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BIOLOGICAL MONITORING USED IN ASSESSMENT OF THE AIR POLLUTANTS

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Abstract

Nowadays, the air pollution has become a major environmental problem due to rapid increase of industrialization and anthropogenic activities which led to climate change. Air pollution is considered as a harmful agent for human health. Different classes of pollutants like gaseous (SOx, NOx) are continuously released in air and perceived/recognized as pollutants. Among the biological models, plants could indicate pollution load in a particular area via alterations in physiological parameters so, there is a need for reliable and sustainable air pollution monitoring and control methods.

Keywords: *air quality, bioindicators, biomonitoring, climate change*

Introduction

Air is the environmental factor that is the fastest medium that support the transport of pollutants into the environment. Air pollution has many and significant adverse effects on human health and it can cause damage on flora and fauna. For these reasons, special attention is paid to the surveillance, maintenance and improvement of air quality (Bakiyaraj & Ayyappan 2014, Bermadinger et al 1988).

Air quality is determined by emissions to air from stationary and mobile sources (road traffic), as well as long-distance transport of air pollutants (Bucur et al 2018).

In Romania, the "air quality" field is regulated by the Air Quality Legislation with the purpose of protecting together the human health and the environment, which are inseparable entities and the existence of human's activity is environmentally dependent.

The regulating measures aimed at maintaining the quality of ambient air where it meets the ambient air quality objectives established by this law and its improvement in other cases (Kozlowski 1980, Neidoni et al 2018). Based on the development of the industries, the adverse effects on the environment have also emerged (Ashmore et al 1978).

The aim of this study was to identify and select certain species of plants for using them in experimental biomonitoring studies. An experimental fumigation closed system with controlled environmental characteristics and pollutant delivery was used to study the effects of air pollution on plants species. The relative degrees of plant injury as a response of plants to pollutants fumigation were assessed.

Materials and Methods

The European Standard, SR EN 16789 - Biomonitoring with superior plants was used as a source of documentation. "Standardized exposure method for tobacco". with the extension of its applicability and for other species of the same family as the Solanaceae (SR EN 16789: 2017; SR EN 14625: 2005; Tingey 1989).
The plant species and varieties used for the nitrogen dioxide test were as follows: P1 - *Nicotiana rustica,* P2 - *Nicotiana tabacum,* P4 - *Petunia hibrida*. Plants were grown from seeds in a standard soil mix. After about four weeks, the seedlings were transplanted into cylindrical vessels with a capacity of about 750 ml containing a fresh soil mixture (Petrescu et al. 2017, Pakeman et al 2000). Three plants of each variety were placed in the fumigation chamber for about 7 days until four-seven-leaf stage (Cozea et al 2018). Only the healthy leaf plants were exposed to ozone in the fumigation system in the enclosed room (Begu et al 2006). The fumigation method in the closed room. In this study, the plant responses to fumigation of nitrogen dioxide were evaluated according to the relative degree of injury of different tobacco varieties (Partha 2014). To assess the susceptibility of three Solanaceae family plants to nitrogen dioxide (NO_2), two rounds of fumigation experiments were performed for seven days each round. Plant pots were watered and covered with plastic bags, before fumigation, the plants were watered to keep the stomata open. The exposure period was 24h/day, 7 days along/exposure. These tobacco varieties were exposed to a series of concentrations of nitrogen dioxide (0.1 ppm first round and 0.2 ppm) in a one-pass growth chamber fumigation system for short periods of time. The method used to fumigate plants in these experiments was actually a naturally illuminated glass chamber. The fumigation chamber had a total size of 100 cm in length × 45 cm in height × 50 cm in width with a volume of 225 liters. The sidewalls were built from glass sheets, except for a single top of Plexiglas, (Figure 1) where the air treatment plant was mounted.
As the air inside the glasshouses has not been recirculated synthetic nitrogen dioxide portions has been added to maintain stable concentrations inside the glasshouses during fumigation periods. Awareness of the susceptibility levels of these plant varieties at the laboratory level is useful for the biological monitoring process of nitrogen in field experiments.
For microscopy Tests, with the role of observing the necrotic plant tissue was used Leica M205 FA stereomicroscope, one of the most advanced, multidimensional stereo imaging system providing unsurpassed image resolution from panorama to the finest microstructure. Zoom range of 7x - 160x, the Leica M205 FA, allows 3D macro-to-micro viewing. Leica's Triple Beam technology for fluorescence illumination enhances image contrast, details, and intensity.

Figure 1. The fumigation glasshouse

Results and Discussion

Biomonitoring is a new field of research for our country as well as the use of sentinel species. The previous research activity in this study involved preliminary stages before this work, regarding biomonitoring with sentinel plants, which had the aim the identification of the species of plants with specific pollutants response, selection of certain plant species and utilization of the species plants selected in field experimental biomonitoring studies.

The research activities of the first stage were aimed to select specific species of plants with a specific response for a series of chemical compounds by tests carried out in test rooms with known pedo-atmospheric conditions, and in terms of concentration the pollutants present in the air as well as the physical parameters (temperature, humidity, lighting level, etc.).

P1-*Nicotiana rustica* was clearly the most sensitive at 0.1 ppm nitrogen dioxide during the 1st exposure, followed by P2-*Nicotiana tabacum* (Figure 2) and P4-*Petunia hybrida* compared to control plants. The scale bar on Leica stereomicroscope represents for Figure a) 10 mm and for Figure b) 5 mm. These plants showed necrosis and chlorosis on the leaves.

The polluting sensitivity of plants was different. Thus, the results of the experiments varied according to the type of plant. After each fumigation round, visible injuries were evaluated as the percentage of injured leaves, and these were determined by visual estimates. The lesions / percentage of damage to plant leaves after two rounds of exposure to *Nicotiana rustica* were about 74% of the final leaf area and about 30% of the area of *Nicotiana tabacum* leaves and about 23% injury to *Petunia hybrida*. In parallel, the control was a set of plants that were similar in number and size to the experimental set were kept under the same conditions but without exposure to pollutants. No major noxious effects were observed when the plants were exposed to concentrations of 0.1 ppm nitrogen dioxide compared to concentrations of 0.2 ppm (Figure 3). Doses of 0.1 ppm did not cause visible leaf damage in any tobacco varieties. Doses higher than 0.2 ppm caused visible damage to the leaves characterized by necrosis and chlorosis and ultimately the irreparable destruction of exposed plants.

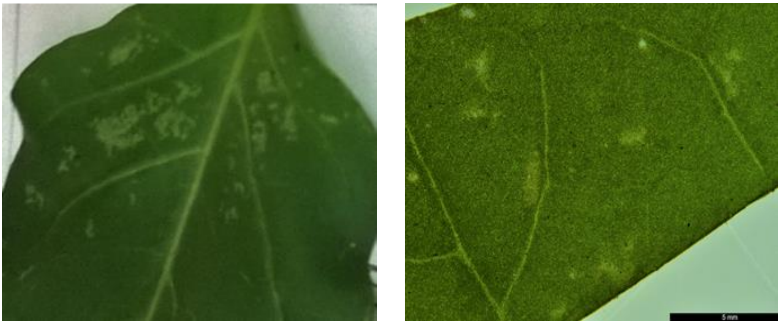

Figure 2. *Nicotiana tabacum* - nitrogen dioxide fumigation effects (a, b)

The air temperature varied within the experiment rooms. The treatments were applied at temperatures between 24°C and 35°C. Dose-response relationships were characterized by estimating the relationship between the dose or exposure level and the severity of exposure-induced effects (Figure 3).

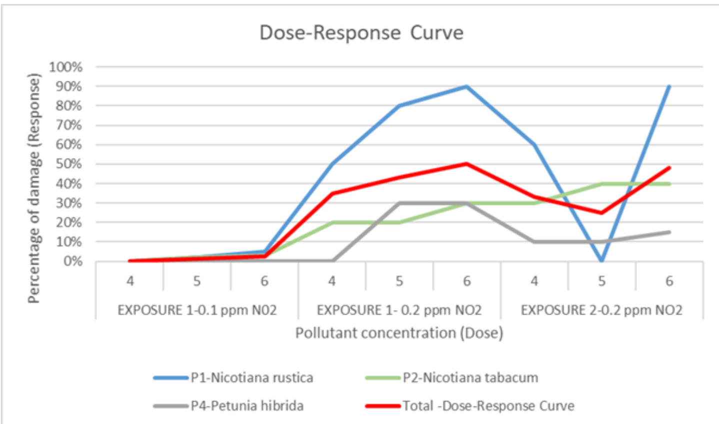

Figure 3. Dose-Response Curve

For the carried out experiments, a correlation between the concentration and the intensity of the pollutants was observed by analysing the response in the case of the exposed plants, the cause-effect relationship. The two different concentrations represented the minimum number to detect a pattern of response and the relationship between the affected leaf area and the pollutant concentration suggests that the injury threshold may be close to the exposure concentration of 0.2 ppm.

Causality was sustained by the presence of a pollutant (or other stressors) immediately before or during observation of structural or functional dysfunctions of the observed community or individuals (Mocuta 2017). Demonstration of noxious effects of pollutants can only proceed using living organisms that either react sensitively to specific pollutants/pollutant mixtures. Such bioindicators also integrate the effects of all environmental factors including interactions with other pollutants or climatic conditions (Velickovic & Perisic 2006). In this way risks from complex pollutant mixtures and even chronic stress below pollutant thresholds can be recorded.

Bioindicator plants like that used in that study have already been used for many years in scientific studies to demonstrate pollutant effects. In some countries such as Germany, Austria, the Netherlands and Italy, some methods have found their way into the routine monitoring of industrial plants by private companies and environmental authorities. At a Europe-wide level the use of bioindicator plants has not been yet established (with a few notable exceptions) (EEA 2017a). One reason for the lacking acceptance amongst decision makers in politics, public administration and private enterprises is the insufficient standardization of the procedures, a fact making it difficult to compare different results obtained from their use (Gombert et al 2006).

The application and validation in the laboratory of the developed models / methods took into account the research on laboratory testing of the method of biomonitoring of air quality with plants. Biomonitoring is considered a valuable method for assessing the level of pollutants for situations where monitoring of air pollution through instruments in inaccessible locations is too expensive (Joshi et al. 2009). The growth was not evaluated. We're not included into count the lesions that were not induced by nitrogen dioxide for this experiment or if the degree of injury that occurs cannot be assessed as being characteristic of this pollutant (Lu et al. 2018). This was very important because the relative sensitivity depends on the level of exposure to the pollutant. A data study was carried out in order to achieve the methodology for approaching the research on biomonitoring the air with sentinel plants to contribute to a standardisation of method applied at a European level and also to demonstrate the noxious effects of air pollutants on living beings in a clear and sustainable manner (SR EN 16789: 2017). The future step is the exposition of bioindicator plants cultivated under standardized conditions to ambient air on the sites in order to assess and to demonstrate the effects of different air pollutants. The tests were carried out in parallel with the selected species with a recognized response, selected in the previous studies in the presence of ozone in the air, and tests with the same plants on nitrogen dioxide (NO_2). The scientific and technical investigations were accompanied by the concept developed within the scope of the study.

During the test period the selected plants were subjected to dioxide gas of two different concentrations of 0.1 and 0.2 (ppm). Two rounds of fumigation were performed. The closed-chamber method that was used for these experiments has facilitated the classification of the susceptibilities of the test plants. Chlorosis, necrosis and variable-point follicular burns, with punctual appearance, were observed.

Variation of symptom expression among tobacco varieties was distinct. These plants showed necrosis and chlorosis of the leaves Thus the wild type of tobacco P1-*Nicotiana rustica* was clearly the most sensitive to nitrogen dioxide, followed by P2-*Nicotiana tabacum* and P4-*Petunia hybrida*. The difference between plant species and different sensitivity at the same pollutant concentration was attributed to the following factor: different stomatal conductance. Although this indicates detoxification, the decomposition products of nitrogen dioxide may affect the photosynthesis of the plant by the phenomenon of chlorosis.

Advantages of Biomonitoring vs. Instrumental Monitoring:

-can provide indications of time variation, accumulation or effect of interaction of certain abiotic factors, and the response of individual living organisms or community organisms to environmental changes.

-the use of bioindicators to monitor populations and, implicitly through extrapolation on ecosystems exposed to pollution phenomena and can provide timely intervention to protect the integrity of the environment and indirectly the health of the human population.

Conclusions

Performing tests plant's response to air pollution (the concentration of compounds in the air) have been monitored changes in leaf integrity, growth rate and plant growth in general, as well as tissue changes.

The biological response integrates the direct influence of the pollutant with the individual responses of each individual species and each individual response, which varies depending on the genotype structure and in close correlation with the other environmental factors that acts on living organisms (temperature, luminous intensity, humidity). Additional information on plant tissue that provided an analysis on effects associated with the intensity of exposure and its action showed by visual inspection the presence of necrosis at the stomata level in Test plants versus Control plants.

In order to be used as bioindicators, the selected species exhibited a characteristic reaction to the action of a certain pollutant, in this case nitrogen dioxide, which is not involved with that produced by other stressors, as demonstrated by these tests and the correlations made.

Effective action to reduce the impacts of air pollution requires a good understanding of its causes, how pollutants are affecting humans, ecosystems, the climate, and subsequently society and the economy. The real advantage of using biological monitoring methods is that they integrate the influence of different factors (the most important being the pollutants), and the answer is the result of their action and the reaction of the living organism.

The research activity in this study involved the completion of some stages that will form the basis of future studies on Biomonitoring with sentinel plants, a stage that will continue to be studied and will offer a starting point of understanding for a better biomonitoring.

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