The potential of *Hedera helix* L. stems and leaves for atmospheric pyrene phytomonitoring

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ABSTRACT

The aim of this work was to investigate the phytomonitoring potentials of *Hedera helix* L. from the Bor′s municipality concerning (atmospheric) Pyrene as a well-known hazardous substance. The results of gas chromatographic-mass spectrometric analysis of the unwashed stems and leaves showed that Pyrene concentrations obviously varied at all selected rural and urban/industrial zones (RZ and UIZ). These first signs of various sources of Pyrene in the investigated area were later supported by the results of the performed Pearson's correlation analysis, which showed that the detected Pyrene concentrations came not only from the vicinal heating and smelting plants, as the main sources of pollution in the whole region but also from the domestic heating in RZ, or forest fires, or controlled fires in the cultivated fields, and finally from the traffic in all zones. The correlation analysis also signalized that at some locations, the detected concentrations (especially in stems) came not only from the atmosphere but also from the soil. The calculated factor R confirmed the sites with the greatest atmospheric impact. Very high R values were calculated for 3 sites (two in UIZ and one in RZ) with the highest value of 1.61. Based on the obtained results, it can be concluded that the investigated stems and leaves, with the applied chemical and statistical analyses, and the calculation of R factor, can serve as a useful tool in the atmospheric Pyrene phytomonitoring.

Keywords: phytomonitoring, pyrene, *Hedera helix* L.

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Introduction

Pyrene (Pyr) is a complex organic compound (Fig. 1) belonging to the class of polycyclic aromatic hydrocarbons (PAHs). It comprises 4 fused benzene rings (Fig. 1) with a molecular weight of 202.30 g/mol. The melting point of this compound is 150.6ˆC, whereas the boiling point amounts to 404°C; Henry's Law Constant, which indicates the chemical's potential to volatilize, is 1.2 (Pa/m^3) (Verbruggen, 2012).

Figure 1. Pyrene structural formula (NIST, Chemistry, WebBook, SRD 69)

As with other PAHs, Pyr has a pretty low solubility in water – 77 µL, resulting in better accumulation in the aquatic organisms and sediments. Its vapor pressure – $2.5 \cdot 10^{-6}$ mmHg is also low. For this compound, Log Kow (that provides an indication of the potential of the organic compound to partition between water and lipids) is 4.96, while log Koc (indicates a compound's potential to bind to organic carbon in soils) is 4.75 (Alagić et al., 2015; Papludis et al., 2022). Toxicological characteristics of Pyr are also noticed. Its toxicity was observed not only in humans but in all animals, too (especially in aquatic species). Its toxicity to plants is not defined yet but it can be supposed that, in some extent, it may by have same toxic effects in these organisms. According to USEPA (United States Environmental Protection Agency) IRIS program (Integrated Risk Information System) for humans, Reference Concentration for Inhalation Exposure (RfC) is not assessed, whereas the amount of its Reference Dose (RfD) for Oral Exposure is $3 \cdot 10^{-2}$ mg/kg-day. In the case of Pyr, the kidneys and the complete urinary tract are the most endangered. Fortunately, there is no evidence of human carcinogenicity (https://iris.epa.gov/ChemicalLanding/&substance_nmbr=445).

Similarly to other PAH compounds, Pyr originates from different anthropogenic and natural (eruptions of volcanoes and fires in forests) sources. In general, PAHs are released from the pyrolysis of hydrocarbons present in waste, fossil fuels (tar, wood, and oil), and biomass. They

are present in grilled food and tobacco smoke, too (Alagić et al., 2015; Papludis et al., 2022, 2023a). Its concentrations in the environment are constantly increasing, and Pyr is included in 16 priority PAHs, known as dangerous pollutants, even in micro-concentrations (Alagić et al., 2016, 2017; Morillo et al., 2007).

The mentioned facts raised human need to track the concentrations of these compounds, including Pyr, in the environment, trying to protect the endangered local populations. One of the most appropriate methods for PAH/Pyr monitoring is a biological method known as bio-/phyto-monitoring, which is mostly based on the monitoring of PAH concentrations in the aboveground plant parts (Reinholds et al., 2015; Sari et al., 2020). It is also known that most plants can absorb this substance from the soil but cannot transport it to the aboveground parts (Alagić et al., 2016, 2017). In this work, the concentrations of Pyr were monitored using unwashed, dried, stems and leaves of *Hedera helix* L. (poison ivy, PI) from the Bor region. The concentrations were detected applying gas chromatographic-mass spectrometric, GC/MS method.

Experimental

The locations in the Bor region that were chosen for the conducted phytomonitoring experiment were as follows:

- Flotacijsko jalovište (FJ), Bolničko naselje (BN), Slatinsko naselje (SN) and Naselje Sunce (NS) in the urban-industrial zone (UIZ), and

- Oštrelj (O), Borsko jezero (BJ) Krivelj (K), Zlot (Z) and Gornjane (G) in the rural zone (RZ).

The distances of the chosen locations from the city heating plant and the copper smelter as the main sources of pollution in the whole region, are as follows: for the site FJ (an abandoned flotation tailings pond) - 0.7 km, for the site BN (near the hospital in Bor) - 1.3 km, and for the two suburb sites SN and NS - 3.2 km, and 3.6 km, respectively. The sites from RZ are located regarding to the Bor town as follows: site O - 4.5 km, site BJ - 7 km, site K - 8 km, site Z - 13 km, and site G - 19 km.

It should be mentioned here that the town of Bor (UIZ) is a relatively novel settlement, with the developed central heating based on coal burning, whereas in RZ, the domestic heating based on different kind of fuels is dominant.

Plant material of PI was collected from the selected sites in a way that was described in Papludis et al. (2023a), together with the way of its preparation and GC/MS analysis. Used apparatus was 7890/7000B GC-MS-MS triple quadrupole device (Agilent Technologies, USA), equipped with a Combi PAL auto sampler and an HP-5MS capillary column. The only distinction was that the leaves and stems were analyzed as unwashed.

The processing of the results of PI samples, i.e., in terms of evaluating the level of atmospheric Pyr in the Bor region, the ratio (factor R) of Pyr concentrations in leaves and stems, was calculated as follows: $R_{leaf/stem} = C_{leaf}/C_{stem}$ (C_{leaf}, and C_{stem} are the concentrations of Pyr in the corresponding plant organs at each location). The amounts of factor R bigger than 1 point to the sites with the greatest atmospheric impact (Oliva and Mingorance, 2006; Papludis et al., 2023b). The relation between the content of Pyr in plant organs and the distance from the main sources of contamination in the entire region (the vicinal heating and smelting plants sited in the mining-metallurgical complex in the town of Bor), the statistical method of Pearson's correlation analysis (Miller, and Miller, 2005; Reinholdset al., 2015; Sari et al., 2020) was performed in IBM SPSS program, version 20.

Results and Discussion

Measured concentrations of Pyr (in µg/kg dry weight) in the stems and leaves of PI are given in Table 1. It shows that almost all investigated locations had very different concentrations of Pyr, which ranged from nd (not detected) in stems from site K to 167.42 µg/kg in the same plant parts from site SN. The lowest leaf concentration was found at site S: 2.41 µg/kg, whereas the highest was in leaves from site O: 18.96 µg/kg. The only sites with similar stem and leaf concentrations were FJ and BN from UIZ. These facts were the first signals that the content of Pyr in the investigated parts from UIZ and RZ was not of the same origin (Alagić et al., 2016, 2017), which was later supported by the results obtained from the performed Pearson's correlation analysis.

Table 1. The concentrations of Pyr (µg/kg, dw) in the leaf and stem samples of PI

Location	Pyr stems	Pyr leaves
FJ	8.73	8.24
BN	8.01	8.45
SN	167.42	9.48
NS	10.26	13.09

O	89.43	18.96
S	68.52	2.41
BJ	8.44	7.19
K	nd	17.01
Z	7.90	16.13
G	4.70	7.56

nd – not detected

More precisely, Pearson′s correlation analysis showed that the concentrations of Pyr in plant stems were in a weak negative correlation with the distance from the main sources of pollution (p = –0.29), which means that with distance increasing, the concentrations of Pyr decreased. However, this low value of the Pearson′s correlation coefficient (generally, less than 0.39; Evans, 1996) has a practical meaning that the main sources of pollution had a very low influence on stem concentrations in UIZ, and RZ. In the other hand, leaf Pyr concentrations were in a positive correlation with the distance from the same sources of pollution but also, with a very low value (p = 0.039); this means that with distance increasing, the concentrations of Pyr also increased but in a very low extent. This fact finally points that the main sources of pollution cannot be treated as the only sources of Pyr in the investigated area - they definitively point on additional sources of Pyr (Alagić et al., 2016, 2017), which may include: the domestic heating in rural areas, forest fires, controled firest in the filds with the cultivated plants, and finally, the developing trafic in all zones. In addition, Pearson′s correlation coefficients also showed that the contents of Pyr in plant stems were in a statistically significant positive correlation (p = 0.740, at the 0.05 level) with the corresponding soil concentrations (given in Papludis et al., 2023a), while leaf and soil contents were in a low positive correlation (p = 0.395). Although the correlation between the pollutant concentration in soil and in the unwashed aboveground part cannot be taken as a confident (only the pollutant concentration in the washed aboveground part can reflect an actual bioaccumulation), these calculated correlation trends may represent an evidence that, in general, stems had a better ability for Pyr assimilation from the soil than leaves, which further explains some cases of locations with higher stem than leaf concentrations (the locations SN, O, S, and BJ), i.e., this may explain that stem Pyr concentrations at mentioned locations, came probably, predominantly from the soil, and not from the atmosphere.

The sites with the greatest atmospheric impact were confirmed by the calculation of factor R, presented in Fig. 2.
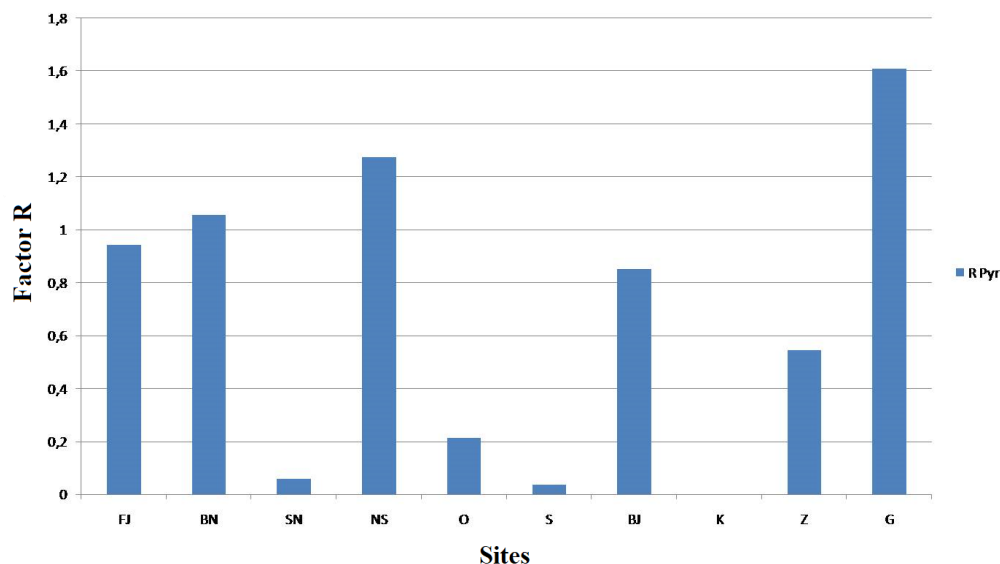
Figure 2. The values of factor R at the investigated sites

R values higher than 1, were calculated for the sites BN, NS, and G (where the highest value were found, 1.61). Also, amounts very close to 1 were found in the case of the sites from UIZ: FJ, and BJ. The lowest R value was found at the site S (0.04), which points that the contribution of atmospheric pollution was the lowest at this place. R factor was not posible to calculate for the site K.

Conclusion

The results for Pyr concentrations in the stems and leaves of PI showed that these concentrations varied at the selected locations from nd in stems from RZ (site K) to 167.42 µg/kg in the same plant parts from the site SN in UIZ. The lowest and the highest leaf concentrations were found at the sites from RZ: S - 2.41 µg/kg, and O - 18.96 µg/kg. The highest R-value was at site G = 1.61, whereas the lowest R-value was found at site S = 0.04.

With the assumption (resulting from the performed Pearson′s correlation analysis) that the pyrene content in the stem is mainly the consequence of accumulation from the soil, whereas the leaf content mainly originates from the atmospheric deposition, the ratio of pyrene concentration in unwashed leaves and stems (R) can serve as an indicator of atmospheric pyrene pollution.

Acknowledgement

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