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Green Biomonitoring Systems for Air Pollution †

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Abstract: Human activities have led to environmental pollution, while industrial growth and the gradual transition to urbanization are the main causes of the growth of different types of environmental pollutants; the continuation of studies on air quality biomonitoring in the context of "green revolution" have renewed the way to use "green tools" using higher plants with specific capabilities as biomonitors.

Keywords: biomonitoring; environmental pollution; enzymes; green revolution

1. Introduction

Urbanization, industry and transport development cause high concentrations of air pollutant emissions, which lead to harmful effects on nature especially for living organisms. After the emergence of awareness of the danger of ecosystems imbalance, it is now necessary to find ways to monitor them, related with international policy efforts to limit air emissions. All these actions have been transposed into plans and agreements at the international level and involved with a global movement known as the "green revolution". Plants can also indicate pollution load in a particular area via alterations in physiological parameters such as pigment degradation, membrane damage, production of antioxidant metabolites and changes in antioxidant enzyme activities. The aim of this first environmental surveillance is to detect possible changes in the functioning and composition of biological systems, which could eventually lead to disorganization, collapse, or move them in an evolutionarily unfavorable direction. Early warnings from specialists allow policy decision makers to take action to remedy the situation, before irreversible negative effects appear. The environment monitoring could be instrumental or biological. Biological monitoring or biomonitoring may replace or supplement instrumental monitoring and at the international level, the use of living organisms as "tools" in determining the quality of the environment in general and air contamination in particular is continuously increasing [1].

As for the pollution "green" indicators, they are of two types: sensitive species, which indicate the presence of a pollutant by the appearance of lesions or malformations, and accumulating species.

The most important reasons for using bioindicators are: demonstrating how the biological effect of pollutants is integrated with the sensitivity of biological sensors and environmental (climatic) factors; through extrapolation of toxic hazard from vegetal to human, showing the toxic potential on humans; and the possibility of standardization of working methods [2].

In this paper, we have conducted our own studies on air-quality biomonitoring with plants in which the aim was to monitor air pollutants as dangerous factors to living organisms because of their high oxidative potential. So, the studies consist of the analysis of antioxidant compounds that are represented as a structural class of chemicals, with a wide range of biological functions, with the role of inhibiting free radicals. Thus, a low

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level of inhibition of free radicals indicates a reduced ability to inhibit them and increased sensitivity to leaf diseases (necrosis). Green plants can also act as indicators of air pollution via the detection of metabolic products of the pollutants in their tissues or measuring the indirect effects of pollutants based on the "built-in" ability to have an antioxidant response system to protect themselves from the damage caused by oxidative stress resulting from pollutant exposure. Only when the balance between the amount of antioxidant compounds and that of free radicals (caused by pollution) is altered, does the activation of the plant's response system take place [3].

A correlation between the level of reactive oxidative system (ROS) in the cell and the activity of enzymes helps us to deduce that the antioxidant mechanism of the plant is responsible for combating oxidative stress due in this case to the stress factor: pollution. Based on the experimental results in the study we developed our knowledge in biomonitoring and we consider that this is the basis for the development of new methods of use and study with applications in environmental assessment.

One of our aims was to change the appearance and biochemical composition of plants exposed to various pollutants compared to those not exposed [4].

In order for them to be used in practice, selection of species capable of providing the information necessary for the purpose of monitoring was a must in chosen plants. Sensitivity and response of plants to air pollutants are variable. The plant species which are more sensitive act as biological indicators of air. The plant material selected was: *Nicotiana tabacum*, *Petunia hibrida*, *Ricinus comunis*, *Trifolium pretense*.

In our case, the aim was to determine the antioxidant capacity of the extracts from the samples of plants exposed to pollutants compared to the control ones, in order to highlight that the degree of inhibition of free radicals decreases in the exposed plants compared to the control ones. Exposure locations used:

1. Site with incinerator, coded Exposure site 1;
2. Site with poultry farm, coded Exposure site 2;
3. Site with slaughterhouse, coded Exposure site 3.
4. Fumigation chambers.

Air pollutants generate reactive oxygen species (ROS) that leads to damage at cellular and molecular levels [5]. Damaging effects induced by oxidative stress may be minimized by various enzymatic and non-enzymatic antioxidant defense mechanisms, as has been suggested previously [6].

The classical methods of biochemistry, the analysis of some antioxidant compounds and combined with those of biomonitoring have led to the completion of knowledge about the ways in which plants act on pollution control.

ROS OXYGEN REACTIVE SPECIES are a normal product of plant cell metabolism. A variety of environmental stresses lead to excessive ROS production, which causes oxidative damage and can eventually cause cell death. The most common reactive oxygen species are: O_2, O_2, H_2O_2, OH. To avoid oxidative damage, higher plants improve their natural antioxidant defense system.

One group of non-enzymatic compounds is the polyphenols. Excess phenols are considered important biomarkers for the phytotoxic effects of heavy metals and other pollutants. Increasing the production of phenolic compounds is a capability of the plant's defense mechanism. Phenolic compounds are of particular interest because of their involvement in the stress response, such as intra and/or interspecific competition or air pollution [7]. Polyphenols are produced in the cytoplasm and form droplets in vacuoles that will later evolve into a single vacuole. If the excess polyphenols degenerate in the cytoplasm, the organs disappear and eventually the release of vacuolar contents leads to the death of mesophilic cells [8].

Thus, in our study, the amount of polyphenols in the exposed plants was analyzed compared to the control plants.

Antioxidant activity is determined in our case by SOD superoxidysmutase activity. The role of antioxidants is to destroy the free radicals of the cell that have a negative effect

on living organisms. A special role in neutralizing the effects of oxidative stress belongs to the enzyme superoxidysmutase (SOD). Superoxidismutase (SOD) is a metal enzyme with subunit structural organization. SOD is the main regulator of oxidation processes in the cell. This enzyme catalyzes the recombination reaction of O_2 radicals.

Biochemical determinations of bioindicator samples can provide important information on increasing pollution levels.

2. Methods

2.1. Determination of the Total Polyphenol Content

The total content of polyphenolic compounds in plant extracts was determined colorimetrically using Folin–Ciocalteu reagent. Folin–Ciocalteu, also called the gallic acid equivalence method (GAE), used for colorimetric analysis of phenolic and polyphenolic antioxidants [9].

A sample of 0.2 mL of plant extract was added to a test tube and mixed with 2 mL of Folin–Ciocalteu reagent; after 5 min of reaction, 1.8 mL of sodium carbonate (7.5%) was added. The absorbance was measured at 750 nm using a UV–VIS spectrophotometer.

The curve was established for analysis using gallic acid. The polyphenolic content was determined using the standard gallic acid calibration curve and expressed in mg of gallic acid equivalents.

2.2. Determination of Antioxidant Capacity by Determining SOD

The method described by Winterbourn et al. is based on the ability of superoxide dismutase to inhibit the reduction of tetrazolium-Nitro Blue Tetrazolium (Nitro BT) salt with superoxide radicals [10]. An enzymatic unit is the amount of enzyme that produces 50% inhibition under standard conditions of speed and reaction temperature. Superoxide radicals are generated in the reaction medium by photoreduction of riboflavin. In parallel, a blank and a sample were performed, except for the enzymatic solution. The blank and the sample were illuminated for 5 min by a neon bulb, after which the extinction was read at 560 nm.

3. Results and Discussion

Following are presented the determination of polyphenols expressed in gallic acid and the following results were obtained (Table 1).

Table 1. Polyphenols expressed in gallic acid mg/g dry substance.

	Nicotiana Tabacum	Petunia Hibrida	Ricinus Comunis	Trifolium Pratense
Control	1.12	0.62	0.45	0.79
Exposure Site 1	2.17	2.49	3.25	1.25
Exposure Site 2	2.76	2.43	5.39	1.43
Exposure Site 3	2.34	2.36	4.38	2.34
H2S Fumigation	1.85	2.79	1.35	2.69
NH3 Fumigation	1.96	2.67	1.27	3.02

Examining the results obtained in the previous table, we can see the high content of polyphenols in plants exposed to pollutants, especially those located in exposure sites and then in fumigation areas compared to the values obtained in climate control tests. This analysis gives us important information about the increase in the amounts of polyphenols in plants (in excess) with the degree of pollution. Table 2 shows the degree of inhibition of free radicals by the SOD method.

Table 2. Antioxidant activity by determining superoxidismutase. Antioxidant Activity Inhibition %.

	Nicotiana Tabacum	Petunia Hibrida	Ricinus Comunis	Trifolium Pratense
Control	84	93	75	98
Exposure Site 1	34	27	68	35
Exposure Site 2	57	42	67	87
Exposure Site 3	68	54	43	96
H2S Fumigation	75	39	74	25
NH3 Fumigation	69	43	87	31

There has been observed a decrease in the ability to inhibit free radicals in plants exposed to pollutants from non-exposed plants (controls), which is explained as follows: free radicals can occur as a result of all existing forms of pollution (contaminating air, water, soil); to balance the situation, the bodies constantly produce certain substances with antioxidant effects.

In the case of pollutants in the environment, the amount of the enzyme superoxidismutase decreases along with the ability of organisms, in our case exposed plants, to defend against free radicals. In this case, there was a sensitization of plant organisms and the appearance of diseases destroying cells.

So, according to the results obtained, an increased content of polyphenols was observed in plants exposed to pollutants, especially in those exposed in the exposure sites and then in fumigation glasshouses compared to the values obtained with the control plants in the climatic chamber.

A decrease has been observed in the ability of plant to inhibit free radicals in plants exposed to pollutants from non-exposed plants (controls) which is explained as follows: free radicals can occur as a result of all existing forms of pollution (contaminating air, water, soil); to balance the situation, the bodies constantly produce certain substances with antioxidant effects.

4. Conclusions

After these determinations, we have observed that:

Polyphenols (expressed in gallic acid) are considered as important biomarkers for the phytotoxic effects of pollutants. Thus, the amount of polyphenols in the exposed plants was analyzed compared to the control plants.

Impregnation of leaf tissues with phenols in higher quantities compared with controls are active responses. This investigation shows how the leaves adapt to increased pollution stress. This analysis gives us important information about the increase in the amounts of polyphenols in plants (in excess) that increases with the degree of pollution.

The antioxidant activity (Winterbourn method) of bioindicators was analyzed, revealing the role of antioxidants in the destruction of free radicals in the cell that have a negative effect on living organisms. Superoxidismutase (SOD) is a metal enzyme with subunit structural organization. SOD is the main regulator of oxidation processes in the cell.

With increased amounts of pollutants in the environment, the amount of the enzyme superoxidismutase decreases along with the ability of organisms, in our case of exposed plants, to defend against free radicals. In this case, there was a sensitization of plant organisms and the appearance of diseases destroying cells.

Based on the results of this study, it is concluded that biochemical and physiological differences can explain the effects of environmental pollution of exposed species.

Antioxidants can be used as a marker of biomonitoring pollution intensity.

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