Aspects Regarding the Use of Some Species of Plants as Bioindicators in Air Quality Assessment

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The study aims to assess the identification of the specific species of plants for selection of certain plant species and utilization of selected one's in experimental biomonitoring studies.The purpose of the proposed study was to develop and apply new techniques, methods and methodologies for air quality assessing in the context of climate change and updating of international environmental studys. The novelty of the active biomonitoring method using plants is an innovative research area for the National Research and Development Institute for Industrial Ecology in terms of the use of sentinel species. The first stage research activity was aimed to identify plant species with specific response to certain environmental pollutants, in our case, ozone. By exposing higher plants (from the Solanaceae family) to various environmental conditions, a direct impact measurement of ground-level ozone was considered - as an indicator of environmental pollution (air) - there was a significant difference relationship between soil level ozone variation and foliar necrosis.

Keywords: air pollution, biomonitoring, foliar necrosis, ozone

Air pollution has become a major environmental problem facing the world today due rapid increase in industrialization and anthropogenic activities. As such, there is need for reliable and sustainable air pollution monitoring and control methods. Biological monitoring of air pollutants can be passive or active. Passive methods observe plants growing naturally within the area of interest [1]. Active methods detect the presence of air pollutants by placing test plants of known response and genotype into the study area [2]. Biomonitoring of air pollution using plants is the method of interest in recent time as it is cost effective, sustainable and environmentally friendly compared to traditional methods [3].

Advantages of Biomonitoring vs. Instrumental Monitoring are: can provide a response to the combined effect of certain pollutants, unlike instruments that measure separately the quantities of each pollutant; is financially sound if insufficient financial resources are available for the installation and maintenance of equipment; is very convenient for cases where large areas are being monitored for a very long time; can provide indications of time variation, accumulation or effect of interaction of certain abiotic factors and the response of individual living organisms or organisms to environmental changes [4].

The use of bioindicators to monitor air pollution and implicitly, ecosystems exposed to pollution phenomena can provide a timely intervention to protect the integrity of the environment and indirectly the health of the human population [5].

Experimental part

Some plant species are highly sensitive to particular air pollutants and show specific responses to pollutants effects by showing specific damage symptoms [6]. Plant species were used to detect and monitor air pollutants correlated with foliar necrosis produces by a high ozone level [7].

For the implementation of this experiment was used the European Standard, EN 16789-Ambient air. Biomonitoring with Higher plants [8]. The reference method for ozone measurement were provided by EN 14625: Ambient air. The standardized method for measuring the ozone concentration [9].

For this fact, we have proposed to initiate the research on the use of sentinel species. The tests were carried out to identify the sensitivity of some plant species of the *Solanaceae* class: P1-*Nicotiana rustica*, P2-*Nicotiana tabacum*, P3-*Nicotiana alata* and P4-*Petunia hibrida*.

The tests included three main steps: i) preparation for monitoring, ii) exposure of the test plants -(P) in two zones: i) industrial area (I) and green peri-urban area, and iii) exposure effect in the tested areas compared to laboratory controls -(M). Seeds used for sowing were of a physical purity of at least 94%, so a germinate capacity over 70%. The optimal temperature germination of seeds was 25-28 degrees C [10, 11].

Selected plants were grown in two-litter pots, with standard soil, in laboratory conditions; suitable irrigation was maintained by capillarity. So, eight-week-old cultivars, six lants from each species, were exposed on the exposure racks, for two weeks in two sites, with differing tropospheric ozone levels, from May to June, 2018. Concentration of ozone levels in the tested areas was also measured [10, 12].

In both field areas, the percentages of leaf affected surface by typical ozone-induced necrosis of the fourth, fifth and sixth oldest leaves were estimated by a single operator, respecting the recommendations proposed [11, 13].

Histo-anatomical analysis of health and affected leaves was used and gives us the opportunity to study the interaction between plants and the environment. So, it has been shown that plants can control their stomata characteristics correlated with environmental conditions, important aspect in monitoring of air pollution. Stomata's, regulate the mechanisms of the leaf gas exchange and significant correlations between ozone level from the two sites and the degree of stomata leaf damages were found [14, 15].

Results and discussions

Comparative study and establishing correlations between conventional and biomonitoring assessment methods

Biomonitoring assessment capabilities offer a wide range of information. The information provided is

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polyvalent, expressing air quality and reproducing the effects of pollution [16].

In our study, visible symptoms such as necrotic areas were not observed on the leaves of the exposed plants [17].

Numerical percentage of foliar injuries on exposure areas

- The exposed panels were compared at the end of the exposure period (14 days) with the Control (M).
- The evaluation was done weekly and the percentage of foliar destruction was estimated. Estimates were done by visual inspection, using a scale of 1 to 5.

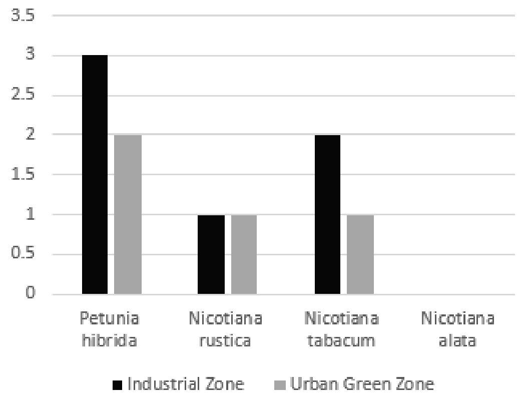

Fig 1. Percentage of foliar injuries for the bioindicator samples

The number of stomata at the end of the exposure period at the sample bioindicator (P1) *Nicotiana rustica* indicated an increase in their density as a protective factor against the unfavourable environmental conditions, thus for the plants exposed in industrial Zone (I) number of stomata/ mm^2 was situated in the range 301 to 314. On the other hand, for the plants exhibited in the urban green UG Zone, the number of stomata/mm^2 was situated in the range 278 to 285 compared with the Control samples (M1), which have a range between 267 and 272. The fact that the number of stomata has increased on the foliar surface in accordance with the degree of pollution it has also been revealed from the observations made in the other samples (P2) *Nicotiana tabacum* and (P4) *Petunia hibrida.*

The histo-anatomical analysis for foliar injuries was also used: stomata, which regulate the mechanisms of entry and exit of leaf gases, provide the opportunity to study the interaction between plants and their environment, thus demonstrating that plants can control their stomata characteristics, in the short term by influencing the opening and closing of stomata to optimize the exchange of CO2 and water vapour.

In the comparative microscopic images between the Samples and the Controls, we observed changes in the stomata characteristics on the sample leaves, that have been demonstrated and correlated with the increased concentration of Ozone in the environment through previous studies (issued in the Standards) and confirmed in this study, so changes in the density, distribution and morphology of the stomata on the foliar surface were also observed, as well as the occurrence of *necrotic* spots on the foliar level, the multiplication and the increase of the bristles, which can be considered important and correlated with the effect of ozone pollution [17-19].

In comparative microscopic images between Samples and Controls, changes in stomata's characteristics were observed in samples; literature studies in the field indicate a direct qualitative and quantitative correlation of changes with increased ozone concentration. Thus, changes in the density, distribution and morphology of the stomata on the foliar surface, as well as the occurrence of *necrotic spots* at the foliar level, the multiplication and dimension increase of the stomata's, are important aspects correlated with the effect of ozone pollution [20, 21]. Measurements in the two tested areas showed high ozone concentrations (93µg /m^3 in the UG area and 96µg /m^3 in I Zone) without exceeding the value limit for human health protection (120µg /m^3) [22, 23].

Conclusions

The results of our research study revealed the following important aspects:

-In (I) area the number of leaves affected by foliar necrosis was much higher compared to the (UG) area; Similarly, the affected foliar surface was higher for plants exposed in (I) area.

-A given response of plants to the action of the polluted environment was in the following order:

Petunia hibrida (Petunia)>*Nicotiana rustica* (Wild Tobacco) > *Nicotiana tabacum* (Tobacco)

-*Nicotiana alata* (Queen of the Night) did not respond properly due to the natural fragility of tissues and foliar necrosis with atypical format for ozone pollution.

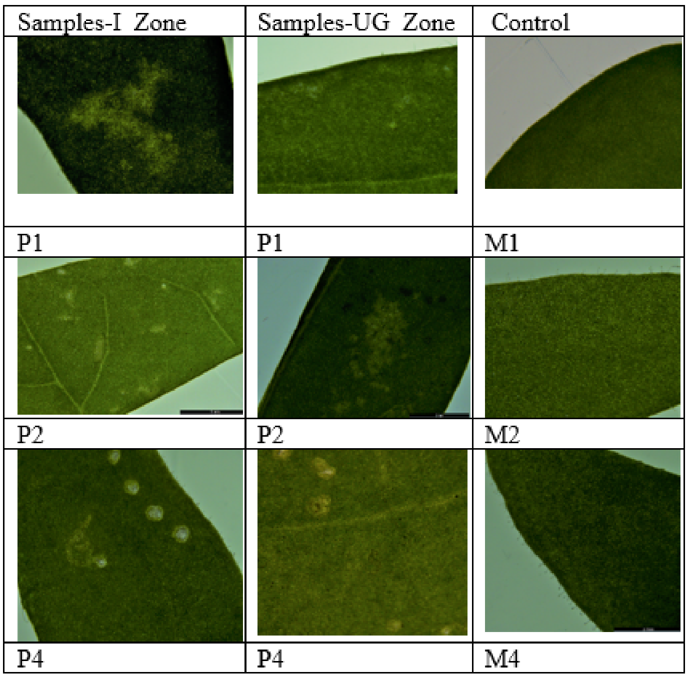

Fig 2. Comparative images obtained at Leica M205 FA microscopy

In conclusion, three bioindicators: *Petunia hibrida* (Petunia), *Nicotiana rustica* (Wild Tobacco) and *Nicotiana tabacum* (Tobacco) were selected to be used in the future research. The current study will be continued by increasing the number and variety of exposure areas, the use of larger plant samples and the monitoring of a larger number of air pollutants. The results obtained will be treated and interpreted statistically to a better understand of the mechanisms underlying the effects of air pollution on plants.

Plants play an important role in monitoring and maintaining the ecological balance by actively participating in the cycling of nutrients and gases and also provide enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollution level in the air environment. Using biomonitoring methods to the plant species is an important approach towards air pollution monitoring and abatement.

Air pollution is one of the major environmental problems that is receiving global attention because of it adverse effects on plants and animals and humans. Hence, there is need of integral efforts toward remediation and monitoring of air pollution. Biomonitoring using plants can be a relevant method because of its sustainability, affordability and eco-friendliness. The plants that are sensitive to a particular pollutant or mixture of pollutants can be used as veritable tools towards mitigating environmental and health problems associated with air pollution.

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