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Short communication

Indirect pesticide contamination of wild flora in the Prespa region, North Macedonia

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Introduction

Excessive pesticide use in agriculture poses a serious environmental risk. Approximately 98% of sprayed pesticides affect non-target species, and 80–90% volatilize into the environment shortly after application, dispersing into soils, rivers, groundwater, and lakes, and appearing in wildlife (Ali et al., 2021). Evidence of pesticide residues in drinking water and food chain products is increasing (Stipaničev et al., 2017).

The Prespa region is an important agricultural site, represented with the biggest plantations of apples in North Macedonia and main source of livelihood for the population in the region. Although systems for sustainable usage of pesticide were established in the previous years, many farmers continue to rely on pesticide spraying systems to protect their crops. Since Prespa region with Golem Grad Island represents an important site with high biodiversity, unmonitored usage of pesticides could pose a danger to the biosystem.

There is no systematic pesticide monitoring system for the region, therefore this study aimed to determine whether there is indirect pesticide contamination of wild flora around Lake Prespa and Island of Golem Grad and to what extent.

Materials and methods

Plant material

Representative samples of wild flora were collected in spring and autumn from 8 locations (Krani; Pretor, Slivnica, Resen (two localities with varying distance from agricultural orchards), Stenje (two locations – lakeshore and road site), and Island Golem Grad). The species of each miscellaneous plants' sample from the different locations were identified and voucher specimens were stored. The samples were consisted of total of 48 different medicinal plant species differing among the locations. Coordinates and distances from orchards were recorded.

Sample preparation and analysis

Sample preparation was performed using the QuEChERS extraction method (EURL, 2015). Pesticide residues were analyzed by gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry, screening for 450 compounds. Concentrations were expressed in ppb calculated for one kilogram of analysed material (μg/kg).

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Statistical analysis

Statistical analyses included Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), Partial Least-Squares Discriminant Analysis (PLS-DA), and heatmap visualization (SIMCA ver. 14.1).

Risk assessment

Maximum residue levels (MRLs) for apples, blackberries, and pine nuts from the EU Pesticides Database were used as indicative reference points, as no standards exist for wild plant species (European Commission).

Results and discussion

A total of 19 pesticides were detected in the representative plant samples on 5 localities for the two sampling periods. In spring samples, 10 pesticides were identified. Pretor showed the highest contamination with 8 pesticides identified, followed by Krani and Resen with 2 pesticides each. In Pretor were found the highest concentrations of pesticides - pyrimethanil and tebuconasole with 80.70 and 135.03 ppb, respectfully. In autumn samples, 12 different pesticides were detected, out of which Resen - 8; Pretor - 5 and Stenje-road - 3 pesticides. Acetamiprid (546.15 ppb), difenoconazole (518.86 ppb), and pyriproxyfen (299.61 ppb) were the most present pesticides residues in autumn samples at the Resen-field site. These concentrations exceed the MRL. Pyrimethanil was the most frequently detected pesticide (in 5 spring and in 4 autumn samples), followed by acetamiprid (in 3 autumn samples). At Slivnica, Stenje – lakeshore and Island Golem Grad samples no pesticides were detected.

According to the identified pesticides and the extent of contamination, PCA and HCA analysis of all samples in both seasons distinguished 3 groups: Group 1 (Pretor-high pesticide load), Group 2 (Resen-field post-spraying in spring / Resen-field in autumn), and Group 3 (low or no residue sites). PLS-DA loading plots showed that pyrimethanil, acetamiprid, tebuconazole, and difenoconazole were strongly associated with the high-contamination sites. Detected pesticides correlated with locally used products: Faban (fungicide, active ingredient pyrimethanil) and Kestrel (insecticide, active ingredient acetamiprid).

Indirect contamination was confirmed at 5 of 8 locations. Although MRL comparison is limited due to lack of standards for wild plants, the results indicate potential ecosystem risk. All of the analysed wild plant species are used in traditional medicine or as natural products, raising concerns about human exposure.

Conclusion

The study demonstrated extensive pesticide contamination of wild plants in the Prespa region, with residues detected at most sampling sites and the highest levels occurring near agricultural areas. Multivariate analyses confirmed clear contamination patterns linked to local pesticide use, particularly pyrimethanil and acetamiprid. The presence of residues in wild medicinal and edible plants highlights the environmental spread of pesticides and raises concerns regarding ecosystem health and potential human exposure.

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