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ENVIRONMENT AND SUSTAINABLE DEVELOPMENT. MECHATRONICS. OCCUPATIONAL SAFETY AND HEALTH AND FIRE SAFETY. SMART MANAGEMENT SYSTEMS. GRAPHIC ENGINEERING. DESIGN. TRAFFIC ENGINEERING. BIOTECHNOLOGY AND HEALTHCARE. MECHANICAL ENGINEERING. ECOTOURISM AND RURAL DEVELOPMENT.

ECOLOGICAL RISK ASSESSMENT OF ASPARTAME AND NEOTAME IN RIVER SEDIMENTS

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Abstract: *Artificial sweeteners are high-priority emerging contaminants that are widespread in surface waters worldwide as a result of municipal wastewater discharges into the aquatic environment. Given that these substances can cause toxic effects on aquatic organisms, they represent a potential risk for the river ecosystem as a whole. Although sediments have not often been the subject of scientific research on pollution with artificial sweeteners, there is a possibility that these substances pose a risk to sediment-dwelling organisms. Aspartame and neotame are sweeteners that have a high sorption affinity for organic-rich particles and partition to sediment in the water-sediment system. In order to determine the possible risk for benthic biota, an ecological risk assessment of detected aspartame and neotame in sediments from five rivers in Serbia was performed. In addition to the risk assessment of individual sweeteners, "the cocktail effect" of their mixture was also estimated. Detected levels were compared with the lowest predicted no-effect concentration (PNEC) values for sediment organisms from the Norman Ecotoxicology Database, based on experimental ecotoxicity data and QSAR model predictions for aquatic organisms.*
Keywords: Artificial Sweeteners, Ecological Risk Assessment, River Sediments, Benthic Organisms

1. INTRODUCTION

Artificial sweeteners are metabolically inert, calorie-free sugar substitutes widely used in food and pharmaceutical products [1]. These substances enter the environment in unchanged form through sewage system and their presence in natural waters has been documented worldwide [2,3]. Some ecotoxicological research has shown that artificial sweeteners can cause toxic effects on aquatic organisms, affecting the growth, behaviour and metabolism [4,5]. For this reason and their unknown long-term environmental fate, they belong to a new class of high-priority emerging contaminants [2]. In scientific literature, ecological risk assessment based on the detected levels of these compounds in river ecosystems has been evaluated only for water organisms [3]. Since sediments represent a functional part of aquatic ecosystems, determination of toxicological risk on benthic biota is a necessary step in a comprehensive overview of river ecosystem contamination with artificial sweeteners.

In this paper, ecological risk of aspartame and neotame was estimated to sediment-dwelling organisms in five rivers in Serbia (the Sava, the Danube, the Tisza, the Morava and the Veliki Lug). These sweeteners were selected for analysis based on their specific physical and chemical properties (high log K_{ow} and log K_{oc} values and low water solubility) [6], with a high sorption affinity for organic-rich particles and partitioning to sediment in the water-sediment system. Detected

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concentrations were compared with the lowest predicted no-effect concentration (PNEC) values for sediment organisms obtained from the Norman Ecotoxicology Database, based on experimental ecotoxicity data and QSAR model predictions for aquatic organisms [7]. In addition to the risk assessment of individual sweeteners, "the cocktail effect" of their mixture was also evaluated. The instrumental analysis of the prepared extracts was performed using liquid chromatography with tandem mass spectrometry (LC–MS/MS), as sensitive and reliable method for the detection of traces of artificial sweeteners in complex environmental matrices.

2. EXPERIMENTAL PART

2.1 Chemicals and reagents

Aspartame and neotame, as high purity (≥ 98%) analytical standards, were purchased from Sigma-Aldrich (Buchs, Switzerland). These sweeteners are approved for use as food additives in the Republic of Serbia (Regulation No. 53/2018) [8]. The solvent methanol (HPLC grade) was obtained from J.T. Baker (Gliwice, Poland). Ammonium acetate was of analytical grade.

2.2 Sampling and sample preparation

Five sediments samples were collected from the following rivers in Serbia: the Sava (SED1), the Danube (SED2), the Tisza (SED3), the Morava (SED4) and the Veliki Lug (SED5). Approximately 1 kg of the sediments was collected in plastic containers and kept in a freezer. After defrosting, the sediment samples were air-dried at room temperature, for several days, in the dark. Samples were then homogenized and sieved through 500 μm pore size sieve. Sediments were prepared for analysis using optimized ultrasonic extraction (USE) method with methanol as extraction solvent. The detailed preparation procedure is shown in a previous study of Gvozdić et al. 2020 [6].

2.3 LC–MS/MS analysis

LC analysis was performed using a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA). Chromatographic separation of analytes was carried out on a Luna® C8 reverse-phase column, 150 mm × 3.0 mm i.d. and 3 μm particle size (Phenomenex, Torrance, USA). The mobile phase consisted of deionized water, methanol, and 0.1 mol L^{-1} aqueous solution of ammonium acetate. MS/MS analysis was performed using LTQ XL (Thermo Fisher Scientific) linear ion trap mass spectrometer, coupled with a HPLC system. Electrospray ionization (ESI) in negative mode was used as the ionization technique. LC–MS/MS operating parameters for the determination of aspartame and neotame were developed and optimized in a previous study [6].

2.4 Ecological risk assessment

Ecological risk to benthic organisms was evaluated based on risk quotients (RQs) calculated by dividing the measured environmental concentration (MEC) of selected sweetener with the corresponding predicted no-effect concentration (PNEC) for sediment organisms [3]. The PNEC values used in the study were obtained from the Norman Ecotoxicology Database as the lowest PNECs, based on experimental ecotoxicity data or predicted by QSAR (quantitative structure–activity relationship) analysis for freshwater organisms [7]. The lowest PNECs for sediment organisms ($PNEC_{sed}$) were calculated using the following equation (Eq. (1)):

$$PNEC_{sed} = PNEC_{freshwater} \times 2.6 \times (0.615 + 0.019 \times K_{oc}) \quad (1)$$

Ecological risk to benthic organisms is classified into four categories: "negligible/no risk" (RQ < 0.01), "low risk" (0.01 < RQ < 0.1), "moderate/medium risk" (0.1 < RQ < 1), and "high risk" (RQ ≥

1) [3,9]. In order to evaluate "cocktail effect" of the mixture of artificial sweeteners detected in each sediment sample, the RQ_{mix} was calculated, based on the concentration-addition method for risk assessment of mixtures, as the sum of the RQ values obtained from the individual sweetener concentrations (Eq. (2), [9]):

$$RQ_{mix} = \sum_{i=1}^{n} RQ_i \quad (2)$$

3. RESULTS AND DISCUSSION

Aspartame and neotame were found in all river sediment samples. The high detected levels of aspartame (Table 1) reflect high consumption of this sweetener in the human diet in Serbia. The highest concentrations of aspartame detected in rivers the Sava (sample SED1, 568 ng/g) and small river the Veliki Lug (sample SED5, 355 ng/g) point to their high contamination by municipal wastewater. On the other hand, neotame was recorded at significantly lower levels which can be explained by the lower use in food and beverages of this relatively new artificial sweetener. Neotame was found at the highest concentrations in the Veliki Lug River (sample SED5, 56 ng/g) and the Danube (sample SED2, 48 ng/g) indicating high sewage input in these water bodies. In predominantly agricultural regions of sampling sites on rivers the Tisza and the Morava (samples SED3 and SED4), levels of neotame were significantly lower (2 and 6 ng/g, respectively).

Table 1. Ecological risk assessment of aspartame and neotame for sediment-dwelling organisms detected in sediment samples of five rivers in Serbia

River	Sample	Aspartame (ng/g)	RQ*	Ecological risk	Neotame (ng/g)	RQ*	Ecological risk
Sava	SED1	568 ± 71	1.47	high risk	12 ± 3	0.57	medium risk
Danube	SED2	117 ± 20	0.30	medium risk	48 ± 9	2.30	high risk
Tisza	SED3	210 ± 31	0.54	medium risk	2 ± 0	0.09	low risk
Morava	SED4	184 ± 16	0.47	medium risk	6 ± 1	0.29	medium risk
Veliki Lug	SED5	355 ± 42	0.92	medium risk	56 ± 10	2.68	high risk

*$PNEC_{sed}$ values (385 μg/kg for aspartame and 20.9 μg/kg for neotame)

Ecological risk assessment based on the detected levels of aspartame and neotame in five rivers in Serbia indicates low ($0.01 < RQ < 0.1$) to a high risk ($RQ \geq 1$) for sediment-dwelling organisms. According to the $PNEC_{sed}$ value for aspartame (385 μg/kg), noticeably high environmental risk ($RQ \geq 1$) was recorded in the Sava River (sample SED1). The RQ values for aspartame in other investigated rivers in Serbia were ranging from 0.30 to 0.92, implying medium risk ($0.1 < RQ < 1$) for benthic biota.

As for neotame, taking into account its $PNEC_{sed}$ value (20.9 μg/kg), high ecotoxicological risk was observed in rivers the Veliki Lug and the Danube, while detected levels in the Sava and the Morava pointed to medium ecological risk. Low risk was determined for sediment from the Tisza, probably as a consequence of lower wastewater burden. In addition, according to the mixture risk approach, calculated RQ_{mix} have shown that the "cocktail effect" of detected sweeteners should be taken into account when assessing the risk to environment. The most pronounced "cocktail effect" was observed for the rivers the Veliki Lug ($RQ_{mix} = 3.60$) and the Danube ($RQ_{mix} = 2.60$), both heavily impacted by untreated municipal wastewater (Figure 1).

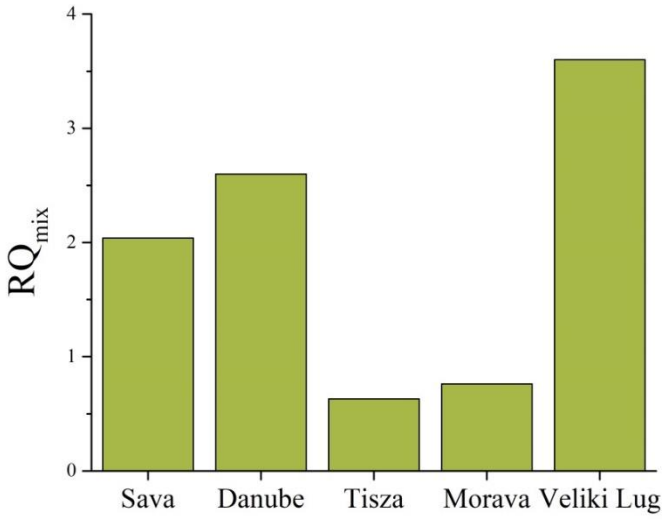

Figure 1. The risk quotients for a mixture (RQ_{mix}) of artificial sweeteners detected in sediments of five rivers in Serbia

The obtained results clearly show that sediments of river ecosystems in Serbia are of great concern in terms of detected artificial sweeteners. Because sediments are a major sink of organic pollutants, they can be a secondary source of pollution in the case of resuspension. Since wastewater treatment in Serbia is generally rare, continuous accumulation of these contaminants in sediments is inevitable. Therefore, investments in the construction of municipal wastewater treatment plants and introduction of new treatment processes, as well as stricter control of sewage pollution in Serbia are necessary.

4. CONCLUSION

The levels of aspartame and neotame detected in rivers the Sava, the Danube, the Tisza, the Morava and the Veliki Lug indicate a significant ecological risk for benthic organisms. The highest ecotoxicological risk was recorded in the Veliki Lug, the Danube and the Sava, as a result of extensive municipal wastewater discharges. This was determined according to risk quotients of the mixture of the two detected artificial sweeteners. The ecological risk was less pronounced in the agricultural regions of the Tisza and the Morava Rivers. The results presented in this study clearly show that untreated wastewater discharges in Serbia affect the quality of river ecosystems and threaten the benthic biota. The estimated high risk to the aquatic environment requires the improvement of municipal wastewater treatment and management and the inclusion of artificial sweeteners in routine monitoring programs.

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