reduction in gas exchange parameters in polluted apricot plants could also be explained by the obstruction of the stomatal opening by dust pollution, which declined the stomatal conductance, further leading to a decrease in photosynthesis.

Figure 4 illustrates soluble sugars and proline in leaves of apricot plants. Results show that soluble sugars and proline contents declined significantly ($p \leq 0.05$) in apricot plants, submitted to air pollution stress, as compared to the values, recorded in control ones. Recent studies show great interest in the use of osmoticum biomarkers as a form of environment monitoring, since the increase or inhibition of the contents of certain osmotic adjustment substances can explain a possible response to the environment stress. In many plant species, proline accumulation under environmental pollution has been correlated with stress tolerance, its concentration shown to be generally higher in tolerant plants than the sensitive ones (Sen et al., 2017). Apart from acting as osmolyte for osmotic adjustment, proline helps stabilizing sub-cellular structures, scavenging ROS, and protecting cellular redox potential under stress conditions. It may also act as protein compatible hydrotrope, alleviating cytoplasmic acidosis and maintaining appropriate $NADP^+$/NADPH ratios, compatible with metabolism (Hayat et al., 2012). Singh et al. (2017) reported an increase of proline content in leaves of *Lagerstroemia speciosa*, grown in polluted conditions. Similarly, Seyyednejad et al. (2009) reported that proline content was higher in leaf tissues of *Callistemon citrinus* around the petrochemical site. However, in *Plantago lanceolata*, proline content was lower for plants grown in contaminated sites, in comparison to those belonging to non-contaminated sites (Nadgórska-Socha et al., 2013), which was in accordance with the results, obtained from the present study. In addition to proline, soluble sugars play a vital role in plants, since they are implicated in different metabolic events and act as molecule signals, regulating different genes, especially those involved in osmolyte synthesis, photosynthesis, and sucrose metabolism. In the present study, reduction in photosynthesis along with the high requirement of energy leads to the reduction of soluble sugars content in stressed apricot plants. The decreased content of proline and soluble sugars in leaves of apricot plants, grown in polluted site, reflected the sensitivity of this species.

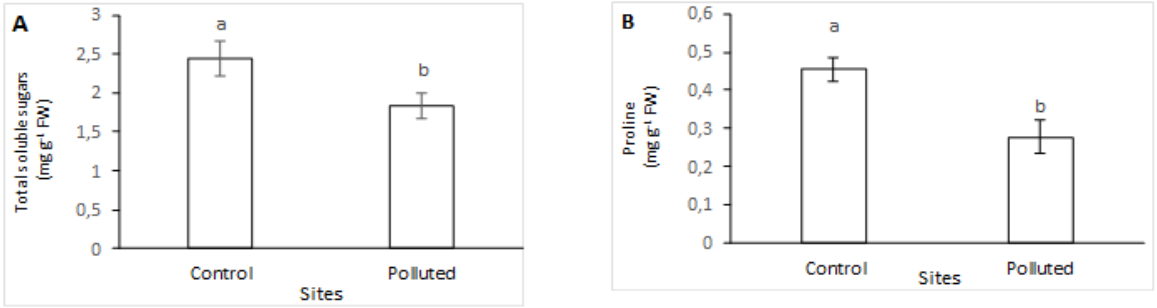

Fig. 4. The effect of air pollution on proline and total soluble sugars content in the leaves of *Prunus armeniaca*. Values represent the means of 3 replications per treatment ± SD. Different letters indicate significant differences among the treatments ($p \leq 0.05$, Ducan test).

CONCLUSION

Due to constant release of toxic substances into air environment, it is necessary to know how they act in the organisms, present there, in order to avoid possible imbalance in the ecosystem. The present study aimed at comparing the suitability of known biomarkers of plant stress in reflecting the level of air pollution. Different biomarkers such as TBARS, gas exchange, photosynthetic pigments, proline, and soluble sugars of apricot (*Prunus armeniaca* L.) leaves changed significantly ($p \leq 0.05$) in polluted area, in comparison to control plants. According to these results, these biomarkers indicated that air pollution in the industrial site of Sfax was quite hazardous and had to be controlled.

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