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AIR POLLUTION EFFECTS ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN *NERIUM OLEANDER* AND *ROBINIA PSEUDO ACACIA* PLANTS IN TEHRAN

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ABSTRACT

The air pollution effects on the activity of antioxidant enzymes were investigated on *Nerium oleander* and *Robinia pseudo acacia* in Tehran. Considering the information obtained from the Department of the Environment of Iran, Sorkh Hesar Park as well as South Azadi were selected as two sampling sites representing the unpolluted and polluted area respectively. A number of plant leave samples were collected from both sampling sites simultaneously. The activity of plant enzymes including peroxidase, catalase and ascorbate peroxidase was investigated using spectrophotometric methods. A higher level of peroxidase and catalase enzymes were measured in both plant samples collected from polluted area. However, this higher level was only statistically significant for the activity of peroxidase enzyme in *Robinia pseudo acacia* plants compare of to the control group ($p < 0.05$). The lower level of ascorbate peroxidase was observed in *Nerium oleander* plant leaves collected from the contaminated sampling site ($p < 0.05$), but though, the activity of this enzyme in *Robinia pseudo acacia* did not change significantly. The overall plant injury symptoms found in this study demonstrated that both *Nerium oleander* and *Robinia pseudo acacia* have a potential to be considered as effective bioindicators to reflect the environmental air quality in polluted areas.

Key words: Air pollution, antioxidant enzymes, *Nerium oleander*, *Robinia pseudo acacia*

INTRODUCTION

Air pollution is one of the most significant environmental concerns in both developed and developing cities. The urban air quality is continuously affected by emissions from both stationary and mobile combustion sources. Mobile sources contribute to the emission of major urban air pollutants including: carbon monoxide (CO), nitrogen oxides (NOx), sulphur oxides (SOx), particulate matter (PM), lead (Pb), photochemical oxidants such as ozone (O_3) and ozone precursors like hydrocarbons and volatile organic compounds (Costa, 2001). Various physical, chemical and dynamic processes may generate air pollutants including particulates and gaseous contaminants that may cause adverse health effects in human or animals, affect plant life and impact the global environment by changing the atmosphere of the earth (Raabe, 1999; Bakand *et al*., 2005; Hayes *et al*., 2007).

While plants can improve the air quality in some extent, air pollution may adversely influence the plant life. Air pollutants such as ozone may inter into plant tissues via stomata and elevate the level of reactive oxygen species (ROS) causing serious damage to the DNA, proteins and lipids (Sharma and Davis, 1994; Hippeli and Elstner, 1996). It is well known that plant cells have several antioxidative defence mechanisms such as tocopherol, carotenoids, glutathione and glutathione reductase enzymes, superoxide dismutase, catalase, ascorbate peroxidase, polyphenol oxidase and particularly peroxidase to protect plants against these oxidative stressors (Kangasjarvi *et al*., 1994; Pell *et al*., 1997; Noctor and Foyer, 1998;

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Sanderman *et al*., 1998). However, the activation of antioxidative defence mechanisms requires a high consumption of energy which may consequently inhibit the plants growth.

In this study, the air pollution effects on the activity of antioxidant enzymes were investigated in *Nerium oleander* and *Robinia pseudo acacia* plants in Tehran. A number of plant leave samples were collected from selected sampling sites simultaneously. The activity of different plant enzymes including peroxidase, catalase and ascorbate peroxidase were investigated using spectrophotometric methods. A number of plant injury symptoms induced by urban air pollution was investigated in selected popular plant clones.

MATERIALS AND METHODS

Site selection

Tehran, a capital city of Iran, is located in large valley-like vicinity surrounded by mountains from three sides. The natural air ventilation of Tehran is further inhibited due to the location of the city at 30-degree north altitude and presence of downward waves. The city has a very low level of rain and the annual thermal inversion in Tehran is expected to be approximately 240 days (Forohar, 1991). A large number of industrial settings and stationary sources of air pollutants are located in the south and south west of Tehran. The wind streams that normally occur from west to east may transport airborne contaminants into the city where it is already polluted by huge number of motor vehicle emissions and mobile sources of contamination. Therefore, considering the information obtained from the Tehran Environmental Protection Agency and the Air quality Control Office, Sorkh Hesar Park and South Azadi were selected as two sampling sites representing the unpolluted and polluted area respectively (Fig. 1).

Sample collection

In this study, the air pollution effects on the activity of antioxidant enzymes were investigated in two popular plant clones including *Nerium oleander* and *Robinia pseudo acacia* (Fig. 2). The leave samples of *Nerium oleander* (the 5th leave of each branch) and *Robinia pseudo acacia* (leaves of middle branches) were collected from both sampling sites simultaneously. The average of four samples was collected for each plant and collected samples were transferred to the laboratory at cold temperature (4ºC).

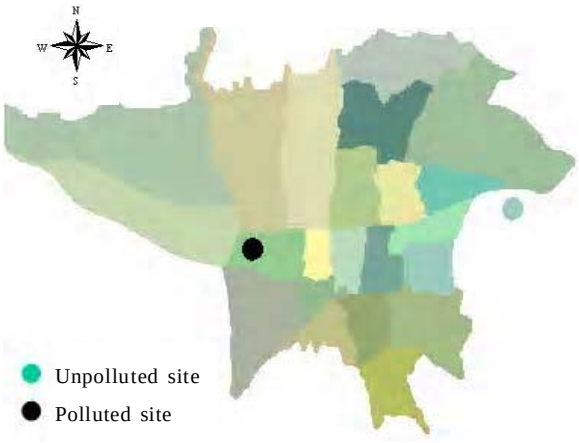
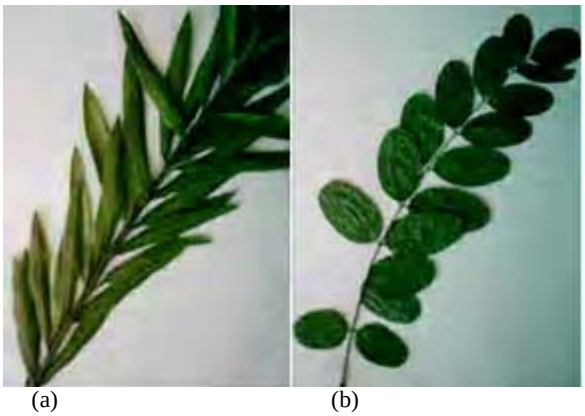


Fig. 1: The map of Tehran representing the selected sampling sites



(a) (b)

Fig. 2: Selected plant clones; a) *Nerium oleander*, b) *Robinia pseudo acacia*

Extraction of proteins

For extracting the proteins, fresh leaves (1 g) were homogenised in Tris- HCl buffer (5 mL; 0.05 M; pH=7.5). The homogenised samples were then filtered and centrifuged at 13000 g for 20 min at 4ºC. The samples of supernatant were kept at 4ºC before protein analysis (Benavids *et al*., 2000).

Measurement of enzymes level

The following spectrophotometric methods were used to quantify the proteins level in collected

samples. The activity of peroxidase enzyme was measured using the acetate buffer (0.2 M; pH=4.8) and hydrogen peroxide (3%) and benzidine (0.04 M) as previously described (Hadadchi, 1986). The activity of catalase enzyme was determined using the Tris (50 mM; pH=7) and hydrogen peroxide (3%) (Kar and Mishra, 1976). The activity of ascorbate peroxidase enzyme was quantified using the phosphate buffer (0.05 M; pH=7) and hydrogen peroxide (3%) and ascorbate 50 µM (Nakano and Asada, 1981). After analysing the samples, the absorbance level was recorded using a spectrophotometer (UNICAM 8625) and the proteins levels were determined accordingly.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2002 and SPSS (version 12.0) Software. Experimental results were expressed as mean ± standard deviation (m ± SD) of four different replicates. One way analysis of variance (ANOVA) was used to compare the average enzyme levels of different sample groups, followed by multiple comparisons and Duncan tests to identify which group was statistically significantly different. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Variation of peroxidase enzyme

The variation of peroxidase enzyme activity in leave samples of both sampling sites is summarised (Fig. 3). The average activity of peroxidase enzyme was increased in both plant samples of contaminated site. The average optical density (OD) and hence the activity of peroxidase enzyme in *Robinia pseudo acacia* samples of contaminated site (53.3 ± 25.38; OD) was statistically significantly increased when compared to the control group (12.66 ± 4.44; OD) ($p < 0.05$). However, the activity of this enzyme in *Nerium oleander* (12.74 ± 7.28; OD) did not changed significantly compare to the control group (9.79 ± 3.37; OD).

Variation of catalase enzyme

The variation of catalase enzyme activity in leave samples of both sampling sites is summarised (Fig. 3). The average activity level of catalase enzyme was increased in both plant samples of contaminated site, but not at statistically significant level. The average activity of catalase enzyme in *Robinia pseudo acacia* samples of contaminated site (3.795 ± 2.96; OD) was increased when compared to the control group (2.046 ± 1.58; OD). The average activity of catalase enzyme in *Nerium oleander* samples of contaminated site (7.30 ± 6.17; OD) was also increased compare to the control group (6.91 ± 3.54; OD).

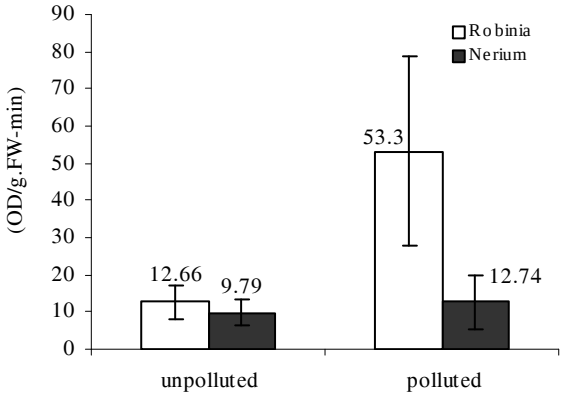


Fig. 3: The variation of peroxidase enzyme activity in leave samples

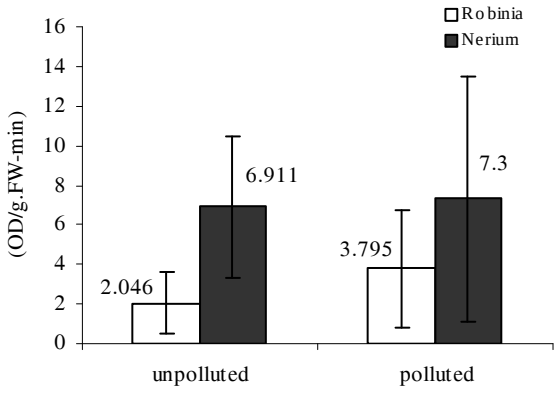


Fig. 4: The variation of catalase enzyme activity in leave samples

Variation of ascorbate peroxidase

The variation of ascorbate peroxidase enzyme activity in leave samples of both sampling sites is summarised (Fig. 4). The average level of ascorbate peroxidase enzyme was decreased in both plant samples of contaminated site. The average activity level of ascorbate peroxidase enzyme in *Robinia pseudo acacia* samples of

contaminated site (12.22 ± 5.6; OD) was decreased compare to the control group (12.81 ± 5.6; OD) but, not at statistically significant level. The average activity of ascorbate peroxidase enzyme in *Nerium oleander* samples of contaminated site (3.05 ± 2.17; OD) was statistically significantly decreased when compared to the control group (9.64 ± 2.16; OD) ($p < 0.05$).

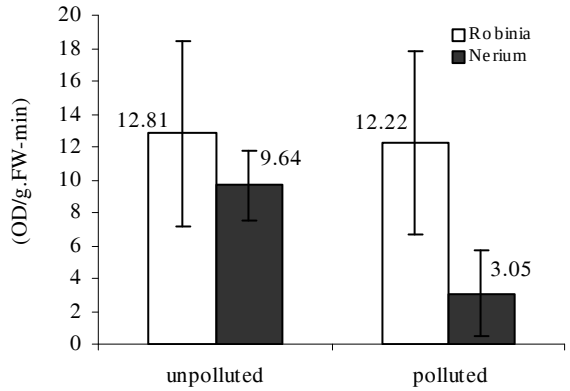


Fig. 5: The variation of ascorbate peroxidase enzyme activity in leave samples

DISCUSSION

Air pollutants are exogenous substances in indoor or outdoor air including both particulates and gaseous contaminants that may cause adverse health effects in human or animals, affect plant life and impact the global environment by changing the atmosphere of the earth (Raabe, 1999). Various physical, chemical and dynamic processes may generate air pollution leading to emission of gases, particulates or mixtures of these into the atmosphere. While great attempts have been made to reduce emissions from both stationary and mobile sources, millions of people today face excessive air pollution in both industrial and urban environments (Costa, 2001; Chauhan and Johnston, 2003). Although air quality standards have been adapted as a guidance for control measures, many Asian, African and South American cities have virtually uncontrolled air pollution (Costa, 2001). Consequently, both local and global environmental problems can potentially arise where such releases are not controlled properly. In this research, a number of plant injury symptoms induced by urban air pollution was quantified in *Nerium oleander* and *Robinia pseudo acacia* in Tehran, the city which is polluted by both mobile and stationary air pollution sources. In plant cells, electrons may be transferred via chloroplast or mitochondrial electron transfer systems. When come into contact with oxygen molecules, these electrons can produce reactive oxygen species (ROS) which is toxic for the plant. Different oxidative stressors such as air pollutants may elevate the level of reactive oxygen species (ROS) causing serious damage to macromolecules of organism such as nucleic acids, proteins and lipids (Mecord, 2000). For examples, a number of air pollutants such as sulphur dioxide, ozone and pesticides like paraquat may induce plant injury via generation of reactive oxygen species. The plant cells have several antioxidative defence mechanisms to protect plants against these oxidative stressors (Kangasjarvi *et al*., 1994; Pell *et al*., 1997; Prasad, 1997; Noctor and Foyer, 1998; Sanderman *et al*., 1998; Dixit *et al*., 2001). These defence mechanisms include both enzymatic (e.g. superoxide dismutase, catalase, peroxidase and ascorbate peroxidase, glutathione reductase) and non enzymatic metabolites (e.g. tocopherol, carotenoids, glutathione and ascorbate). A number of plant injury symptoms induced by urban air pollution was quantified in *Nerium oleander* and *Robinia pseudo acacia* plants. A higher level of peroxidase enzymes was measured in both plant samples collected from polluted area (Fig. 3). However, this higher level was only statistically significant for the level of peroxidase enzyme in *Robinia pseudo acacia* plants when compared to the control group ($p < 0.05$). Although the average level of catalase enzyme was increased in both plant samples of contaminated site, no statistically significant differences were observed when compared to the control groups (Fig. 4). The lower level of ascorbate peroxidase was observed in *Nerium oleander* plant leaves collected from the contaminated sampling site ($p < 0.05$; Fig. 5). Though, the activity of this enzyme in *Robinia pseudo acacia* did not changed significantly.

Both structural and anatomical analysis of collected plant samples were also investigated in details and the publication is underway. In overall,

the higher amount of visible structural damage was observed in *Robinia pseudo acacia* plant leaves in comparison with *Nerium oleander* plant samples. It can be suggested that the higher structural damage observed in *Robinia pseudo acacia* may be related to the high activity of the peroxidase enzyme measured in *acacia* plant samples of contaminated site.

The activity of peroxidase and superoxide dismutase enzymes of *Ficus microcarpa* plant have been studied in traffic polluted area (Li, 2003). The higher activity of peroxidase enzyme was reported in polluted area. However, no statistically significant changes were reported in the activity of superoxide dismutase enzyme in *Ficus microcarpa* plants. The activity of peroxidase enzyme has been investigated in Populus plants exposed to ozone air pollutant and the higher activity of peroxidase enzyme was reported due to exposure to ozone (Bernardi *et al*., 2004). It has been identified that the level of peroxidase and ascorbate peroxidase in plants with larger leaves was higher than plants with the smaller leaves.

However, different results of biological endpoints observed in plant clones may provide an indication of the possible metabolic processes responsible for the adverse effects of air pollution in plants. The overall plant injury symptoms found in this study may reveal, in some extent, the environmental consequences of inadequately controlled urban air pollution in Tehran. While air quality standards have been adapted, appropriate control measures need to be implemented to avoid the severe consequences of excessive air pollution. Our finding suggested that the *Nerium oleander* and *Robinia pseudo acacia* plant clones have a potential to be considered as effective bioindicators to reflect the environmental air quality in cities such as Tehran polluted by both traffic and industrial airborne contaminants.

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REFERENCES

Bakand, S., Winder, C. Khalil, C., Hayes, A., (2005). Toxicity assessment of industrial chemicals and airborne contaminants: Transition from *in vivo* to *in vitro* test methods: A review. Inhal. Toxicol., **17** (13): 775-787.

Benavids, M. P, Marconi, P. L., Gallego, S. M., Comba, M. E., Tomaro, M. L., (2000). Relationship between antioxidant defence systems and salt tolerance in solanum tuborosum, Australian. J. Plant. Physiol., **327**: 273-278.

Bernardi, R., Nali, C., Ginestri, P. C., Pugliesi, I. C., Lorenzini, G., Durante, M., (2004). Antioxidant enzyme isoforms on gels in two poplar clones differing in sensitivity after exposure to ozone, Biol. Plantarum., **48** (1): 41-48.

Chauhan, A. J., Johnston, S. L., (2003). Air pollution and infection in respiratory illness. British. Medical. Bulletin., **68** (1): 95-112.

Costa, D. L., (2001). Air pollution. In: Casarett and Doull's toxicology: the Basic Science of Poisons, sixth edition. Klaassen, C. D. (Ed). McGraw-Hill, N. Y. USA., 979-1012.

Dixit, V., Pandey, V. and Shyam, R. (2001)., Differential antioxidative responses to cadmium in roots and leave of pea (pisum sativum). J. Exp. Bot., **52** (338): 1101-1109.

Forohar, F. (1991). Air pollution of Tehran and the ways of prevention. Tehran. Iran.

Hadadchi, G. H. (1986). Biochemistry and plant physiology, Jahad Daneshgahi, Tehran. 1986.

Hayes, A., Bakand, S. and Winder, C., (2007). Novel in vitro exposure techniques for toxicity testing and biomonitoring of airborne contaminants. In: Drug Testing In Vitro-Achievements and Trends in Cell Culture Techniques,Wiley-VCH, Berlin, 103-124.

Hippeli, S., Elstner, E. F., (1996). Mechanisms of oxygen activation during plant stress: biochemical effects of air pollutants, J. Plant. Physiol., **148**: 249-257.

Kangasjarvi, J. Talvinen, M. and Karjalainen, R. (1994). Plant defence systems induced by ozone. Plant. Cell. Environ., **17**: 783-94.

Kar, M. and Mishra, D. (1976). Catalase, peroxidase, polyphenol oxidase activities during rice leaf senescence. Plant. Physiol, **57**: 315-319.

Li, M. H., (2003). Peroxidase and superoxide dismutase activities in fig leaves in response to ambient air pollution in a subtropical city, Archives. Environ. Contamination. Toxicol., **45**: 168–176.

Mecord, J. M., (2000). The evolution of free radicals and oxidative stress, American. J. Medicine., **108**: 652-659.

Nakano, Y., Asada, K., (1981). Hydrogen peroxide is scavenged by ascorbate- specifics peroxidase chloroplasts, Plant. Cell. Physiol., **22**: 867-880.

Noctor, G. and Foyer, C. H. (1998). Ascorbate and glutathione: Keeping active oxygen under control, Annual Review of Plant Physiology and Plant Molecular Biolology., **49**: 249-276.

Pell, E. J., Schlagnhaufer, C. D., Arteca, R. N., (1997). Ozone-induced oxidative stress: Mechanisms of action and reaction, Physiol. Plantarum., **100**: 264-273.

Prasad, M. N. V., (1997). Trace metals, In: Plant Ecophysiology. Prasad, M.N.V. (Ed). Wiley, N. Y. USA., 207- 249.

Prasad, M. N. V., (1997). Trace metals, In: Plant Ecophysiology. Prasad, M.N.V. (Ed). Wiley, N. Y. USA., 207- 249.

Raabe, O. G., (1999). Respiratory exposure to air pollutants. In: Air pollutants and the respiratory tract. Swift, D. L. and Foster, W. M. (Eds). Marcel Dekker INC, N. Y. USA., 39-73.

Sandermann, Jr. H., Ernst, D., Heller, W. and Langebartels, C., (1998). Ozone: an abiotic elicitor of plant defence reactions. Trends. Plant. Sci., **3**: 47-50.

Sharma, Y. K. and Davis, K. R. (1997). The effects of ozone on antioxidant responses in plants. Free. Radical. Bio. Med., **23**: 480-488.