Biomonitoring climate change and air quality assessment using bioindicators as experimental model

Andreea COZEA
National Research and Development Institute for Industrial Ecology INCD ECOIND, 71-73 Drumul Podu Dambovitei Street, code 060652, Bucharest, Romania
andreea.cozea@yahoo.com

Elena BUCUR
National Research and Development Institute for Industrial Ecology INCD ECOIND, 71-73 Drumul Podu Dambovitei Street, code 060652, Bucharest, Romania
poluare.aer@incdecoind.ro

Andrei VASILE
National Research and Development Institute for Industrial Ecology INCD ECOIND, 71-73 Drumul Podu Dambovitei Street, code 060652, Bucharest, Romania
poluare.aer@incdecoind.ro

Catalina STOICA
National Research and Development Institute for Industrial Ecology INCD ECOIND, 71-73 Drumul Podu Dambovitei Street, code 060652, Bucharest, Romania
catalina.stoica@incdecoind.ro

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 gaseous pollutants (SOx, NOx) are continuously released in air and perceived/recognized as pollutants. Among the biological models, plants could give us information related with pollution range in a monitored area, analyzing injuries caused on the leaves surfaces. The aim of this study was to identify and select certain species of plants for using them in experimental biomonitoring studies. An experimental fumigation system with a closed fumigation glasshouses maintained under defined conditions with environmental control and pollutant delivery was used to study the effects of air pollution on plants species. The relative degrees of injury as a response of plants to pollutants fumigation were assessed. Considering the sensitiveness degree to pollutants, the indigenous tobacco Nicotiana rustica proved to be the most sensitive followed by cultivated tobacco Nicotiana tabacum and least sensitive Petunia hibrida. Future researches will focus on field biomonitoring.*

Keywords: climate change, air quality, biomonitoring, bioindicators, experimental model.

Introduction

Due to the fact that humans and the environment are inseparable entities results that the existence of human's activity is environmentally dependent. Environmental factors can be affected as a result of their use by humans, so there is pollution, an implicit aspect of life, in which some resulting products from the life activity, become residues that can produce inconveniences and in fact the good living depending on their nature and quantity. Based on the development of the industries, the adverse effects on the environment have also emerged.

Effects of atmospheric pollution with gaseous, liquid or solid waste may endanger the health of humans, animals and plants. Air is the carrier of many pollutants that it spreads rapidly over the entire surface of the Earth (Bermadinger et al., 1988). Among these we mention: Nitrogen dioxide (NO_2) - largely resulting from combustion processes; Sulfur dioxide (SO_2) -emitted when burning sulfur- Effects of atmospheric pollution on gaseous, liquid or solid waste or on products that may endanger the health of humans, animals and plants. Air is the carrier of many pollutants that it spreads rapidly over the entire surface of the Earth (Bermadinger et al., 1988).

Among these we mention: Nitrogen dioxide (NO_2)-largely resulting from combustion processes; Sulfur dioxide (SO_2) is emitted when burning fuels containing sulfur; Ground-level ozone (O_3) is formed by chemical reactions (triggered by the sun's rays) involving pollutants emitted into the air, including those from transport, natural gas extraction, landfills and chemicals; Benzoprene (PaB) from incomplete fuel combustion; particulate matters (PM) are suspended particles in biological or chemical air classified as pollutants. (Burton, 1986)

One of the most representative air pollutants is nitrogen dioxide (NO_2). Negative influences on living organisms were associated also to NO_2 pollution.

The damaging effects of air pollutants on plants are better known (Ivanescu and Toma, 2003) Over time, monitoring studies have identified two types of injury, foliar lesions, due to exposure to NO_2 producing: chronic lesions caused by the presence of NO_2 at low concentrations over a long period of time (weeks) and acute lesions by exposure to a short period (hours) with pollutant at a high concentration. (Zeevaart, 1970)

Laboratory research activities focused on activities aimed at tests conducted in fumigation test rooms with known atmosphere, in terms of the concentration of pollutants present and of the physical parameters (temperature, humidity, level of illumination). The Tests plants were performed in parallel with the Control plants, selected in the first study phase, and in described test the same plants selected and using as pollutant synthetic nitrogen dioxide (NO_2). Thus, the plant's response to air pollution (the concentration of compounds in the air) was identified and quantified compared to a Control batch kept under normal pollution conditions. Changes in leaf integrity, growth rate, and plant growth in general, as well as tissue changes, were monitored. The activities carried out were completed by drawing up the dose-response curve from which the minimum applied pollutant concentration was deducted to obtain a change or induce the expected symptoms at the level of the individuals in the study, resulting in a methodology for approaching the research on biomonitoring the air with sentinel plants (Maciuca, 2003).

In establishing a cause-effect relationship using a dose-response curve, it is important because determine how a dysfunction or a structural change based on the observed action of the pollutants is the resultant of the action of a certain pollutant factor through a physical or biological process. Understanding the damaging mechanism of air pollutants is with importance in the environmental protection. This kind of experiments are all the more valuable as the action of the cause-effect relationship mechanism and can be demonstrated both in the natural environment and in the induced environment with controlled experiments and also we can apply easily an environment monitoring system with low cost.

Literature review

For inducing controlled experiments several types of different fumigation systems are described in the literature to study the effects of air pollution on plants. Were used fumigation glasshouses, upper opening rooms or field fumigation systems and sometimes closed rooms with controlled inside atmospheric conditions. These fumigation systems were used for the experimental reproduction of atmospheric conditions. Experimental studies were conducted to monitoring the plants introduced in these rooms and have been conducted for more years, and some previous works are described in literature representing important information for the present experiments (Azadi, 2004) (SR EN 16789, 2017).

So, in the present study we have been use bioindicators for pollutant monitoring. A bioindicator is a biological organism or bias that indicate the presence of a particular pollutant by the identification of typical symptoms or quantifiable manifestations (Joshi et al., 2009). The biological response integrates the direct influence of pollutants with the individual response patterns of each species and each individual, which varies depending on the structure of the genotype as well as in close correlation with the other environmental factors that act on living organisms. The action of various atmospheric pollutants on plants has been investigated by many researchers (EEA, 2017a). At the ultrastructural level there are numerous changes in the vegetative structure. They accompany morphological changes such as chlorosis and necrosis that consist of destruction of chlorophyll pigments, degeneration of cellular organs (Kozlowski, 1980).

Biological monitoring - biomonitoring is important because the information taken through plants reflects the effects of environmental pollutants. These effects can be highlighted at organizational levels appropriate to the scale of analysis. The information provided by the environmental response can be hierarchically organized on levels of complexity (Burton, 1986).

Economic impact- The EU 2020 Strategy established the interdependence between environmental, economic and social policies. In the economical context of globalization, a debated aspect is environmental policy, as a specialized entity on national and international level in assessing and determining the negative influences of pollution on the environment.

The EU's environmental policy has emitted pollution limits regarding environmental protection by implementing measures at Member State level coming with imposed pollution Standards. Thus, starting from in field pollution measurements, has been established a series of Environmental Protection Strategies, which does not affect the economic growth. (Repez, 2016) These environmental policies are sustained by strategies also supported by periodic instrumental pollution degree monitoring and the establishment of compliance with existing standards. (OECD, 2016).

Biomonitoring can replace or complement instrumental monitoring by providing other types of data, using the response of indicator organisms (plants) to existing or experimentally created environmental conditions. Biomonitoring can provide indications of time variation, pollutant accumulation or the combined effects of certain factors on monitor organisms. One aspect to be considered is the difficulty, often the impossibility, of a specific reaction to a particular pollutant, because in the natural environment rarely acts as a unique source of pollution that emit a single category of substances (Tingey, 1989).

Therefore, to eliminate this inconvenience in this study, "Controlled Exposure " have been assessed. This type of test is designed to compare the observed effects on healthy plants that are exposed to the action of a particular pollutant and the effects observed in the natural environment in which it is assumed to act and that pollutant alone or in correlation with other stress factors. For these tests, growth chambers/greenhouses were used in the case of small plants (young plants or small herbaceous plants).

The Air quality in Europe report series from the EEA presents regular assessments of Europe's air pollutant emissions, concentrations and their associated impacts on health and the environment. (Bruyninckx, H. (2017), www.eea.europa.eu)

The annual limit value for nitrogen dioxide (NO_2) used as pollutant in our study have exceeded concentrations across Europe. Around 10% of all the reporting stations recording concentrations above that standard in 2015 in a total of 22 of the EU-28 and three other reporting countries. 89 % of all concentrations above this limit value were observed at traffic stations. (https://skupnostobcin.si/)

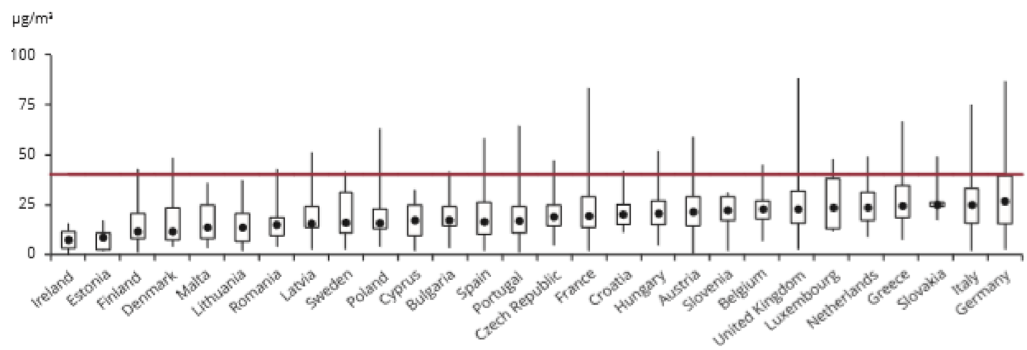

Figure 1NO2 concentrations in relation to the annual limit value in 2015 in the EU-28

Notes: The graph is based on the annual mean concentration values for each country, the lowest, highest and median values (in µg/m^3) at the stations are given. The rectangles mark the 25th and 75th percentiles. At 25 % of the stations, levels are below the lower percentile; at 25% of the stations, concentrations are above the upper percentile. The limit value set by EU legislation (equal to the WHO AQ guideline) is marked by the red line. The graph was correlated with the country situation that depends on the number of stations considered.

Source: (https://skupnostobcin.si/) (EEA, 2017a).

The effect of a pollutant on a biomonitor is strongly determinate by the environmental climate before, during and after exposure to the NO_2 as well as the type of exposure intakes (Pakeman et al, 2000). Soil humidity and availability of nutrients, photosynthetic radiant flow, relative humidity and temperature. There are principles that may affect stomata opening, which determines the amount of airborne gaseous pollutant absorbed by a leaf, and consequently the magnitude of the effects and the growth of the plants and the appearance of the visible lesions (Partha, 2014) (Azadi, 2004).

The results were measured and correlated with the intensity of the pollutant agent that purchase the plants or organs, as well as the exposure time applied in each case. The results of these tests were expressed by the dose-response curves from which the minimal applied concentration was deduced to obtain an expected changes or induce the expected symptoms at the level of the individuals (plants) under study. In order to fulfill the criterion of the gradual correlation of the cause with the effect, it is necessary to have a quantitative relation between the concentration of the pollutant agent and the intensity of the observed dysfunctions in the individual's study (Velickovic and Perisic, 2006).

The proposed aim of that paper were included in general objective of the study such was the starting of the research activities regarding the development and applying the methods and methodologies regarding air pollution assessment in the global climate changes context and for Environmental International Regulations updates.

Methodology

Biomonitoring is a new field of research for our country as well as the use of sentinel species. 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.) (Gombert et al., 2006).

For the implementation of this experiment, 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.*

Materials and methods performed: 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. Three plants of each variety (according to experiments) were placed in the fumigation chamber about 7 days (four-seven-leaf stage). Although the plants were grown in the laboratory environment, they were checked and only the healthy leaf plants were exposed to ozone in the fumigation system in the enclosed room. (Azadi, 2004)

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. 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. These tobacco varieties were exposed to a series of concentrations of nitrogen dioxide in a one-pass growth chamber fumigation system for short periods of time. Doses of 0.1 ppm did not cause visible leaf damage in any tobacco varieties. (Azadi, 2004) 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. 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 pexiglass, where the air treatment plant was mounted.

Figure 2. The fumigation glasshouse
Source: Authors' own research

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. (Cozea et al., 2018)

As the air inside the glasshouses has not been recirculated, enough 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 dioxin monitoring process of nitrogen in field experiments. 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. (Azadi, 2004) 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. This was very important because the relative sensitivity depends on the level of exposure to the pollutant.

Results and discussions

In this section the author presents the main findings of his/her research. It is important to use critical thinking in order to analyze realistically the results obtained, and how the research hypotheses have been validated or not. Also, it is important to compare results of present research with results obtained in similar research, by using the literature in the domain. If the paper presents a theoretical model or theory, the discussion should be about the way the new conceptual contribution can be applied and how it compares with some similar models or theories.

Variation of symptom expression among tobacco varieties has been distinct. Thus P1-*Nicotiana rustica* was clearly the most sensitive to nitrogen dioxide, followed by P2-*Nicotiana tabacum* and P4-*Petunia hybrida*. These plants showed necrosis and chlorosis in the leaves.The polluting sensitivity of plants was different. Thus, the results of the experiments varied according to the type of plant. 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. 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*. Plants were being well ventilated before each experiment to optimize the stomata's opening. In parallel, 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.

The air temperature varied within the experiment rooms. The treatments were applied at temperatures between 24°C and 35°C. It was not possible to determine if the temperature had an effect on the plant's response to the pollutant, but the approximate linear relationship between the pollutant concentration and the injuries suggests that the temperature did not a major effect in this case. (Azadi, 2004)

Data analysis revealed that: the environmental factors that increase the rate of gas transfer at the stomata level automatically determine a take-up of an increased amount of pollutants and a more intense effect on the plant (intensification of metabolism); differences of genotype that exist between populations belonging to the same family cause the occurrence of physiological reactions to different stresses that give different sensitivities to these (necrosis and chlorosis);there was a greater degree of damage to the growth peaks than to the basal leaves as a result of their higher metabolism resulting in a higher sensitivity of the upper leaves and growth peaks to the effects of pollution; Significant differences were found between epidermis morphology from control plant leaves and plants exposed to nitrogen dioxide fumigation; stomata were gradually blocked by stomata closing and producing necrosis, so the leaves die sooner than normal;

Dose-response relationships are characterized by estimating the relationship between the dose or exposure level and the severity of exposure-induced effects.

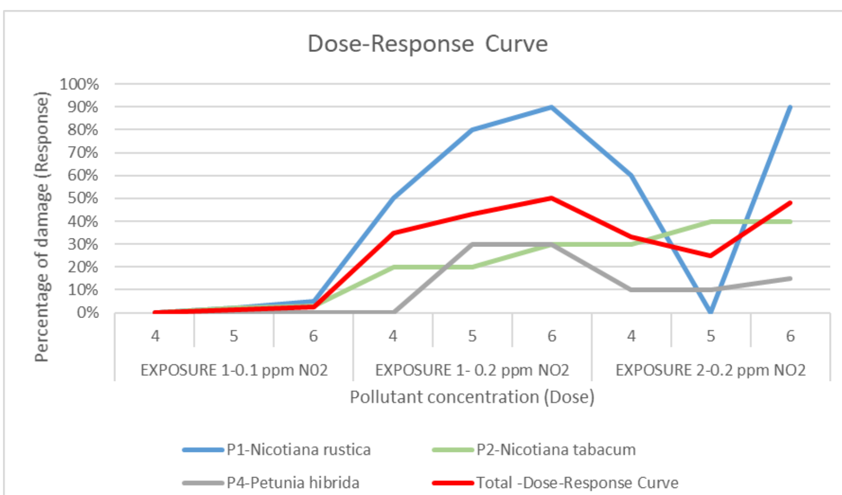

Figure 3. Dose-Response Curve

Source: Authors' own research results

For the carried out experiments, a correlation between the concentration and the intensity of the pollutants were observed analyzing the response in the case of the exposed plants, the cause-effect relationship being thus argued. The two different concentrations represent the minimum number to discern a pattern of response, and the relationship between the leaf area and the pollutant concentration suggests that the injury threshold may be close to the exposure concentration of 0.2 ppm.

Causality is 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.

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.

Conclusion

During the test period the selected plants were subjected to dioxide gas of 2 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 primarily at growth peaks.

Variation of symptom expression among tobacco varieties was distinct. 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.* These plants showed necrosis and chlorosis of the leaves. The biological response was not only influenced by the duration of the exposure and the concentration of the pollutant(s), but also by the relative sensitivity of each organism.

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. At this stage most of the pollutant was decomposed into the cell by relatively harmless oxidation of the components of the cell wall of the stomata, they were gradually blocked by the closure of the ostiole, thus causing necrosis. The membrane lysate and the chlorotic or necrotic symptoms usually appear as effects of rather intense pollution.

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).

Histological analysis of plant tissue that provided additional information on the effects associated with the intensity of exposure and its action showed by visual inspection the presence of necrosis at the stomal 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 plans to reduce the impact of air pollution require an assessment of its causes, how pollutants affect the environment, and implicitly, 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 chapter that will be studied in a future

References

Azadi, M. (2004) Assessment of ozone effects on five varieties of tobacco via fumigation method, *International Journal of Environmental Science and Technology*, 1(2), 81-88.

Bermadinger, E., Grill, D., & Golob, P., (1988). Influence of different air pollutants on the structure of needle wax of spruce (Picea abies (L.) Karsten). *Geojournal*, 17, 289–293.

Bruyninckx, H. (2017), Air pollution Retrieved from https://www.eea.europa.eu/

Burton, M. A. S., (1986). Biological monitoring of environmental contaminants (plants). *MARC Report Number 32*. Monitoring and Assessment Research Centre, King's College London, University of London.

Cozea, A., Bucur, E., Lehr,C.B., Pascu,L.F.,& Tanase G.h. (2018) Bioindicators in air quality control, SIMI 2018, 20-21 september, 2018, Bucuresti, ISSN-L: 1843 – 5831, 2018, DOI: 10.21698/simi.2018.ab31, 80-81

EEA, 2017a, 'Air Quality e-Reporting Database', European Environment Agency (http://www.eea. europa.eu/data-and-maps/data/aqereporting-2) accessed 19 July 2018.

Gombert, S., Asta, J. & Seaward, M.R.D. (2006) Lichens and tobacco plants as complementary biomonitors of air pollution in the Grenoble area (Isere, southeast France). Ecological Indicators. 6 (2), 429-443.

Ivanescu, L., & Toma, C., (2003). *Influenta poluarii atmosferice asupra structurii plantelor*. Ed. Fundatiei Andrei Saguna, Constanta.

Kozlowski, T.T., (1980). Impacts of air pollution on forest ecosystems. *BioScience*, 30, 89–93.

Maciuca ,A., (2003). Aspecte privind utilizarea bioindicatorilor in supravegherea ecosistemelor. *Bucovina forestiera*, 9(1), 53-58.

Joshi, N., Chauhan, A. &. Joshi, P.C. (2009) Impacts of industrial air pollutants on some biochemical parameters and yield in wheat and mustard plants. Environmentalist, 29, 98-104.

OECD, 2016, The economic consequences of outdoor air pollution, OECD Publishing, Paris (http://dx.doi. org/10.1787/9789264257474-en).

Partha, P. (2014) Biomonitoring with special reference to visible damages in different plant species due to air pollution. *Inter. Letters of Nat. Sci.* 11(1), 32-37.

Pakeman, R., Osborn, D., & Hankard, P. (2000) Plants as biomonitors of atmosphere pollution: A review of their potential use in integrated pollution control. Environmental Agency Technical Report, 319.
Retrieved from https://skupnostobcin.si/
Repez F., (2016) Protectia nediului-necesitate,reglementare si implementare, Editura Centrului ethnic-editorial al armatei, Bucuresti, 130-134
Tingey D.T., (1989). Bioindicators in air pollution research - applications and constraints. in: biologic markers of air pollution stress and damage in forests, Committee on biological markers of air pollution damage in trees. National Research Council, National Academy Press, Washington D.C.
Velickovic M., Perisic S., (2006). Leaf fluctuating asymmetry of common planta in as an indicator of habitat quality, *Plant Biosystems,* 140(2),138-145.
The European Standard, SR EN 16789, 2017, - Biomonitoring with superior plants. “Standardized exposure method for tobacco"
Zeevaart, A.J. (1970) Some effects of fumigating plants for short periods with NO_2, *Environmental Pollution,* 11 (2), 97-108