THE USE OF INVASIVE SPECIES AS WATER CONTAMINATION BIOINDICATORS: *SINANODONTA WOODIANA* CASE

Aleksandra Petrović[1], Vojislava Bursić[1], Gorica Vuković[2], Dušan Marinković[1], Ivana Ivanović[1], Bojan Konstantinović[1], Nikola Puvača[3]

[1]*Department for Environmental and Plant Protection, Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Trg Dositeja Obradovića 8, Serbia*
[2]*Institute of Public Health of Belgrade, 11000 Belgrade, Bulevar despota Stefana 54A, Serbia*
[3]*Faculty of Economics and Engineering Management, University Business Academy, 21000 Novi Sad, Cvećarska 2, Serbia*
e-mail: petra@polj.uns.ac.rs

Abstract

Freshwater mussels are considered as the suitable bioindicators of ecosystems health, both, aquatic and surrounding terrestrial. These species have high filtration rates, providing an important link between the water column and the benthic zone, and also playing an important role in the nutrient cycling, substrate stability, bioturbation, and controlling the levels of suspended solids. The aim of this study is to obtain preliminary results of *Sinanodonta woodiana* use, as a potential bioindicator for pesticide residues detection in the river Danube. LC-MS/MS method indicated the qualitative and quantitative presence of six pesticide residues: chlorotoluron, diuron, linuron, metolahlor, terbuthylazine and acetamiprid in *S. woodiana* soft tissue. Biomonitoring with mussels has several potential applications. It may be used to complement or supplement the traditional water and sediment monitoring programs.

Introduction

The freshwater bodies, lakes and rivers in Europe, are under heavy contamination pressure by different organic and inorganic pollutants and contaminants. In order to preserve these aquatic habitats, the use of bioindicators and biomonitors is more than obligatory in monitoring programmes. Almost any organism could indicate the processes of the environment degradation, but some organisms perform this function much better than the others. The high-quality bioindicators should be sensible but tolerant, capable for bioaccumulation, widely distributed, easy to sample, identify and analyze.

Freshwater mussels are usually considered as the suitable bioindicators and biomonitors of ecosystems health, both, aquatic and surrounding terrestrial. These species have high filtration rates, providing an important link between the water column and the benthic zone, and also playing an important role in nutrient cycling, substrate stability, bioturbation, and controlling the levels of suspended solids. The abundance, diversity and species richness of freshwater mussels are severely decreasing worldwide, where nearly half of the species are thought to be currently threatened. Consequently, the logic question has arisen: if the mussels are excellent bioindicators, but most species are currently endangered, is it justify and economically effective to use invasive mussels, such as *Sinandonta (Anodonta) woodiana* (Lea, 1834) as the water contamination bioindicator?

S. woodiana, Chinese pond mussel, is a native species of East Asia and lives in a large area from the River Amur, through China to Cambodia. In the early 1970s, different species of carp fish, infested with glochidia, have been excessively imported to European countries for biological control of organic debris, freshwater plants and mosquitos. In this way, *S. woodiana* spread out and established stabile populations in almost all European countries, including some Indonesian islands, Dominican Republic, Costa Rica and recently USA [1]

[2]. In some countries, for example Italy, Chinese pond mussel was introduced intentionally, related to some commercial activity, such as the production of artificial pearls in Tuscany [3] and as a "bio filter" for garden ponds, commercially sold in some garden centers [4].

More than 140 billion kilograms of fertilizers and large quantities of pesticides are used and disposed annually in the agricultural sector, creating huge sources of diffuse pollution in freshwater ecosystems. The presence of these contaminants and pollutants in aquatic and terrestrial ecosystems has become a globally important issue. The river ecosystem is a subject to increased stress associated with diverse human activities. Modern agriculture contributes to the soil and water pollution, as it often involves the use of different pesticides, which results in a gradual increase of their residues and metabolites in the aquatic environment and consequently in aquatic organisms.

According to [5], the mussels may cumulate, without any distinct physiological effects, significant amounts of pollutants in both, soft tissues and shells. The amounts of the actual accumulation may not be indicative of real pollutant emissions only, but they also may indicate the mobilization and transfer within the trophic webs of the long-term pollutant accumulations that are contained in the aquatic ecosystems bottom sediments.

The aim of this study is to obtain preliminary results of *S. woodiana* use, as a potential bioindicator for pesticide residues detection in the river Danube.

Experimental

Mussel collection

The mussels were collected from two localities on the river Danube: Petrovaradin and Sremski Karlovci. The transect method was used for mussel sampling, along the 200 m of the coast and 5 m from the coast, at a depth of 0.10 to 1.5 m. The mussels were collected by hand or by sieving the sediment using a 1 m^2 mesh (diameter 25x25 mm). Individuals longer than 7 cm were separated and only *S. woodiana*, which is an invasive and allochthonous species. The mussels were washed in tap and distilled water, the shells were removed, and complete soft tissues were prepared for further analyses.

Pesticide residues detection

A validated LC-MS/MS method was used for qualitative and quantitative determination of pesticide content in the mussel soft tissue. The validation was done in accordance to SANTE/11813/2017 by triple quadrupole mass spectrometer (Agilent 6410B Triple Quad Mass Spectrometer, USA) in positive electrospray ionization using multiple reaction monitoring mode (MRM). The method was validated for accuracy, precision, linearity, limits of detection and limits of quantification (LODs and LOQs). The extracts were obtained using the acetonitrile-based QuEChERS preparation technique. The calibration was performed in MMC. The calibration range was from 0.01 to 0.5 μg/ml. The obtained R^2 was higher than 0.99 for all the studied pesticides. The LODs were belowe 0.005 mg/kg and the LOQs were set on 0.01 mg/kg. For the recovery, the samples were spiked with the analytes at three concentration levels (0.05, 0.1 and 0.2 mg/kg).

Results and discussion

The average recoveries for all analytes were in the range from 70.1 to 91.3% (RSDs 3.77 – 10.12%) (Table 1.). The obtained mean values of the responds were with RSD <20%. An efficient, sensitive and reliable LC–MS/MS(ESI), has been developed and applied for the detection of 26 pesticides in mussel soft tissue.

Table 1. Average recoveries

Pesticides	R^2	Average recoveries ± RSD (%)
Carbendazim	0.9941	80.7±3.77
Metribuzin	0.9990	91.2±6.42
Chloridazon	0.9998	89.4±5.71
Chlortoluron	0.9936	81.5±6.91
Chloroxuron	0.9995	90.8±5.17
Desethyl atrazine	0.9994	86.4±4.87
Sebutilazin	0.9990	87.2±10.12
Dimefuron	0.9993	80.4±8.26
Diuron	0.9998	72.5±5.83
Etidimuron	0.9998	98.8±7.13
Isoproturon	0.9997	70.1±9.11
Metobromuron	0.9996	72.4±6.47
Metamitron	0.9989	71.3±5.63
Metazachlor	0.9999	91.3±6.53
Methabenzthiazuron	0.9997	83.5±7.91
Metolachlor	0.9997	70.7±6.42
Propazine	0.9998	80.1±7.70
Simazine	0.9974	82.4±6.47
Terbuthylazine	0.9988	73.4±7.61
Terbuthylazine-desethyl	0.9996	82.7±5.17
Acetamiprid	0.9918	81.4±5.88
Imidacloprid	0.9997	82.2±4.90
Clothianidin	0.9926	89.1±8.26
Thiamethoxam	0.9990	86.7±5.51
Thiacloprid	0.9967	81.4±5.72
6-Chloronicotinic acid	0.9948	80.7±6.24

QuEChERS method indicated the qualitative and quantitative presence of six pesticide residues: chlorotoluron, diuron, linuron, metolahlor, terbuthylazine and acetamiprid in *S. woodiana* soft tissue (Table 2.).

Table 2. Pesticide residues in mussel soft tissues (mg/kg)

Pesticide residues	Locality	
	Petrovaradin	Sremski Karlovci
Chlorotoluron	0.013	0.016
Diuron	0.006	0.003
Linuron	0.002	0.002
Metolachlor	0.010	0.005
Terbuthylazine	0.003	0.003
Acetamiprid	0.003	-

Similarly to pesticide residues, the mussels' ability to accumulate heavy metals appears to be a taxonomic feature that depends on both, individual requirements for bio-elements and behavior, as well as on the related metabolic processes in a given environment [6]. Recorded differences in the pollutant concentrations of specific mussel species living in the same environment are the result of the selective elements uptake with food and the variable regulation of their concentration levels in soft tissues and shells [7].

S. woodiana represent an excellent bioindicator species, as it is easy for handling regarding the size (it could grow up to 30 cm) [8], short juvenile stage, as this species reproduces already in the first year of life, having reached the shell length of 3-4 cm [9] and long life span, as the individuals can live between 12 – 14 years [10]. The species is a habitat generalist found in heavily modified and artificial habitats, tolerant to high siltation rates [1] and prefers habitats with higher temperatures (the optimal thermal conditions vary within 10 and 35ºC) [11]. Larvae, glochidia are released from May to August (mainly in June and July), and this species can develop 2 – 3 larval stages per year, compared to the native species which reproduce once a year. Parasitic period lasts between 5 and 15 days, depending on the water temperature [10].

As spatial bioindicators and biomonitors, mussels are superior to fish, which are very mobile and in some cases migratory. Furthermore, as a monitoring medium, mussels are also superior to the sediment, because they can be more easily standardized. While the sources of biological variability in mussel samples can be controlled, the factors, such as particle size distribution and organic content, which strongly affect the contaminant adsorption to the sediments, cannot [12].

Biomonitoring with invasive mussel species has several potential applications. They may be used to complement or supplement the traditional water and sediment monitoring programs, principally to provide information on bioavailability, simultaneously preserving the native mussel species populations. Residues in mussels can also be used to define the impact zone of potential point source pollution and can serve as a feedback mechanism for determining the effectiveness of pollution control measures as they are implemented. These organisms constitute the major portion of the benthic biomass in many areas and they significantly modify the sediment by burrowing, respiratory and excretory activities, thus altering the profiles of contaminants in bottom sediment and contributing to their transformation [12].

Conclusion

European rivers are contaminated with pesticides and in almost half of the river basins, the levels of chemicals that can harm fish, invertebrates and algae have been measured. A significant improvement in water quality has been declared a goal for all EU Member States and provided by the provisions of the Water Framework Directive. However, new scientific research has shown that this goal was not achieved due to the high level of the toxic substances in the water. The Danube, with its fascinating ecosystems that provide from recreation and fishing to river drinking water for millions of people, are polluted by chemicals from cities, agriculture and industry. Such a chemical "cocktail" seriously damages aquatic organisms, but also poses a potential risk to humans, other animals and environment.

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