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LEAD ACCUMULATION AND RESPONSE OF ANTIOXIDANT ENZYMES OF *LIGUSTRUM JAPONICUM* IN URBAN ATMOSPHERE OF BAKU CITY

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The use of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX), as bioindicators of lead pollution in urban atmosphere of Baku city was investigated using the Ligustrum japonicum. At higher lead concentrations, CAT, SOD, APX and GPX exhibited remarkable induction with an increase in the concentration of lead as a defense mechanism against the metal stress comparing with those of control samples. Based on the above study, it is evident that Ligustrum japonicum can be used as a bioindicator to monitor the effect of heavy metals on the antioxidant content.

Key words: antioxidant enzymes, metal content, urban pollution, biomarkers.

Introduction

In an attempt to define and measure the effect of pollutants on an ecosystem, biomarkers have attracted a lot of interest. The underlying principle of the biomarker approach is the analysis of an organism's physiological or biochemical response to pollutant exposure. This concept is derived from the idea that a toxic effect manifests itself at the subcellular level before it becomes apparent at higher levels of biological organization. The measurement of biochemical responses to chemical contaminants may serve to improve the assessment of biologically significant exposures to toxic chemicals and enhance the ability to assess the risk of effects on the health and survival of toxicant exposed populations. When compared to chemical residue analysis, biomarkers have the advantage of being more relevant biologically [17]. Environmental pollution by metals has become one of the most important problems in the world. Environmental poisoning by heavy metals has increased in the last decades due to extensive use of heavy metals in agriculture, chemical and industrial processes, becoming a threat to living organisms

[13, 10]. The presence of excessive amounts of both essential and non-essential trace elements in the environment can cause serious problems to all organisms. The mechanisms of cytoplasmic toxicity are similar in all organisms; however, different plant species and varieties show a wide range of variability of metal tolerance [10, 22]. Most plants show a high degree of sensitivity toward heavy metals, resulting in reduced growth and other visible symptoms of metal toxicity. Heavy metals generally cause damage to plants, either directly or indirectly, through reactive oxygen species (ROS) formation. ROS damage cell membranes, nucleic acids and chloroplast pigments [11, 1]. Redox metals, such as copper or iron, may directly produce free radicals [25], whereas cadmium is a non-redox metal unable to participate in free radical formation, but is capable of inducing oxidative stress in cells [23]. Plants show metal-induced alterations in both the activities of antioxidative enzymes and the level of soluble antioxidants [7]. Plants cope with oxidative stress by using antioxidant enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APOX, EC 1.11.1.11), catalase (CAT, 1.11.1.6), glutathione reductase (GR, EC 1.6.4.2), peroxidase (POD, EC 1.11.1.7), glutathione peroxidase (GPX, EC 1.11.1.9), and glutathione S-transferase (GST, EC 2.5.1.18) [2,11,16], as well as by non-enzymatic compounds such as cysteine (Cys), reduced glutathione (GSH), carotenoids, ascorbate (ASC), _-tocopherol, etc. [8].

Among the various air pollutants, the effects of certain heavy metals on cellular systems have received a great deal of attention in recent decades due to the increasing exposure of living organisms to these metals in the environment. Many studies showed that heavy metals with toxic levels were reported to affect a variety of biological processes in plants [4]. Causes' enhancement production of reactive oxygen species (ROS) in plant cells via number of routs after exposure of the plant to environmental stresses.

The aim of this paper was to evaluate the potential of the antioxidant/ detoxification enzymes of *Ligustrum japonicum* for use as biomarkers of lead pollution in air.

Materials & Methods

Plant material

The evergreen tree which commonly occur in all three examination areas, were selected. This widespread tree species, namely *Ligustrum japonicum* (Oleaceae). This plant abundant in urban and rural areas of Azerbaijan, because they are quite tolerant against climatic influences due to their modesty and adaptability. Three investigation areas have been chosen. The 1st one is located in the industrial zone of Baku (Absheron peninsula), the 2nd is characterized by high traffic (Airport) and the 3rd zone represents a rural area (botanical garden of Baku State University). Trees of approximately the same age were chosen, Care was given to avoid the collection of leaves characterized by insect infestation, presence of honeydew, bird dropping, pesticide

treatment, chlorosis or necrosis, coarse, and anomalous dust cover. Leaf samples were collected and dried in the laboratory [20].

Analytical method

Metal analysis

After drying and homogenization, the samples were placed in polythene bags and stored in a refrigerator at 4 ºC. 1 g of a sample was digested in an open quartz tube. 10 ml of concentrated HNO_3 (Merck, pro analysis) was added and the mixture was left at room temperature overnight. Then it was heated at 50^{o}C for 2 h and subsequently heated at 160º C for 4 h. The solution was filtered through a Whatman type 589/2 filter and the filtrate was diluted to 25 ml volume with double de-ionized water. These final solutions were analyzed for heavy metal concentrations using a Perkin Elmer atomic absorption spectrophotometer 850 at the.

Enzymes Extraction

Frozen (–70 °C) leaf material (0.5 g) were homogenized in 6 ml of 0.1 M potassium phosphate extraction buffer (pH 7, containing 100 mg insoluble PVP and 0.1 mM EDTA) with Ultra Turrax. The homogenate was centrifuged for 5 min at 6000 × g and 4 °C. The supernatant was filtered through a Whatman GF/A glass fiber disc with a vacuum filtration system and stored at –70 °C [21].

Catalase (CAT, EC 1. 11. 1. 6)

CAT activity was assayed at 20 °C in a 3 ml reaction volume containing 2.8 ml 50 mM potassium phosphate buffer (pH 7 not containing EDTA), 120 µl enzyme extract and 80 µl of 0.5 M H_2O_2 Activity was determined by UV spectrophotometer at 240 nm [1] CAT activity was calculated as µmol H_2O_2 min^{-1}.

Superoxide dismutase (SOD EC 1. 15. 1. 1)

SOD activity was determined according to [3]. The reaction mixture (3 ml) contained potassium phosphate buffer (pH 8, 0.025% Triton X-100 and 0.1 mM EDTA), enzyme extract, 12 mM L-methionine, 75 µM nitroblue tetrazolium chloride (NBT) and 2 µM riboflavin. NBT reduction ratios were measured with a spectrophotometer adjusted to 550 nm.

Ascorbate peroxidase

APX activity was assayed using the method described by [14] A reaction mixture consisting of 100 µl supernatant, 17 mM H_2O_2 (450 µl; Fisher Scientific), and 25mM ascorbate (450 µl; Fisher Scientific) was then assayed for 3 min at 290 nm. Activity was measured as disappearance of ascorbate. One unit of enzyme activity was defined as a decrease in absorbance of 0.001 min–1 at 290 nm.

Guaiacol Peroxidase

GPX activity was measured using the method described by [12]. A reaction mixture consisting of supernatant (100 µl), 17mM H_2O_2 (450 µl; Fisher Scientific), and 2% guaiacol (450 µl; Fisher Scientific) was then assayed for 3 min at 510 nm.

Results & Discussion

Site	CAT (unit g^{-1} FW)	SOD (unit g^{-1} FW)	PX (unit g^{-1} FW)	APX (unit g^{-1} FW)	Pb (ppm)
Control	38.5344	2.472	3.792	54.378	52.53
Absheron (Industrial zone)	40.976	2.4144	9.234	201.57	105.74
Airport (high traffic zone)	43.7088	2.8656	6.462	213.576	80.44

In higher plants, heavy metals induce oxidative stress by generation of superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^{-}), and singlet oxygen ($^1O_2$), collectively termed ROS [6]. ROS can rapidly attack all types of biomolecules such as nucleic acids, proteins, lipids, and amino acids, leading to irreparable metabolic dysfunction and cell death. Therefore, the induction of antioxidant enzymes including SOD, CAT, GPX and APX is an important protective mechanism to minimize oxidative damage in polluted environments. The results of the present study highlighted the response of the antioxidant enzymes to stress imposed by different species of heavy metals. These results are in coincidence with [5, 9] who reported the potential use of antioxidant enzymes as good biomarkers for metal stress.

The data recorded in table 1 show that, the highest concentration value of Pb was recorded at airport region (with high traffic load) following by those of Absheron region (industrial area) while the lowest value was recorded at botanical garden (control site) as follow:-

At control site: 52.53 ppm. At Absheron region: 80.44 ppm. At airport region: 105.74 ppm. In agreement with these results, table 1 shows that, all antioxidant enzymes (CAT, SOD and APX) exhibited highest activity values at airport region (with high traffic load) following by those of absheron region (industrial area) while the lowest values are recorded at botanical garden (control site) as follow:-

At control site: 11.1008, 2.472, 3.792 and 54.378 unite g-1 FW for CAT, SOD, PX and APX respectively. At Absheron region: 41.976, 2.4144, 9.234 and 201.57 unite g-1 FW for CAT, SOD, PX and APX respectively. At airport region: 43.7088, 2.8656, 6.462 and 213.576 unite g-1 FW for CAT, SOD and APX respectively. In this concern [18] reported that antioxidant enzymes had higher affinity for decomposing H_2O_2 that formed under different types of stress. Pb is known to induce oxidative stress through over-production of reactive oxygen species [19]. Although an important role of Zn in living organisms is related to its antioxidant properties [15], an excess of Zn caused severe effects on biomass production, and resulted in oxidative stress. Enzymes such as SOD, CAT, GPX, and APX can be activated against ROS in several organisms following heavy metal stress [24].

Table 1: Activity of antioxidant enzymes (CAT, SOD, PX and APX) in leaf tissue of *Ligustrum japonicum* **(**unit g^{-1} FW**)** at different lead concentrations (ppm).

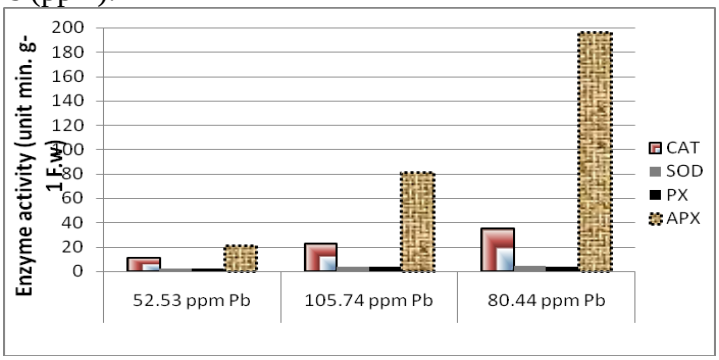

Figure1. CAT, SOD, PX and APX activity in *Ligustrum japonicum* exposed to increasing concentrations of Pb (ppm) at control site, Absheron (industrial zone) and Airport (high traffic zone).

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АНТИОКСИДАНТНЫЕ ФЕРМЕНТЫ LIGUSTRUM JAPONICUM В КАЧЕСТВЕ БИОМАРКЕРОВ ЗАГРЯЗНЕНИЯ АТМОСФЕРЫ БАКУ

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РЕЗЮМЕ

Использование антиоксидантных ферментов, а именно: супероксид дисмутазы (SOD), каталазы (КАТ), аскорбатпероксидазы (APX) и глутатионпероксидазы (GPX) в качестве биомаркеров загрязнения свинцом в городской атмосфере Баку было исследовано с помощью более высокой концентрации свинца. CAT SOD, APX и GPX демонстрируют значительную индукцию при увеличении концентрации свинца по отношению к контрольной группе. Исходя из *Ligustrum japonicum* этого исследования, очевидно, что *Ligustrum japonicum* может быть использован в качестве биоиндикатора для мониторинга влияния тяжелых металлов на содержание антиоксидантов.

Ключевые слова: антиоксидантные ферменты, тяжелые металлы, загрязнение, биоиндикаторы.

LIGUSTRUM JAPONICUM BİTKİSİNİN ANTİOKSİDANT FERMENTLƏRİ BAKI ŞƏHƏRİNİN ATMOSFER HAVASININ ÇİRKLƏNMƏSİNDƏ BİOMARKER KEYFİYYƏTİNDƏ

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XÜLASƏ

Ligustrum japonicum vasitəsilə Bakı şəhərinin atmosfer havasının ağır metallardan qurğuşunla çirklənməsini tədqiq edərkən antioksidant fermentləri: superoksiddismutazalar (SOD), katalazalar (KAT), aksorbatperoksidazalar (APX) və qlutationperoksidazalar (GPX) istifadə edilmişdir. Qurğuşunun daha yüksək miqdarında CAT, SOD, APX və GPX fermentləri yoxlama qruplara nisbətən əhəmiyyətli dərəcədə induksiya nümayiş etdirmişdir. Bu tədqiqatdan irəli gələrək, ağır metalların antioksidantların tərkibinə təsirinin monitorinqi keçirildikdə Ligustrum japonicum bioindikator kimi istifadə oluna bilər.

Açar sözlər: antioksidant fermentləri, ağır metallar, bioindikator, şəhər çirklənməsi.

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