# University of Pannonia

## Doctoral School of Chemistry and Environmental Sciences


**PHYTOTOXICITY OF ATMOSPHERIC PARTICULATE MATTER**

*PhD Thesis*


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## Introduction

Atmospheric particulate matter (PM) can be divided into three main categories based on size as coarse, fine, and ultrafine particles (UFPs) with aerodynamic diameters of 2.5 to 10 μm (PM10), <2.5 μm (PM2.5), and <0.1 μm (PM0.1), PM2.5 (particles less than 2.5 μm in diameter) and PM10 (particles less than 10 μm). The sources of different compounds could be both anthropogenic and natural, including vehicular emissions, industrial activities (Caumo et al. 2023), biomass burning (Cipoli et al. 2023), secondary atmospheric reactions as well as natural sources (e.g., dust storms, volcanic activity, forest fires, and sea spray) (Filonchyk and Peterson 2024).

PM exerts its harmful effects through physical processes, as these particles may directly block stomatal openings. However, the major risk is the presence of potentially harmful contaminants bound on the surface, such as metals (Kelly and Fussel 2012), organic compounds including polycyclic aromatic hydrocarbons (PAHs) (Kim et al. 2013). During the vegetation period, plants are mainly exposed to two sources: open burning of waste and vehicular emissions.

The environmental risks of open burning of domestic waste have become a worldwide problem. Practices, however, are different. Especially in the developing regions of the world, it might be a common disposal practice. Open burning of waste is more common in rural areas (Lemieux et al. 2000), quite frequently together with garden (green) waste. These potentially toxic emissions pose a certain threat to ecosystems, both to natural vegetation and agroecosystems. In addition to phytotoxic effects, bioaccumulation of both organic and inorganic components might occur, jeopardising ecosystem health and as these components most possibly move up the tropic chain, consumers' health will be at risk. PM emitted during waste burning might also contain a wide range of toxic heavy metals such as As, Cd, Cu, Cr, Ni, Pb, Se and Zn (Valavanidis et al. 2008).

Traffic-related PM mass concentrations have shown a diminishing tendency in Europe due to more stringent regulations coupled with technological improvements, however, pollution from traffic, especially emission of diesel-powered vehicles, poses a serious environmental concern in Europe (Matthias et al., 2020).

Both PAHs and heavy metals increase the production of reactive oxygen species and induce intracellular oxidative stress, which is thought to be the main mechanisms of phytotoxicity. Plants growing in polluted habitats have shown a wide variety of symptoms including both physiological and morphological traits (Bell et al. 2011). Biochemical end-points such as the presence and concentration of antioxidant enzymes and the concentration of photosynthetic pigments have been long in use (Rabe and Kreeb 1979). It should be noted, however, that most studies have been conducted in the field, while tests under controlled laboratory conditions are much less available.

## 1. Objectives

Plants, being sessile organisms, have to endure the chemical stress posed by air pollutants. Considering potential sources, winter heating will probably affect only coniferous species, while traffic-related emissions and open burning of waste will expose herbaceous and deciduous species both in natural and agricultural ecosystems.

During my research the main goal was to investigate the potential phytotoxic effects of these emissions, based on a standard protocol, the No. 227 OECD GUIDELINE FOR THE TESTING OF CHEMICALS: Terrestrial Plant Test: Vegetative Vigour Test. As the Guideline follows a step-by-step and clearly defined test protocol, individual assays are repeatable and reproducible, fulfilling basic quality assurance criteria. Repeatability and reproducibility make it possible to compare the sensitivities of different plant species, which will in turn provide basic data for extrapolating these results to wider plant communities.

Both traffic-related and waste burning emissions contain significant amount of polycyclic aromatic hydrocarbons. As such, phytotoxicity test results were intended to compare with analytical measurements, to determine if the distribution pattern of PAHs might support potential toxicity of the samples.

Specific objectives were as follows:

i. to evaluate the combined effect of air pollution and drought stress comparing the sensitivity of different tomato varieties/ landraces using diesel emission samples, in order to assess the possible impact of road traffic and drought on agricultural ecosystems.
ii. to follow under laboratory conditions the transfer of PAHs via wet deposition assessing both the air-serial parts and the soil-root-aerial parts pathways, using rocket (*Eruca sativa* Mill.) as model species.
iii. to evaluate the potential phytotoxic effects of waste burning comparing two widely accepted test species, *Eruca sativa* Mill. and *Lactuca sativa* L. (lettuce).

iv. to evaluate the potential phytotoxic effects of waste burning comparing selected ornamental plants commonly used in European (Hungarian) horticulture.

## 3. Materials and Methods

### 3.1. Sampling and sample preparation

Two different anthropogenic sources were evaluated: traffic-imposed emission and emissions generated during waste burning.

#### *3.1.1 Diesel exhaust sampling*

$PM_{10}$ samples from the exhaust of a 20 year old, light-duty vehicle (environmental standard: Euro3) were taken. The vehicle was operated at idling for 6 x10 minutes. Sampling was performed using a KÁLMÁN $PM_{2.5}$ sampler (flow rate 32 $m^3$ $h^{-1}$). A closed premise was placed approximately 1 meter from the tailpipe, emitted particles were collected on quartz filters (Whatman QMA, diameter 150 mm).

#### *3.1.2 Domestic waste burning*

Waste samples were selected to represent the average composition of domestic waste reported by the (Hungarian) National Statistics Agency.

Experimental burning was performed in a domestic cast-iron residential stove (type: *Servant S114*, rated at: 5 kW) connected to a 11.8 m high lined stack with inner diameter of 150 mm. The average combustion temperature was 310 °C (±56 °C), however, temperature moved in a relatively wide range between 200-450 °C. In case of plastic samples it was occasionally as high as 600 °C. PM10 samples were collected on quartz filters (Advantec QR-100 Ø150 mm).

### *3.1.3 Sample preparation*

Each filter was cut into small pieces and placed in beaker with 1000 mL high purity water for 24h at room temperature and stirred several times. The extract was filtered through 0.45 µm pore size filter (GN-6 Membrane, 0.45 µm Hydrophilic mixed cellulose esters) and stored in 40 ml vials at -20 C until use.

## 3. 2. Analytical measurements

Measurements of polycyclic aromatic hydrocarbons and heavy metals were carried out by courtesy of the Testing Laboratory of the ELGOSCAR-2000 Environmental Technology and Water Management Ltd. accredited by the National Accreditation Authority, registration number NAH-1-1278/2015.

The following standard protocols were applied: MSZ (Hungarian Standard) 1484-6:2003 for PAH measurements, using Agilent 6890GC 5973E MSD GC-MS and EPA 6010C: 2007 for heavy metal determinations, using ICP-OES Thermo iCAP 6300.

3.3 Phytotoxicity testing

3.3.1 Plant material used

List of test species and field(s) of application are given in Table 1.

| Plant name | Field of application | Source |
|---|---|---|
| *Sinapis alba* L | Bioaccumulation of PAHs from diesel emission<br>Bioaccumulation and phytotoxicity of waste burning emissions | Szentesimag Co. |
| *Lactuca sativa* L. | Bioaccumulation and phytotoxicity of waste burning emissions | Szentesimag Co. |
| *Eruca sativa* Mill. | Bioaccumulation of PAHs from diesel emission | Szentesimag Co. |
| *Lycopersicon esculentum* Mill. 'Roma' | Combined effects of air pollution and drought | commercial distributor |

| | | |
|---|---|---|
| *L. esculentum* 'Mobil' | Combined effects of air pollution and drought | |
| *L. esculentum* 'LugasF1' | Combined effects of air pollution and drought | |
| *L. esculentum* 'Tápiószelei' | Combined effects of air pollution and drought | National Center for Biodiversity |
| *L. esculentum* 'Gulácsi' | Combined effects of air pollution and drought | |
| *Gaillardia aristata* Pursh | Phytotoxicity of waste burning emissions | Rédei Kertimag Co. |
| *Calendula officinalis* L. | Phytotoxicity of waste burning emissions | Rédei Kertimag Co. |
| *Alcea rosea annua* L. | Phytotoxicity of waste burning emissions | Rédei Kertimag Co. |
| *Ipomoea purpurea* (L.) Roth | Phytotoxicity of waste burning emissions | Rédei Kertimag Co. |
| *Mirabilis jalapa* L. | Phytotoxicity of waste burning emissions | Rédei Kertimag Co. |
| *Cucurbita pepo* L. | Phytotoxicity of waste burning emissions | Rédei Kertimag Co. |

### 3.3.2 Cultivation of test plants and treatments

Test plants were cultivated and treated according to the protocol specified by the No. 227 OECD GUIDELINE FOR THE TESTING OF CHEMICALS: Terrestrial Plant Test: Vegetative Vigour Test. The Guideline has been adopted to test the potential phytotoxic effects of atmospheric PM and to assess bioaccumulation of atmospheric PM-bound PAHs. (Teke et al. 2020).

3.3.4 End-points used

Phytotoxicity was assessed based on the following end-points: biomass reduction, concentration of photosynthetic pigments and peroxidase levels.

Photosynthetic pigment contents were measured using the spectrophotometric method (Bag N et al., 2012). The sample was 0.2 g from each plant's leaf. (10-10 plants in each group) The sample was homogenized with 15 mL of 80% acetone (Fisher Chemicals, ≥99.8%, ACS; VWR International Ltd., Debrecen Hungary), and centrifuged at 4500 rpm for 10 min (Mistral 2000 MSE, DJB Labcare Ltd. Newport Pagnell, Buckinghamshire, UK). After that, the supernatant was separated and diluted with 80% acetone to a final volume of 25 mL. Absorbance was measured at 470 nm, 647 nm, and 670 nm for photosynthetic pigment measurement using a UV-VIS spectrophotometer. (Metertech SP8001, ABL&E-JASKO Hungary Budapest, Hungary).

The following equations were used for the final calculation:

Chl-a = [9.78xE663 – 0.99xE645] [V/1000xW]

Chl-b = [21.4xE645 – 465xE663] x ]V/1000xW]

Car = [4.69xE440 – 0.268x(5.13xE663 + 20.41xE645] x [V/1000xW]

Where E: extinction values at wavelengths; V: final volume (25 mL); W: mass of sample (0.2 g)

In case of peroxidase measurements, the sample was made from 0.2 g of each plant's leaf, which was frozen and homogenized in an ice-cold mortar and pestle in phosphate buffer (50 mmol/L, pH 7) containing 1 mmol/L EDTA and 0.5 mmol/L Phenylmethylsulfonyl fluoride (PMSF). After preparing, these samples were centrifuged for 20 min at 15.000 x g at 4 $^{0}$C. Peroxidase activity (A 654) was measured according to the protocol described in Imberty et al. with minor modifications.

Tetramethylbenzidine (TMB) peroxidase activity (A 654) was measured following th e protocol described in Imberty (Imberty, A et al, 1984) with minor modifications.

Steps of phytotoxicity testing are illustrated in Fig. 1.

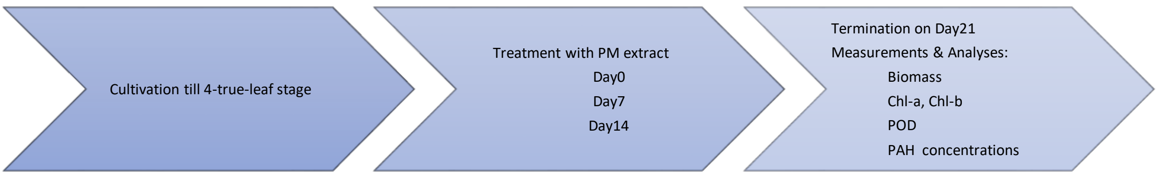


Figure 1. Steps of the phytotoxicity testing procedure

### 3. 4. Statistical analysis

Statistical analyses were performed using the RStudio (RStudio Desktop April 1, 1106) program, ggfortify package (https://CRAN.R-project.org/package=ggfortify). Statistical analysis was carried out using RStudio (RStudio Desktop 1.4.1106) program and R 4.0.0 program (http://cran.r-project.org/src/base/R-4/R-4.0.0.tar.gz) Rcmdr package. Statistical significance was defined as $p \leq 0.05$.

## • Results

Table 2 shows the response of used tomato varieties/landraces to air pollution, drought or to the combination of these factors. As a general conclusion, water stress had more pronounced effect than chemical treatment. Water stress might mitigate phytotoxicity of atmospheric pollutants via stomata closure which in turn reduces uptake of these pollutants.

Comparing sensitivity of different end-points, reduced biomass and biochemical markers as carotene or POD seem more sensitive indicators than photosynthetic pigments Chl-a and Chl-b. Preliminary results indicate the higher resistance of landraces toward abiotic stress, the commercial variety 'Roma' showing the highest while the regional landrace 'Gulácsi' showing the lowest sensitivity.

Table 2 Response of the tested varieties. WW: Well-watered; RW: Reduced watering. PM-: no treatment; PM+: treatment with the PM extract

Roma

| | Biomass | Chl-a | Chl-b | Carotene | POD | Stomata |
|---|---|---|---|---|---|---|
| TG2 RW/PM- | No difference | No difference | No difference | Increase | Increase | Increase |
| TG3 WW/PM+ | Decrease | No difference | No difference | Increase | Increase | No difference |
| TG4 RW/PM+ | Decrease | No difference | No difference | Increase | Increase | Increase |

Mobil

| | Biomass | Chl-a | Chl-b | Carotene | POD | Stomata |
|---|---|---|---|---|---|---|
| TG2 RW/PM- | Decrease | No difference | No difference | No difference | Increase (marginally significant) | Increase |
| TG3 WW/PM+ | Decrease | No difference | No difference | No difference | Increase | Increase |
| TG4 RW/PM+ | Decrease | No difference | No difference | No difference | No difference | Increase |

Lugas

| | Biomass | Chl-a | Chl-b | Carotene | POD | Stomata |
|---|---|---|---|---|---|---|
| TG2 RW/PM- | No difference | No difference | No difference | No difference | No difference | No difference |
| TG3 WW/PM+ | Decrease (marginally significant) | No difference | No difference | No difference | Increase (marginally significant) | No difference |

| | | | | | | |
|---|---|---|---|---|---|---|
| TG4 RW/PM+ | Decrease | No difference | No difference | No difference | No difference | Increase |

Tápiószelei

| | Biomass | Chl-a | Chl-b | Carotene | POD | Stomata |
|---|---|---|---|---|---|---|
| TG2 RW/PM- | No difference | No difference | No difference | No difference | Increase | Increase |
| TG3 WW/PM+ | No difference | No difference | No difference | No difference | No difference | Increase |
| TG4 RW/PM+ | No difference | No difference | No difference | No difference | No difference | Increase |

Gulácsi

| | Biomass | Chl-a | Chl-b | Carotene | POD | Stomata |
|---|---|---|---|---|---|---|
| TG2 RW/PM- | No difference | No difference | No difference | No difference | Increase | Decrease |
| TG3 WW/PM+ | No difference | No difference | No difference | No difference | No difference | No difference |
| TG4 RW/PM+ | No difference | No difference | No difference | No difference | Increase | Decrease |

Assessing the phytotoxicity of emissions generated during open burning of municipal solid waste, test plants showed marked differences in sensitivity. Of the 6 plants, *M. jalapa* showed the highest sensitivity, indicating damage by decreased biomass, chlorophyll b and carotenoid levels, demonstrating increased antioxidant activity by the increased level of peroxidase.

Species-specific sensitivity was also experienced when waste burning emissions were assessed on two quasi-standard test species, *Lactuca sativa* and *Sinapis alba*. While lettuce showed moderate phytotoxic effects, mustard was less responsive as only peroxidase level reduction indicated the deleterious effect of the treatments. In general, POD was the most sensitive end-point.

In these tests, phytotoxic effects could be associated with the heavy metal and PAH content of the extracts used for the test, as samples contained in relatively high concentrations the reportedly phytotoxic Cd, Sb, Zn, as well as PAHs such as fluoranthene, phenanthrene and pyrene.

Treatment of Eruca sativa with diesel emission extract samples revealed that atmospheric PAHs expose plants via two different pathways, via the soil-root-aerial parts pathway and the atmosphere-aerial parts- root pathway. Measuring PAH accumulation in different parts of E. sativa, it way established that PAHs deposited on the soil would be accumulated in the roots and gradually move to above-ground parts of the plant. This will also result in the transfer of higher molecular weight PAHs, though in much lesser concentrations. Considering accumulation in the leaves – which are the edible parts of leafy vegetables – direct atmospheric deposition via precipitation is the main pathway. Rocket can accumulate even higher molecular weight PAHs in considerable quantities.

- ## Conclusions

The No. 227 OECD GUIDELINE FOR THE TESTING OF CHEMICALS: Terrestrial Plants Test: Vegetative Vigour Test was applied to evaluate phytotoxic effects elucidated by diesel emission and emissions generated burning of domestic waste. In concordance with previous studies, biomass reduction and peroxidase enzyme activity proved much more sensitive end-points than phytosynthetic pigments. In c responses, a strong species.specific sensitivity was experienced. The protocol proved adequately sensitive and reliable to assess the damaging nature of waste burning in close proximity of vegetation.

Assessing the combined effects of tomato varieties/landraces to air pollution, drought or to the combination of these factors revealed the higher resistance of local landraces. The study highlights the importance of careful selection of landraces in order to cope with environmental stress in a changing climate and also to make a step towards a sustainable agriculture.

# 6 New scientific points

**Thesis points:**

1. Sensitivity of five different tomato varieties/landraces such as ‘Roma’, ‘Mobil’ and ‘Lugas’ (commercially available varieties) as well as ‘Gulácsi’ and ‘Tápiószelei’ (regional landraces) was compared to the combined effect of air pollution and drought stress on tomato (*Lycopersicon esculentum* Mill.). Water stress had more pronounced effect while treatment with the PM extract triggered response only in case of the three commercial varieties. In general, higher resistance of landraces toward abiotic stress was established, the commercial variety ’Roma’ showing the highest while the regional landrace ’Gulácsi’ showing the lowest sensitivity to individual and combined treatments.
2. I established species-specific accumulation potential comparing lettuce (*Lactuca sativa* L.) and white mustard (*Sinapis alba* L.) plants, lettuce showing substantially higher bioaccumulation of low molecular weight (LMW) PAHs in edible parts.
3. Simulating wet deposition of atmospheric polycyclic aromatic hydrocarbons (PAHs) in rocket (*Eruca sativa* Mill., family Brassicaceae), atmospheric uptake of PAHs proved the main pathway, especially for higher molecular weight PAHs. Results also demonstrated that

both soil-aerial parts and aerial parts-root transfers are low, especially in case of HMW PAHs.

4. Phytotoxic responses to emissions generated during experimental burning of municipal waste were evaluated comparing two commonly applied test plants (*Lactuca sativa* and *Sinapis alba*) and six ornamental plants (*Gaillardia aristata, Calendula officinalis, Alcea rosea annua, Ipomoea purpurea, Mirabilis jalapa* and *Cucurbita pepo*). Strong species-specific sensitivity was experienced, *L. sativa* and *M. jalapa* being the most responsive, in concordance with reported studies.
5. In case of diesel emission and waste burning generated PM samples, growth inhibition and peroxidase enzyme activity proved the most responsive end-points, in concordance with reported studies.


## • Acknowledgments

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# 7 List of publications

## 7. 1. Papers related to the dissertation

- Hubai, K., Kováts, N., Eck-Varanka, B., Tumurbaatar, S., & Teke, G. (2024). Accumulation of Atmospheric PAHs in White Mustard–Can the Seeds Be Affected?. *Bulletin of Environmental Contamination and Toxicology*, *112*(5), 76. Q2, IF: 2.7
- Tumurbaatar, S., Kováts, N., & Hubai, K. (2024). Phytotoxicity Testing of Atmospheric Polycyclic Aromatic Hydrocarbons. *Atmosphere*, *15*(9), 1143. Q2, IF: 2.5
- Tumurbaatar, S., Kováts, N., Eck-Varanka, B., Teke, G., & Hubai, K. (2025). Assessing Risk of Emissions Generated During Illegal Waste Burning: Phytotoxicity and Bioaccumulation. *Atmosphere*, *16*(2). Q2, IF: 2.5
- Hubai, K., Eck-Varanka, B., Tumurbaatar, S., Teke, G., & Kováts, N. (2025). Contribution of Atmospheric Fallout to the Soil–Root–Leaf Transfer of PAHs in Higher Plants. *Applied Sciences*, *15*(8), 4407. Q2, IF: 2.5

## 7. 2. Congress attendances

- Kováts, N., Hubai, K., Eck-Varanka, B., Tumurbaatar, S. (2024). Can landraces better cope with environmental stress? 12TH IEES CONFERENCE, 2024, Chania, Greece

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