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Effects of pesticides to benthic invertebrate community in the Serbian Danube stretch using the SPEAR index

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Abstract Most pesticides in water are originated by surface runoff, or leaching through soil affecting different biological communities in aquatic ecosystems. The Serbian Environmental Protection Agency (SEPA) conducts regular surface water monitoring in the entire territory of Serbia. The present study aimed to provide data on effects of pesticides to non-target freshwater organisms (benthic invertebrates) using the SPEAR index at 9 sampling stations of the Serbian Danube stretch: Bezdan, Bogojevo, Novi Sad, Zemun, Smederevo, Banatska Palanka, Tekija, Brza Palanka, and Radujevac, over a six-year period (2014-2019). During 2014 extreme rainfall and floods were registered in Serbia, and in 2017/2018 extreme drought, respectively. Since the SPEAR index has been primarily developed for small to medium streams, the main goal of the study is to test the use of the SPEAR index in large rivers, such as the Danube River. Most commonly detected pesticides in higher concentration in the water of the Serbian part of the Danube River are: metolachlor, atrazine, terbuthylazine, desethylterbuthylazine, terbutryn and isoproturon. Benthic invertebrate community composition and structure varied from the Danube River entering to Serbia (Bezdan) to its leaving (Radujevac). As conclusion, lower SPEAR index values were calculated at sampling stations situated in the Danube River backwater zone, whilst the SPEAR index values were higher along the main course of the Danube River in Serbia. Therefore, the Danube River backwaters can act as refugia for benthic invertebrates being exposed to a lowered pesticide pollution.

Keywords: Species at Risk index, macroinvertebrates, pesticide exposure, bioindicator

1 Introduction

Pesticides are frequently detected in surface waters, sometimes at levels exceeding ecotoxicological guidelines (Bighiu et al., 2020). Pesticide residue contamination of surface water in agricultural areas can have adverse effects on the ecosystem (Lundqvist et al., 2019). Most pesticides in water are originated by surface runoff, or leaching through soil affecting different biological communities in aquatic ecosystems. Commonly detected pesticides in higher concentration in the water of the Serbian part of the Danube River are metolachlor, atrazine, terbuthylazine, terbuthylazine-desethyl, terbutryn and isoproturon. The "Species at Risk" index for pesticides ($SPEAR_{pesticides}$) is a pesticide-specific bioindicator based on biological traits sensitive to pesticide effects (Liess et al., 2008; Hunt et al., 2017).

Water pollution is a widespread problem all over the world, and the use of pesticides, as a necessary product for obtaining higher yields, also contributes greatly to water pollution (Liess et al., 2008; Rico et al., 2016).

Pesticides are chemical or biological products used to control and destroy harmful organisms. As any use of pesticides has a negative effect on the ecosystem in which it is applied, the rational use of pesticides is very important in the development of agriculture. Since pesticides have the ability to infiltrate underground water, the aim is to reduce the use of pesticides, as well as to remove their residues.

The $SPEAR_{pesticides}$ index is here applied for benthic invertebrates to identify the pesticide stress on water biota. Species of this organism group is already commonly used as bioindicators (e.g., Marković et al. 2012; Janecek et al, 2018) to assess the ecological status of waters, as e.g., by methods developed for the Water Framework Directive (e.g., Novaković 2012, 2013, Ihtimanska et al. 2014) or for toxicity tests (Nowell et al., 2014).

2 Material and methods

The Serbian Environmental Protection Agency (SEPA) conducts regular surface water monitoring in the entire territory of the Republic of Serbia. Multi-habitat sampling procedure was applied for the collection of benthic invertebrates by a hand net (25x25 cm, 500 µm mesh size) in the period summer/autumn within the Annual Water Quality Monitoring Programmes.

The present study aimed to provide data on effects of pesticides to non-target freshwater organisms (benthic invertebrates) using the $SPEAR_{pesticides}$ index at 9 sampling stations of the Serbian Danube stretch: Bezdan, Bogojevo, Novi Sad, Zemun, Smederevo, Banatska Palanka, Tekija, Brza Palanka, and Radujevac, over a six-year period (2014-2019).

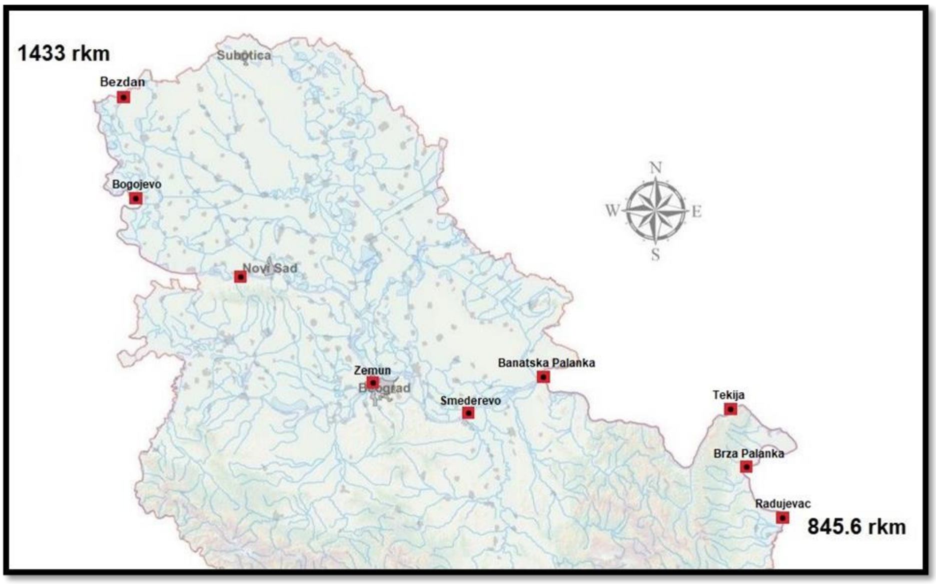

Figure 1: Map of the nine sampling stations along the Serbian Danube stretch.

Figure 2: The Tekija sampling station in the Danube Iron Gate. Photo: B. Novaković.

During 2014 extreme rainfall and floods were registered in Serbia, and 2017/2018 extreme drought. Water chemistry and biological samples were processed in the Sector of National Laboratory of the Serbian Environmental Protection Agency (SEPA). The .

SPEAR$_{pesticides}$ index is calculated using the ASTERICS software v. 4.04 (AQEM, 2002). A map of sampling stations is provided (Figure 1). One of the sampling stations, the Tekija, situated in the Danube Iron Gate, is shown in Figure 2.

Table 1: The SPEAR$_{pesticides}$ index values per year at nine sampling stations along the Danube River in Serbia. Values larger than the mean index are highlighted in bold blue indicating those analyzed stations and years which tend to have higher risk of pesticide exposure effects to non-target freshwater organisms (benthic invertebrates) than the remaining measurements in our study.

Station/Year	2014	2015	2016	2017	2018	2019
Bezdan	1.12	2.21	2.80	3.31	3.05	3.48
Bogojevo	1.81	2.87	2.77	3.46	3.82	3.65
Novi Sad	2.34	**7.21**	**8.26**	**7.68**	**8.63**	**7.12**
Zemun	2.56	4.97	3.08	4.37	5.09	4.21
Smederevo	3.02	**6.68**	**11.65**	**10.21**	**9.02**	**11.22**
Banatska Palanka	2.87	**8.21**	**12.54**	**14.15**	**11.21**	**12.34**
Tekija	0.00	1.02	1.81	2.82	3.84	3.03
Brza Palanka	4.36	**9.21**	**13.38**	**12.31**	**14.23**	**16.55**
Radujevac	3.80	5.56	**8.45**	**8.21**	**8.72**	**10.52**

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Additionally, measurements of the analyzed pesticides in surface water samples were provided to get more precise data on pesticide concentration of the Danube River water in the investigated period. Determination of the selected pesticides concentration in the extract of a surface water sample, without derivatization was done using the gas chromatography technique with a mass detector. For pesticide extraction the EPA Method 3535A: 2007 - Solid Phase Extraction-SPE was used. The pesticide analysis was done using the Agilent Technologies Gas Chromatograph 7890B/5977A MSD/µECD system hardware with PCU and MassHunter Software. The EPA Method 8270D: 2007 Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS) was used for pesticide determination. Solid Phase Extraction (SPE) - leakage a water sample through a Waters HLB 200 mg/6 mL column, pre-conditioned with methanol. After drying the column under vacuum, elute the analyses with methanol and concentrate, replacing the extract solvent with hexane. Analyses in the extract are separated by gas chromatography and identified and quantified on a mass spectrometer.

The following pesticide concentration were measured in surface water samples: metolachlor, an herbicide and represents a derivative of aniline, atrazine, terbuthylazine, desethylterbuthylazine, terbutryn-chlorinated herbicides from triazine class, and isoproturon, a selective, systemic herbicide used to control annual grasses and broadleaf weeds in cereals from class of phenylureas.

3 Results and discussion

The calculated SPEAR$_{pesticides}$ index values at sampling sites of the Danube River stretch in Serbia are provided (Table 1).

The annual mean of the pesticide concentration (sum over the selected six pesticides: metolachlor, atrazine, terbuthylazine, desethylterbuthylazine, terbutryn and isoproturon, in µgL^{-1}) are presented for the sampling stations of the Danube River stretch in Serbia in Figure 3. The highest pesticide concentration in surface water was detected at the Zemun and Smederevo sampling stations in 2016, and the Banatska Palanka/Danube River sampling station in 2014.

Since the SPEAR$_{pesticides}$ index has been primarily developed for small to medium streams, the main goal of the study is to test the use of the SPEAR$_{pesticides}$ index in large rivers (Schäfer et al., 2012; Hunt et al., 2017; Bighiu et al. 2020), as here the Danube River.

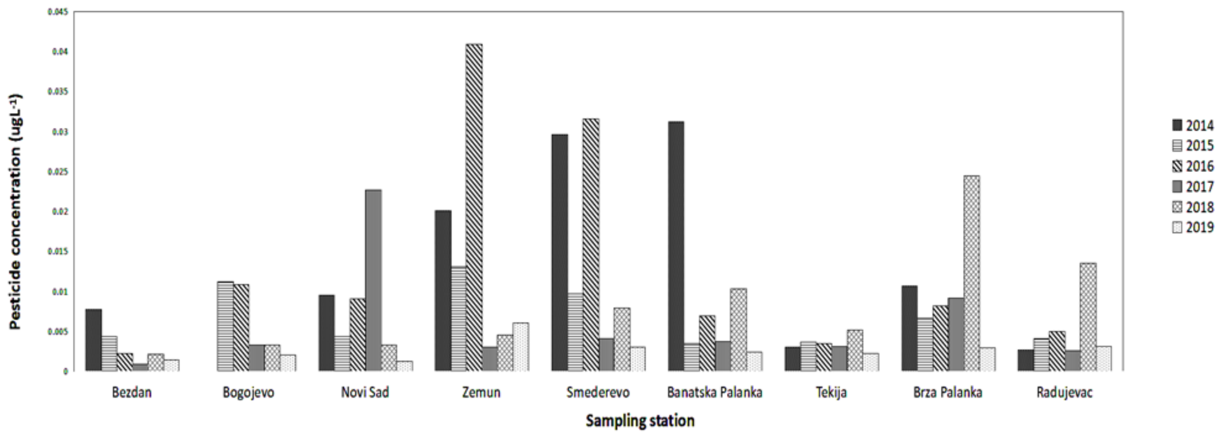

Figure 3: Mean annual concentration (µgL^{-1}) as sum over the most commonly detected pesticides at sampling stations along the Serbian Danube stretch during the six-year study period from 2014 to 2019.

Benthic invertebrate community composition and structure varied from the Danube River entering to Serbia (Bezdan) to its leaving (Radujevac). The lowest summed pesticide concentration, and also the lowest values of $SPEAR_{pesticides}$ index were found at the sampling stations Bezdan, Bogojevo and Tekija. Here, the benthic invertebrate community seems to mirror primarily the riverbed substrate structure, and is thus rather shaped by habitat condition than by pronounced pesticide stress. Habitat conditions are critical to provide shelter for animals of benthic invertebrate community, which mainly depend on hydromorphological conditions (e.g., hydrological connectivity and water velocity associated with wash out and species spreading effects, substrate characteristic of certain grain and pore size to balance between habitat stability and structural habitat turnover, e.g., Rico et al., 2016; Humpesch and Fesl, 2005; Fesl, 2002). Further, the balance between feeding strategist among benthic invertebrates sharing a habitat can be in response to substrate as well if using substrate as trophic source (Ihtimanska et al., 2014; to specify here: sandy fine sediments are essential for active filter feeders, Janecek et al., 2018; Marković et al., 2021).

Highest values of $SPEAR_{pesticides}$ index were found at the the stations Smederevo, the Banatska Palanka and the Brza Palanka (Table 1), which are associated with increased pesticide exposure as shown in Figure 3. Further, slightly evaluated summed up pesticide concentrations were confirmed by a moderate enhanced $SPEAR_{pesticides}$ index value. Thus, over the whole data range measured along the Serbian stretch of the Danube River, values of $SPEAR_{pesticides}$ index and of pesticide concentrations tend to correspond to each other. Hence, our study about $SPEAR_{pesticides}$ in a river ecosystem confirms the applicability of pesticide bioassessment developed for streams studies, which have shown that an exposure to elevated pesticide concentrations above a certain threshold affects benthic invertebrate composition (Hunt et al. 2017; Schäfer et al., 2012). In addition, lower $SPEAR_{pesticides}$ index values were calculated at sampling stations situated in the Danube River backwater zone, whilst the $SPEAR_{pesticides}$ index values were higher along the main course of the Danube River in Serbia. In this view, backwater provides shelter not only in view hydromorphological habitat conditions (less impact of floods and changed sedimentation regime, e.g., Brouder et al., 1999; Humpesch and Fesl, 2005; Janecek et al., 2018) but also reduces the stress of being exposed to pesticides according to our study. Beside differences among stations, which mirror local human impact and specific habitat conditions along river stretches, also the temporal coherence of weather situation was assessed during our six-year study. In year 2014 with extreme flood waves, lower $SPEAR_{pesticides}$ index values were calculated for all sampling stations, which was mainly due to very low benthic invertebrate diversity at all sampling stations. Obviously, hydrodynamic stress has an overwhelming effect here, shaping the community structure of benthic invertebrates against other influences. Such sensitivity shaping benthic invertebrate community against superimposed climate or weather effects, however, must not always become visible. In 2017/2018, at the majority of sampling stations, extreme drought did not affect benthic invertebrate diversity, and the $SPEAR_{pesticides}$ index, respectively.

4 Conclusions

Assessing the effect of pesticide exposure on benthic invertebrates by $SPEAR_{pesticides}$ index among sampling stations of the Danube River in Serbia along years, suits to identify both, the vulnerability of on-target biota against pesticides (1) at specific local habitat conditions, and (2) temporal coincidence with superimposed climate and weather driven changes, such as extreme flood events and draughts. Chemical water analyses revealed the highest pesticide concentration at the sampling stations Zemun and Smederevo in year 2016, which correspond to higher values of $SPEAR_{pesticides}$ index. In general, lower $SPEAR_{pesticides}$ index values were calculated at sampling stations situated in the Danube River backwater zone, whilst the $SPEAR_{pesticides}$ index values were higher along the main course of the Danube River in Serbia. Thus, our study provides evidence that the Danube River backwaters act as refugia against pesticide exposure supporting more tolerance benthic invertebrate taxa. A coincidence between some extreme events and sensitivity to pesticide exposure could also be found, but holds not true for all records along our six-year study. During 2014 extreme rainfall and floods were registered in Serbia affecting benthic invertebrate community composition and structure. Thus, in 2014 the $SPEAR_{pesticides}$ index values were lower as it is expected. As contrary, in

2017/2018, at the majority of sampling stations, extreme drought did not affect benthic invertebrate diversity, and the $SPEAR_{pesticides}$ index, respectively.

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