Response to Dr. Anita Lepossa's review

I would like to thank the Reviewer for taking the time to review my thesis entitled: "Phytotoxicity of atmospheric particulate matter" and for the useful comments and suggestions. I deeply apologize for the typing and editing mistakes mentioned in the Review. In my response I am addressing explicit remarks and questions.

Remarks:

- We do not get a specific description of the process of applying the extract in chapter 5.1.3, and 6.1.1., or whether the 5mL extract dose mentioned in chapter 3.1.3. as the treatment was used on an occasion or in total?

In the Material and Methods section, chapter 5.1.3, the diesel emission extract was applied at 5 ml dose per pot per treatment. According to the guideline, three consecutive treatments are done on Day0, Day8, and Day15. Treatment started when plants reached 4 true leaf stages.

In Chapter 6.1.3, the diesel emission extract was applied at 1.5mL per pot. It has 3 consecutive treatments on Day0, Day8, and Day15.

- It would have been good to provide the dry mass in addition to the % material composition of the incinerated waste.

Experimental burning was conducted by the HUN-REN–PE Air Chemistry Research Group. Samples were forwarded to us, unfortunately we did not receive data about the dry mass composition of the incinerated waste.

- The concentration of Polycyclic Aromatic Hydrocarbons (PAHs) differed by an order of magnitude across the extract. What would be the reason for this?

The reason of different orders of magnitude across the extracts is used different types of plastics in the experiments. Most possibly, the polyurethane content could determine total PAH emsission as this plastic has reportedly high PAH emissions (Kováts et al. 2022).

Kováts, N., Hubai, K., Sainnokhoi, T. A., Eck-Varanka, B., Hoffer, A., Tóth, Á., ... & Teke, G. (2022). Ecotoxic emissions generated by illegal burning of household waste. *Chemosphere*, *298*, 134263.

- The Tables presenting the examined composition of the extracts should be placed in the Material and Methods section, not among the Results. The BCF calculation equation is described in Chapter 3.1.9; however, it is not included in the statistical analysis. The author reports the results of the stomatal surface area test on tomatoes only as summary statements, providing no factual data.

Measurement of stomatal characteristics was performed by dr Bettina Eck-Varanka, co-author of the manuscript 'Combined effects of air pollution and drought stress in tomato landraces', submitted to Industrial Crops and Products (under review). As such, I only included the summary based on these data. In the manuscript, factual data can be found as follows:

Surface area of stomata showed significant response (Tukey HSD) in case of all varieties tested, though response was treatment and variety specific. In 'Roma', only water stress elucidated significant increase (WW/PM- and RW/PM-: $p<0.0000$; WW/PM- and WW/PM+: $p=0.972$; WW/PM- and RW/PM+: $p<0.0000$). In 'Mobil', however, all treatment groups showed significant response, increase in stomatal area (WW/PM- and RW/PM-: $p<0.0000$; WW/PM- and WW/PM+: $p=0.0001$; WW/PM- and RW/PM+: $p<0.0000$). Similar pattern was found in case of 'Tápiószelei' (WW/PM- and RW/PM-: $p<0.0000$; WW/PM- and WW/PM+: $p<0.000$; WW/PM- and RW/PM+: $p<0.0000$).

In case of 'Lugas', only the less water supply/PM treatment elucidated significant increase in comparison to the control (RW/PM+: $p=0.4344$). 'Gulácsi', however, showed an opposite tendency, as significant decrease was experienced in the test groups exposed to water scarcity (WW/PM- and RW/PM-: $p<0.0000$; WW/PM- and WW/PM+: $p=0.086$; WW/PM- and RW/PM+: $p=0.001$).

- What is your opinion, whether in case of *Gailardia aristata, Calendula officinalis*, or *Ipomoea purpurea*, the resulting significant difference in peroxidase activity could also be caused by differences in concentration resulting from species differences?

Indeed, morphology will most influence exposure to air pollution. Exposure might be determined by leaf area or specific characteristics such as presence of hairiness in the case of *C. officinalis* or relatively thin cuticula such as the case of *I. purpurea*.

- How can you explain the POD reduction in response to stress in these three species, in contrast to the results of your previous studies with lettuce and mustard, and even though all other examined parameters showed typical of stress?

In the two experiments reported, two different samples were used, collected during two different burning experiments. Toxic compound content of the samples might have been associated with resulting responses.

- Considering discrepancies in the 2nd new scientific results, in the thesis these results were not discussed deeply as they fell out of the topic of the dissertation, reporting only bioaccumulation and not phytotoxic responses. Therefore, the final version of the thesis booklet did not contain this point.
- With regard to Thesis point 3, the treatment implied that the soil was sprayed with the extract (Miglietta et al. 2015). Spraying was done close to the shoot of individual plants (1.5 ml/plant). During the treatment the aerial parts of the plants were covered.

Miglietta ML, Rametta G, Manzo S, Salluzzo A, Rimauro J, Di Francia G (2015) Methodological issues about techniques for the spiking of standard OECD soil with nanoparticles: evidence of different behaviours. J Nanopart Res 17:1-12.

- With regard to Thesis point 4, effects of the extracts of the applied composition can be specified as follows:

Strong species-specific sensitivity was experienced, *L. sativa* and *M. jalapa* being the most responsive, in concordance with reported studies. Both species showed the highest number of responses, growth impairment, decreased chlorophyll b and elevated level of POD in case of *L. sativa,* decreased biomass, chlorophyll b and carotenoid levels in case of *M. jalapa.* In general, phytotoxic effects could be associated with the heavy metal and PAH content of the extracts used, triggering antioxidative responses. Oxidative stress is supposed to have negative influence on photosynthetic machinery which in turn affects growth.

Questions:

The four-true-leaf stage described in the OECD guideline probably corresponds to different ages across plant species, as developmental rates vary among species. *Were plants of the same age used in the lettuce and mustard experiments, and was there a difference in the treated leaf surface area? Could the higher PAH accumulation seen in lettuce be caused by the larger leaf area absorbing more PM extract during treatment? Based on your results and the health limits, could the treated lettuce from your study be safe to consume?*

Answers:

The growth stage of treated plants was comparable as the OECD 227 Guideline prescribes the starting of treatment at the 2 or 4-leaf stage. We always used 4-leaf stages. Treated leaf areas were depending on the species. In general, morphological characteristics such as area, shape of the leaf, its hairyness, presence or absence of cuticular wax will highly influence the response of the test specimen. Plants with broader leaves will most possible take up proportionally larger doses. Although lettuce is the most impacted, human health risk is associated with annual consumption. Lettuce cultivated outdoors in gardens is mostly consumed seasonally.

Sincerely yours,

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Veszprém, 2025.01.07