PARAMETERS RESULTING FROM NON-INVASIVE AND INVASIVE BIOMONITORING RESEARCH ON PLANTS

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Abstract. *Currently, air pollution is one of the main environmental problems, and urbanization is considered one of the most dangerous changes that an ecosystem can undergo. Biological monitoring of urban habitats is needed to determine both atmospheric pollutants and their effects on organisms. Biomonitoring is a common and feasible method for air quality assessment in different environments. Plants are organisms constantly exposed to air, thus they are the primary receptors of all particles and pollutants in the atmosphere. In monitoring plants in urban environments, it is essential to identify and evaluate the right parameters to give us a complete and detailed picture of their state of health and evolution. This review paper aims to mention the parameters that respond as bioindicators in the monitoring of plant organisms to reveal relevant data regarding the environmental quality of their habitat, considering both invasive and non-invasive methods. Biomonitoring techniques using non-destructive methods are becoming increasingly important due to their advantages over chemical-analytical methods. In vivo methods, which are based on both imaging and spectroscopic approaches, are recently increasingly optimized due to the advantages they offer in the field. In order to achieve the most exhaustive classification of plant parameters, various evaluable plant characteristics, morphological, morphometric, biochemical, and physiological aspects were considered, thus providing a solid basis for data analysis and interpretation.*

Keywords*: biomonitoring, non-invasing methods, air pollution, plant parameters, urban habitat*

INTRODUCTION

Air pollution is one of the primary environmental issues nowadays. The deterioration of air quality is largely caused by urbanization, which has been cited as one of the most drastic changes an environment can undergo (PETROVA, 2011). Air pollution is influenced by industrialization, economic development, energy consumption, and increased traffic, specific elements of human intervention processes, whose negative effects are most strongly felt in urban environments.

Reducing pollutant emissions is the most efficient way to decrease air pollution. There is no device (mechanical or chemical) capable of completely attenuating pollutant emissions at the source. Once pollutants are released into the atmosphere, plants can only absorb or metabolize them, removing them from the atmosphere (RAI & PANDA, 2014).

Biological monitoring of urban habitats is necessary because the determination of single pollutants in the atmosphere does not provide relevant information about the additive effects of chemicals on living organisms (SIMON et al., 2016). Additionally, synergistic interactions between certain pollutants cause increased damage to plant health, making the examination of biological indicators relevant and necessary.

Most techniques for chemical-analytical monitoring of air quality elicit a response when samples are exposed to one or more atmospheric pollutants. Furthermore, physico-chemical methods are costly and require a thorough technical knowledge of the applied analytical techniques. In contrast, plant-based biomonitoring techniques are gaining importance as they are not hindered by many of the aforementioned disadvantages. However, a major disadvantage of implementing a biomonitoring approach is the considerable field sampling effort required to achieve sufficient spatial coverage and, depending on the parameters anticipated, the subsequent laboratory analysis needed.

Biomonitoring is a common method for the efficient and inexpensive assessment of urban air quality (ALLAJBEU et al., 2017). Since plants are constantly exposed to air, they are the primary receptors for both gaseous pollutants and atmospheric particles. In terrestrial plant species, the enormous leaf surface acts as a natural filter for pollutants, especially particles (PETROVA et al., 2022).

Therefore, morphological characteristics and biochemical parameters affected by atmospheric pollutants are particularly useful for estimating pollution levels. High levels of air pollution affect plant morphological (leaf number, leaf area, stomatal number, stomatal structure, flowering, growth, and reproduction), biochemical (enzymes, proteins, pigments, ascorbic acid, and sugar content), and physiological (pH and water content) parameters (MOLNÁR et al., 2018).

This synthesis aims to mention parameters that act as bioindicators in monitoring plant organisms to unveil pertinent data regarding the environmental quality of their habitat, both using invasive and non-invasive methods.

Within plant monitoring in urban environments, it is essential to identify and evaluate suitable parameters that provide a comprehensive and detailed understanding of their health and evolution. For an efficient and precise approach, we will classify parameters into several key categories, encompassing morphological, morphometric, biochemical, and physiological aspects. This classification will enable us to assess various plant characteristics, from their structure and form to physiological and metabolic responses, thus providing a solid foundation for data analysis and interpretation.

MORPHOLOGICAL PARAMETERS

In a 2016 study establishing the Air Pollution Tolerance Index (APTI) for three species located in a heavily trafficked area of India, Pradhan and colleagues analyzed, in addition to the biochemical parameters required for calculating the APTI index, the relationship between leaf dust accumulation and other morphological parameters of the species. According to Varshnay and Mitra (1993), dust particle accumulation depends on the following morphological and morphometric parameters: internode distances, petiole length, leaf area, orientation, margin, folding and arrangement of the leaf, trichomes density, type, and length.

This study considered morphological leaf characteristics such as leaf arrangement (whether alternate, opposite, verticillate, or rosette), leaf margin appearance, orientation (erect, semi-erect, or horizontal), pubescence (presence or absence of trichomes), prominent veins (absent or present), and morphometrics such as internode length (greater or lesser than average), petiole length (greater or lesser than average), and leaf blade size (large, medium, or small).

The aspect of the leaf. Morphological parameters are observable with the naked eye and, in the case of monitoring individuals, may represent plant symptoms caused by atmospheric pollutants. Acute damage occurs when a plant is highly sensitive to a particular pollutant or is exposed to high levels of a pollutant over a short period. Acute damage is defined as damage limited to certain areas, which may result in dark, pigmented spots on a leaf. Some symptoms of acute lesions include yellowing, whitening, dwarfing, or cessation of development and growth without other visible symptoms (NOUCHI, 2002).

Miao and colleagues (2022) conducted measurements of morphological parameters of corn, considering plant height (PH), the thickness of the long axis of the stem (STLA), and the thickness of the short axis of the stem (STSA). These parameters efficiently reflect growth, resistance to root lodging, and information about crop yield. In this study, terrestrial laser scanning (TLS) was utilized, enabling rapid measurements of crop phenotypic parameters.

Banerjee and colleagues (2022) conducted a comparative analysis using scanning electron microscopy (SEM) on the leaf surface of *Ficus benghalensis* and *Terminalia arjuna* at 2 sites, S1 (control) and S2 (heavily polluted). The leaf cuticle, situated above the epithelial cells, is divided into a soluble portion of waxy lipids, which can be crystalline or amorphous, and an insoluble cuticular polymer. The chemical composition of the wax consists mainly of hydrocarbons, esters, and low molecular weight paraffins, which decrease with increasing air pollution.

MORPHOMETRIC PARAMETERS

Berezhnoy and colleagues (2020) explored approaches to address the issue of precise and objective recording, as well as the assessment of morphometric parameters of in vitro plants. Assessing the impact of different nutrient medium compositions at various stages of plant development can aid in selecting the correct nutrient medium and obtaining high-quality planting material disadvantages but manual measurements come with disadvantages.

Today, various hardware and software systems exist for photographing plants and evaluating their morphometric parameters. Researchers have proposed a prototype of an automated system for photographing plants from different angles, creating a 3D volumetric model of the plant, and calculating morphometric parameters at various stages of plant development to limit image distortions from methods using glassware for measurements, where image distortions arise.

Studying the morphological, morphometric, physiological, characteristics of plants influenced by climate change has become a global concern recently. Temperature fluctuations influence the duration of the vegetation period and the biomorphological characteristics of plants (ASLAM et al., 2021). The leaf represents one of the most significant features for identifying specific species in plant morphology, among other organs. Leaf morphometric parameters can be useful in eco-physiological studies to estimate a plant's tolerance to the environment (VERGUN et al., 2022).

Morphometric parameters used by Vergun and colleagues (2022) for measurement in the vegetation phase of *Crambe* spp. included plant height, leaf length and width, petiole length, and panicle length. Flower length and width, corolla length, and petal length and width were used as morphometric parameters for flowers. Fruit length and diameter were used for fruit measurement. The thousand-seed weight was also weighed on analytical balances. Data were collected at the beginning of vegetation, in the budding phase, in the flowering phase, and during fruiting.

BIOCHEMICAL PARAMETERS

PIGMENTS

The growth and development of plants are influenced by biotic and abiotic environmental factors. The variation in natural pigment content (such as chlorophylls, anthocyanins, carotenoids) in plants is sensitive to environmental conditions. In most cases, the variation in chlorophyll content is monitored, as this photosynthetic pigment is found in all green cells, along with anthocyanins, which are the most diverse and widespread secondary metabolites in the flavonoid class in plants.

To measure variations in pigment concentrations in plant organisms, both invasive and non-invasive methods can be used. Current approaches for measuring pigments such as chlorophyll or carotenoids are summarized in the table below, according to Munné-Bosch and Villadangos, 2023.

Table 1

Approaches for measuring pigments in plants

Method	Time needed	Costs	Accuracy	Simplicity
SPAD	Instant	+	++	+++
Spectrophotometry	3h	++	+++	+++
Spectroradiometry	1h	++	++	+++
HPLC	6h	+++	+++	+
Remote sensing	Instant	+++	++	++

Leaf chlorophyll content is an important physiological parameter that serves as an indicator of the stress level to which plants are subjected (ATLASSI PAK et al., 2009), as well as of senescence and nutritional status, especially regarding nitrogen content (LOH et al., 2002). The conventional method for measuring chlorophyll in plant leaves is spectrophotometry, which extracts the pigment from leaf tissue. However, in some cases, analytical estimation is not feasible due to the invasive nature, or the time required for such measurements (especially in the case of many samples).

Therefore, nowadays, a variety of portable devices (chlorophyll meters) are widely used to estimate the gross chlorophyll content in a non-invasive manner (HLAVINKA et al., 2013).

SPAD is a portable device that measures total chlorophyll through atomic absorption spectroscopy. The Photochemical Reflectance Index (PRI), which can be measured through remote sensing, is a good indicator of excess energy dissipation dependent on the xanthophyll cycle. Remote sensing can also be used to measure total chlorophyll in plant leaves, as well as many other physiological parameters related to the detection and monitoring of drought-induced stress (MUNNÉ-BOSCH & VILLADANGOS, 2023).

Chlorophyll fluorescence (CF) is red and far-red light produced in the photosynthetic tissues of plants when excited by natural or artificial light in the visible spectrum. Measuring chlorophyll fluorescence can be a non-invasive method and can be performed rapidly. Using a portable instrument, the degree of damage to the photosynthetic apparatus can be studied under natural conditions, in the field, in the natural habitat of monitored plants.

In a study evaluating the adaptation response to urban pollution of three ornamental tree species, Petrova and colleagues (2022) analyzed three types of biochemical markers: photosynthetic pigments, free proline, and guaiacol peroxidase (GPX) activity. The pigments were determined spectrophotometrically at different wavelengths for carotenoids, chlorophyll a, and chlorophyll b, after extraction with 90% acetone. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were calculated for each sample. Additionally, biochemical parameters such as the chlorophyll a to b ratio and the ratio between chlorophyll pigments and carotenoids were used.

Anthocyanin pigments play an important role in seed dispersal, pollination, organ development, as well as in the adaptation of plants to various biotic (pathogens) and abiotic (drought, nutrient deficiency, insolation) changes (ŠAMEC et al., 2021).

Anthocyanins are commonly synthesized by plants and are responsible for the red, blue, and violet colors of most berries, fruits, and vegetables (ONGKOWIJOYO et al., 2018). The presence of anthocyanins in vegetative organs, flowers, and fruits is beneficial for plant species. According to Menzies et al. (2016), there are two hypotheses regarding the benefits of anthocyanins in leaves: 1. anthocyanin pigments serve as visual warnings to discourage approaching herbivore species, and 2. they protect leaves from a variety of abiotic stress factors.

Properties of these pigments suggest that anthocyanins play an important role in enabling plants to adapt to various environmental constraints, thus representing important parameters in assessing the condition of plant organisms under certain environmental conditions.

Carotenoids such as lutein, β-carotene, and zeaxanthin are involved in dissipating excess energy associated with the xanthophyll cycle, and their content can also decrease due to severe photo-oxidative stress (MORALES, 2021).

The degradation of carotenoid pigments can be caused by oxidation and isomerization, reactions that favor the degradation of red and yellow colors in plants (MIRANDA et al., 2021). Factors such as oxygen, light, heat, peroxide, metal ions, and enzymes are correlated with these degradation reactions (PROVESI & AMANTE, 2015).

Since measuring individual carotenoids with high-performance liquid chromatography (HPLC) requires specialized equipment and is also quite time-consuming and costly, measuring total chlorophyll and carotenoids by spectrophotometry, along with calculating the total carotenoids/chlorophyll unit ratio, can be a cheaper, cost-effective, and rapid alternative for reliably monitoring drought-induced stress.

LEVEL OF AIR POLLUTANTS

Air pollutants cause changes in the composition of the atmosphere and affect organisms in the environment. Based on their formation, two groups have been identified, primary and secondary. The first group is emitted directly from the source (SO2, NO, CO, etc.), while the others are formed in the atmosphere as a result of chemical reactions between air constituents and primary pollutants (SO3, O3, etc.) (ADAK & KOUR, 2021).

The effects and risks of air pollution on vegetation are largely determined by environmental parameters, parameters that should be included in research involving plant tolerance monitoring. Three main parameters, such as plant adaptability, exposure to pollutants, and environmental parameters, cannot be ignored, as they ultimately regulate the behavior of plant tolerance to air pollution.

Gupta and colleagues conducted a study based on the development of a portable Raman spectrometer for rapid and in vivo spectral analysis of plant metabolites. The aim was to diagnose plant stress, such as nutrient deficiency in the presence of drought before visible symptoms and crop loss occur. The Raman leaf clamp probe was very useful for the early diagnosis of nitrogen deficiency in *Arabidopsis thaliana*, Pak Choi (*Brassica rapa chinensis*), and Choy Sum (*Brassica rapa var. parachinensis*).

The nitrogen content of plants (PNC) is an important indicator used to characterize the nitrogen nutritional status of crop plants (FAN et al., 2022). Rapid and efficient determination of nitrogen content is important for evaluating crop growth and selecting applied fertilizers (FU et al., 2021). Although traditional field surveys and destructive sampling methods can obtain more precise PNC information, they are time-consuming and not feasible. To meet the current needs for large-scale, rapid, and efficient monitoring of crop growth conditions in precision agriculture, remote sensing technology is rapidly developing, offering a new option for efficient, non-destructive, and real-time monitoring of the PNC status of crops.

In contrast to sensors such as hyperspectral and multispectral sensors, high-definition digital cameras are inexpensive and have high spatial resolution, simple data processing, and stable performance (LU et al., 2021). The use of inexpensive digital cameras for monitoring the nitrogen nutrition status of crops has gradually become preferred by many researchers.

PLANTS METABOLITES

To protect themselves from environmental stress factors, both biotic and abiotic, plants synthesize secondary metabolites. In higher plants, secondary metabolites are substances synthesized from primary ones (carbohydrates, lipids, and amino acids) (GRUDNICKI & IANOVICI, 2014).

Awan et al. concluded in 2017 that plant growth regulators such as ABA, JA, and ethylene are involved in regulating the plant's response to abiotic stress. Cytokinins are also capable of improving seed germination by mitigating stress factors such as salinity, drought, heavy metal presence, and oxidative stress. Falkowska et al. found in 2011 that gibberellic acid (GA3) plays a significant role in the growth and metabolism of *Chlorella vulgaris* microalgae exposed to heavy metal stress and its ability to adapt to a polluted aquatic environment at a low level. Meanwhile, gibberellins lead to improved Zea growth under saline soil conditions by enhancing nutrient levels and membrane permeability (TUNA et al., 2008).

In the past, traditional biochemical analyses have been used to obtain information about the distribution and dynamics of plants metabolites. For example, immunohistochemistry using anti-hormone antibodies has been used to determine hormone distribution in plant cells and tissues (FORESTAN & VAROTTO, 2013).

Mass spectrometry combined with gas or liquid chromatography has been used to identify and quantify plant hormones with high precision and sensitivity (GEMPERLINE et al., 2016). In the last two decades, advances in fluorescence microscopy technologies and biosensor engineering have provided new tools for monitoring plant hormones with minimal invasiveness and cellular or even subcellular resolution (WAADT, 2020).

BIOMONITORING THROUGH APTI and API

APTI, the Air Pollution Tolerance Index, is an integrated tool for assessing plant response to a diverse range of pollutants through biochemical and physiological parameters, as evaluating a single parameter is not reliable to obtain a clear picture of pollution impact. Khanoranga and Khalid (2019) suggest that APTI can be effectively used to identify bioindicators (plant species tolerant and sensitive) to be used in polluted environments. Some plants are tolerant, while others are sensitive in the same environmental condition. The Air Pollution Tolerance Index (APTI) is used to identify plant species tolerant to different concentrations of air pollutants (KARMAKAR et al., 2021).

The index is mainly based on four biochemical parameters of leaves: ascorbic acid content, total chlorophyll content, leaf extract pH, and relative water content (SAHU et al., 2020). Based on APTI, sensitive plant species can act as a bioindicator, and tolerant plant species help reduce the overall pollution burden and can also be used for the development of green urban areas. The Anticipated Performance Index (API) is the combination of APTI values and relevant biological and socio-economic characteristics (plant habitat, structure, plant type, leaf structure, and economic value). The API is an update of APTI and is used as an indicator for evaluating the capacity of predominant species to clean atmospheric pollutants (OGUNKUNLE et al., 2015). Both methods guide urban greening and planting initiatives in polluted environments.

PHYSIOLOGICAL PARAMETERS

Variation in water content during plant growth is one of the most important monitoring parameters in plant studies. Numerous experimental methods are available to determine the actual water content of live plant components (leaves, stems, and roots), including dendrography, infrared light spectrum imaging analysis, spectrometry, and radar systems (YANG, 2021). These experiments are complex and often use costly materials. Several methods have been developed to directly measure stem water content, such as thermography-based systems, microneedle sensors (BAEK et al., 2018), and the Standing Wave Ratio (SWR) system.

Leaf water content is one of the most common physiological parameters limiting photosynthesis efficiency and biomass productivity in plants (JIN et al., 2017). Therefore, for biomonitoring studies, it is important to quickly and preferably non-destructively determine or predict this parameter. This parameter is highly significant and studied in plant growth in agriculture.

Generally, the classical approach for this parameter involves calculating the difference between fresh matter weight and dry weight. These determinations are invasive and time-consuming. Thus, detecting water stress under drought conditions is a major objective for field remote sensing. Plant water stress determination via remote sensing has been proposed using Near-Infrared (NIR) and Mid-Infrared (MIR) light indices (Hunt and Rock, 1989). The natural variation of Relative Water Content (RWC) under water stress is about 20% for most plants, and therefore, indices derived from NIR and MIR reflectance cannot be used to remotely detect water stress. Recently, Near-Infrared spectroscopy analysis has been widely studied for measuring water content (JIN et al., 2017).

Photosynthetic activity: there is ample evidence documenting changes in the photosynthetic activity of plants cultivated in polluted environments. Decreases in photosynthetic processes result from damage caused by biochemical reactions in plant leaves (MULENGA et al., 2020). Measuring photosynthesis is crucial for quantifying and modeling productivity from leaf to regional ecosystem levels in managed and natural ecosystems. Gas exchange measurement method from leaf to ecosystem level is a classic and commonly used one, but recent improvements in environmental control in commercial systems have led to faster data collection and higher quality.

Respiration and Transpiration: Prolonged exposure of plants to sulfur dioxide and heavy metals leads to the development of visible symptomatic lesions in tissues and physiological disturbances. Respiration can be either stimulated or inhibited depending on the degree of tissue damage. Repair processes utilize energy from adjacent undamaged tissues to necrotic ones, thereby increasing transpiration rate in different zones (GUPTA & SARKAR, 2016).

Plant respiration in natural vegetation is often measured using gas exchange either as CO2 efflux or O2 uptake in the dark, although both approaches capture signals from other biochemical processes such as carboxylation by PEP carboxylase (O'LEARY et al., 2019). CO2 flux measurements are commonly used in the field due to difficulties in measuring small changes in O2 concentration (HELM et al., 2021).

Stomatal Function: Exposure to air pollutants undermines the ability of stomatal cells to do its movements due to the accumulation of heavy metals and suspended particles on leaf surfaces (MULENGA et al., 2020). Thus, changes in stomatal function reduce plant growth by altering the production and translocation of photosynthetic pigments.

Temperature: Thermographic images illustrate the heat emitted by bodies. Thermography is primarily used when monitoring large land surfaces over time. Changes in plant temperature can be attributed to several factors, both in response to pathogen infection and abiotic stress. Although this method is ideal for field monitoring in agriculture on large fields and is non-invasive, it is an indirect and nonspecific detection method (ROPER et al., 2021).

CONCLUSIONS

Biological monitoring of urban habitats is necessary to assess the additive effects of pollutants on living organisms. Biomonitoring techniques that use non-destructive methods are becoming increasingly important due to their advantages over chemical-analytical methods.

Efficient monitoring of plants in an environment with the risk of abiotic factors, especially air pollution, requires the identification and evaluation of suitable parameters.

For an efficient and precise approach to biomonitoring, various characteristics of plants must be evaluated, including morphological, biochemical, and physiological aspects, thus providing a solid foundation for data analysis and interpretation.

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