THE EFFECTS OF ELEVATED SUMMER TEMPERATURES ON CONTENT OF PESTICIDE RESIDUES IN SNR „OBEDSKA BARA“

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Abstract

A special nature reserve Obedska Bara is a world famous swamp area which has been, throughout its long history, a true home for plants and animals and a center of attention of many explorers, scientists and nature lovers. It appeared more than several thousand years ago and since then many things have changed. Given that Obedska Bara is surrounded with agricultural land, there is a possibility that the swamp could be polluted with pesticides. The aim of this study was to highlight the influence of elevated summer temperatures on the content of pesticide residues in SNR „Obedska Bara“. Sampling was carried out at the end of July and August 2017. Pesticide analysis of 90 pesticides was performed by using LC/MS-MS detection. The obtained recoveries were from 69.8 – 114.7% with the relative standard deviation of 2.28 – 11.7% for all investigated pesticides. The obtained limits of quantification - LOQs for all twenty-one investigated pesticides were 0.010 μg/L. The results of pesticide analysis in a water sample from Obedska Bara indicate the presence of 8 pesticides. Just one of them was detected at levels exceeding maximum allowable concentrations –MACs.

Introduction

Once a famous ornithological reserve and today a special nature reserve, Obedska Bara-forest complex is located along the river Sava in southern Srem, Vojvodina, Serbia. In the area of the reserve three-level protection regime is established on the total area of 9820 ha. Obedska bara has been known around the world since the mid-nineteenth century, since when the stories of it as of "paradise" for birds have been spreading. It was proclaimed the protected area in 1994, however the first data about its protection is “since 1874“. Thus, it is the oldest protected area in the country, and also one of the oldest natural resources in the world. "Golden Age" of Obedska swamp lasted throughout the 19th and early decades of the 20th century. Due to its extraordinary natural values, Obedska Bara is on the list of wetlands of the Ramsar Convention from 1977, as the first in our country, and it was declared in 1989 an internationally important bird area (IBA) as well. Obedska bara is known for the variety of wetland and forest habitats, many species of mammals, fish, amphibians, reptiles, insects and extreme wealth of flora, fish fauna and especially of bird fauna.

The degradation of water quality caused by an anthropogenic influence is a problem which is present even at some SNRs in the Province of Vojvodina, Serbia [1, 2]. The pollution problem of aquatic environments has become the main topic of discussion of many scientists and experts from the field of environmental protection. Unfortunately, this problem was noticed relatively late (at the end of 1980’s), after the chemicals in the water caused the disappearance of many aquatic organisms. The special attention is devoted to pesticides which can migrate to surface and groundwater, after the application to plants and soil [3]. Pesticides can get into the

water directly during treatments, by atmospheric precipitation, by desorption from aquatic organisms and sediments, by incorrect use of sprayers, when washing the machines and protective clothing after the pesticides application, and by waste packaging of pesticides [4]. When getting into the water, pesticides can be transformed by physical, chemical and biological processes. A large amount of toxic and persistent compounds can be bound to the solid matter and become an integral part of the sediment. The polluted sediment, due to resuspension, becomes a basic and permanent source of these pollutants and the largest potential source of water quality risks [5, 6].

Keeping in mind all above mentioned, the regular monitoring of pesticides presence in Obedska Bara is required.

Material and methods

Water sample collection and preservation. Water samples from Obedska Bara were collected at the end of July and August 2017, from two locations Obrež (N 44° 73' 421" E 19° 99' 061") and Kula (N 44° 73' 811 E 19° 99' 123"). The water sample was taken from the boat, at the central and ultimate positions of the swamp.

The water was collected in amber glass bottles (1 L) by plunging at the depth of 50 cm and closing with the lid under the water surface. The sample was transported to the laboratory in handy cool boxes and kept at 4 ºC until the analysis.

Pesticide analysis. Pesticide analysis of 90 pesticides was performed with an Agilent 1200 HPLC system equipped with a G1379B degasser, a G1312B binary pump, a G1367D autosampler and a G1316B column oven (Agilent Technologies, Waldbronn, Germany). Chromatographic resolution was achieved with Zorbax XDB C18 analytical column of 50×4.6 mm and 1.8 μm particle size (Agilent Technologies) maintained at 30 °C. The analytical separation was performed using a gradient program starting with 90% mobile phase B and progressing to 5% mobile phase B at 15 min, with methanol as mobile phase A, and water as mobile phase B, both containing 0.1% formic acid. The flow rate was maintained at 0.5 mL min^{-1}. The tandem mass spectrometry analysis was carried out with an Agilent 6410 Triple Quadrupole mass spectrometer equipped with an multimode source (Agilent Technologies, Palo Alto, CA, USA). The following ionization conditions were used: electrospray ionization (ESI^+) positive ion mode, drying gas (nitrogen) temperature 325 °C, drying gas flow rate 5 L/min, nebulizer pressure 40 psi and capillary voltage 3000 V. The dwell time was 100 ms. The data acquisition and quantification was conducted using MassHunter Workstation software B.03.01 (Agilent Technologies 2010). The method was validated according to SANTE/11945/2015 document. The limits of detection (LODs) were determined as the lowest concentration giving a response of three times the average baseline noise. The signal/noise ratio (S/N) in the obtained chromatograms for the LOD estimation was calculated by MassHunter Qualitative Software. LODs were defined as analyte peaks giving the S/N ratio of 3 extracted from the less intense (confirmation) MRM transition, calculated using an extract of Milli–Q water (250 mL) spiked at the 50 ng/L level. The linearity was checked using matrix matched standards (MMS) at concentrations from 10 to 200 ng/mL. The recovery was checked by enriching a blank sample (250 mL of tap water) with the mixture of pesticide standards of 10 μg/mL to get the final mass concentration of 20, 100 and 200 ng/L, with the addition of the internal standards carbofuran-D3, atrazine-D5 and isoproturon-D6 (mass concentration 10 μg/mL).

The sample preparation was performed with Bond ElutPlexa (60 mg, 3 mL) which were conditioned with 3 mL of methanol and 3 mL of HPLC - grade water. After conditioning, 250

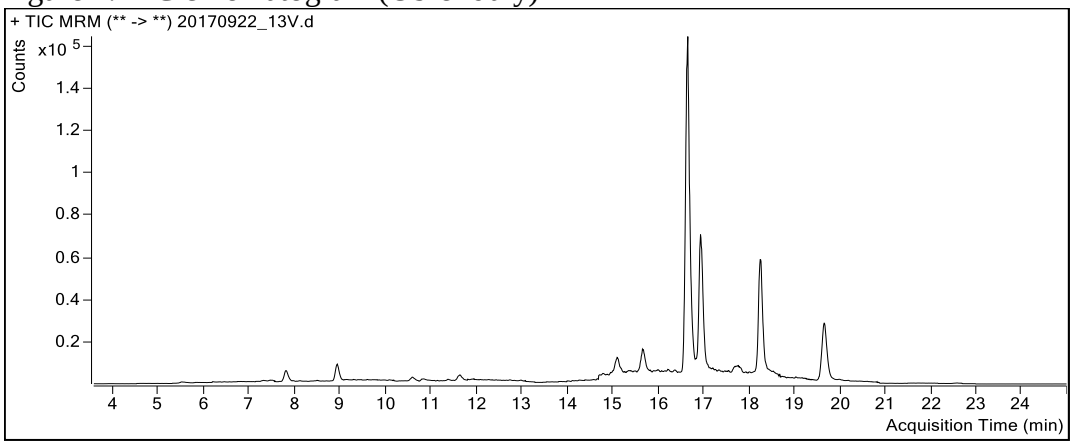
mL volume of water was enriched onto the cartridge with the flow rate settled between 3 and 10 mL/min. The cartridge was then flushed with 5 mL of HPLC - grade water. Pesticides were eluted from the sorbent with 5 mL of methanol and collected in the 10 mL amber glass vial. The solvent was evaporated under a gentle stream of nitrogen in a Techne-Dry block and the residue was dissolved in 0.25 mL of initial mobile phase composition. An extract volume of 10 µL was injected into the LC/MS-MS for the detection.

Results and discussion

The developed LC-MS/MS chromatographic procedure exhibits excellent linearity (R^2>0.99) in the 10 – 200 ng/mL range with satisfactorily precision (RSD<15%). Detection limits were defined for a ratio of S/N of 3 from the less intense (confirmation) MRM transition, calculated using a extract of Milli–Q water (250 mL) sample spiked at the 50 ng/L level. The accuracy and precision were determined via recovery experiments, spiking reagent water at 20, 100 and 200 ng/L, at six replicates per level. The obtained recoveries were from 69.8 – 114.7% with the relative standard deviation of 2.28 – 11.7% for all investigated pesticides.

The obtained limits of quantification - LOQs for all twenty-one investigated pesticides were 0.010 µg/L.

Figure 1. TIC chromatogram (Obrez July)



In Table 1. the concentration detected of the different pesticides are presented.

Table 1. Detected pesticide in the water samples (µg/L)

Pesticide	Kula (July)	Obrež (July)	Kula (August)	Obrež (August)
metamitron	0.009			
metolachlor	0.017	0.015	0.092	**0.134**
terbuthylazine	0.039	0.006	0.03	0.036
Desethyl-terbuthylazine	0.005	0.004		0.005
acetamipride	0.005	0.006	0.087	0.041
imidacloprid	0.038	0.01	0.006	0.008
klothianidin	0.004	0.004	0.001	0.002
thiamethoksam	0.012	0.028	0.003	0.006

Directive 2013/39/EC [8] brings us environmental quality standards (EQS) 21 pesticides. The presence in the environment of the pesticides that are not encompassed in this Directive is regulated by maximum allowable concentration (MAC) of 0.1 μg/L (sum 0.5 μg/L).

The results of pesticide analysis in a water sample from Obedska Bara indicate the presence of 8 pesticides, which are all detected in the sample Kula from July. Just one of them (*Table 1*) was detected at levels exceeding maximum allowable concentrations -MACs (0.1 μg/L) according to the Decision 495/2015/EC and Directive 2008/105/EC [9]. The highest concentration of pesticides (metolahlor 0.134 μg/L) was detected in the sample from Obrež sampled in August.

Conclusion

Elevated temperatures during summer months are able to increase a degree of degradation and volatilization of pesticides, but based to our findings and results we are not able to confirme that clames.

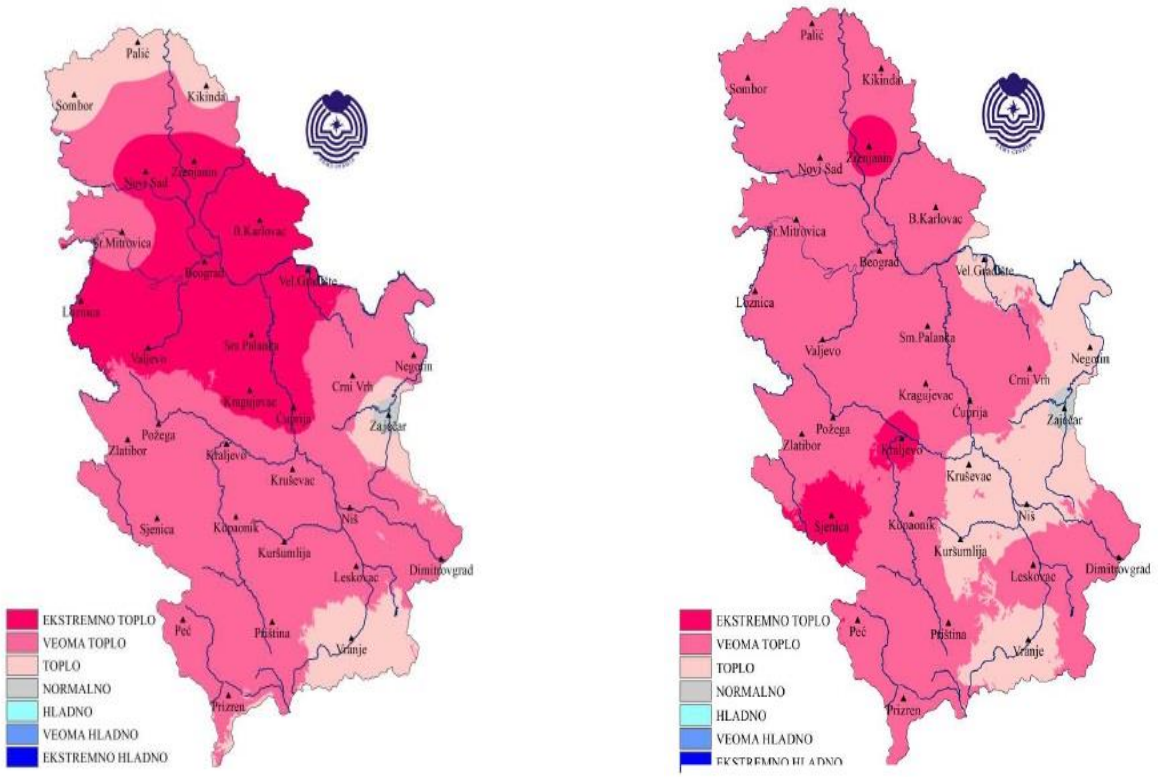


Figure 2. average temperatures during July (left) and August (right)

Acknowledgement

The authors acknowledge the financial support of the Ministry of Education and Science, Republic of Serbia, Project Ref. III 43005

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