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VEŠTAČKI ZASLAĐIVAČI ACESULFAM I SUKRALOZA: OD SASTOJAKA OTPADNE VODE DO ZAGAĐUJUĆIH MATERIJA PODZEMNIH VODA

ARTIFICIAL SWEETENERS ACESULFAME AND SUCRALOSE: FROM WASTEWATER CONSTITUENTS TO GROUNDWATER CONTAMINANTS

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Acesulfam i sukraloza su nenutritivni zaslađivači koji se široko koriste kao zamene za šećer u hrani, piću i farmaceutskim proizvodima. Kao metabolički stabilna jedinjenja, oni su sveprisutni sastojci komunalnih otpadnih voda koje se na kraju ispuštaju u vodenu sredinu. Oni pripadaju novoj klasi emergentnih zagađujućih materija u životnoj sredini sa potencijalno štetnim efektima po vodene organizme. Acesulfam i sukraloza se ne uklanjaju u tretmanima otpadnih voda, otporni su na degradaciju u životnoj sredini, zbog čega se smatraju idealnim indikatorima kontaminacije kanalizacionim vodama. U cilju procene uticaja ispuštanja neprečišćenih otpadnih voda na kvalitet vode za piće u beogradskom vodoizvorištu, ispitivane su koncentracije veštačkih zaslađivača u kanalizacionim, prijemnim rečnim i odgovarajućim podzemnim vodama. Uzorci su prikupljeni iz tri kanalizaciona ispusta u Beogradu, zajedno sa tri uzorka prijemne rečne vode iz Save i Dunava. S obzirom na potencijalnu mobilnost dva zaslađivača kroz akvifer, uzeta su i dva uzorka podzemnih voda. Svi uzorci vode su ekstrahovani i analizirani pomoću metode tečne hromatografije sa tandem masenom spektrometrijom. Koristeći određene koncentracije dva zaslađivača u rečnim i podzemnim vodama, kao i predviđene koncentracije bez efekta (PNEC) za vodene organizme, izvršena je procena ekološkog rizika. Rezultati su pokazali visoke koncentracije acesulfama i sukraloze u svim uzorcima otpadnih voda (2786–7488 ng L^{-1} i 1836–4756 ng L^{-1}, respektivno), obilnost u svim uzorcima rečne vode (51–76 ng L^{-1} i 68–206 ng L^{-1}) i značajne nivoe u uzorcima podzemnih voda (20 i 73 ng L^{-1}, i 17 i 25 ng L^{-1}). Takođe je utvrđeno da koncentracije dva zaslađivača u rečnim i podzemnim vodama ne predstavljaju ekotoksikološki rizik za vodene organizme. Međutim, samo prisustvo sastojaka otpadnih voda u podzemnim vodama izaziva zabrinutost za kvalitet izvora vode za piće.

Ključne reči: *acesulfam; sukraloza; kanalizaciono zagađenje; rečna voda; podzemna voda; procena ekološkog rizika*

Acesulfame and sucralose are non-nutritive sweeteners widely used as sugar substitutes in food, beverages, and pharmaceuticals. As metabolically stable compounds, they are ubiquitous constituents of municipal wastewaters ultimately discharged into the aquatic environment. They belong to a

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new class of emerging environmental contaminants with potentially harmful effects on aquatic organisms. Acesulfame and sucralose are not removed in wastewater treatment, are resistant to degradation in the environment and, therefore, regarded as ideal indicators of sewage contamination. In order to assess the impact of untreated wastewater discharge on the quality of drinking water sources in Belgrade, the concentrations of artificial sweeteners acesulfame and sucralose in sewage wastewater, receiving river water and corresponding groundwater were investigated. The samples were collected from three sewage canals in Belgrade, along with three samples of receiving water from the Danube and the Sava rivers. Considering the potential mobility of the two sweeteners through the aquifer, two groundwater samples were also taken. All water samples were extracted and analyzed by liquid chromatography–tandem mass spectrometry method. Using the measured concentrations of two sweeteners in river and groundwater, as well as predicted no-effect concentrations (PNEC) for aquatic organisms, ecological risk assessment was performed. The results showed high concentrations of acesulfame and sucralose in all wastewater samples (2786–7488 ng L^{-1} and 1836–4756 ng L^{-1}, respectively), abundance in all river water samples (51–76 ng L^{-1} and 68–206 ng L^{-1}) and significant levels in groundwater samples (20 and 73 ng L^{-1}, and 17 and 25 ng L^{-1}). It was also determined that concentrations of the two sweeteners in river and groundwater do not pose ecotoxicological risk to aquatic organisms. However, the mere presence of wastewater constituents in groundwater raises concerns about the quality of drinking water sources.

Key words: *acesulfame; sucralose; sewage contamination; river water; groundwater; ecological risk assessment*

1. Introduction

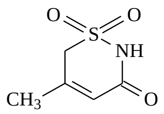
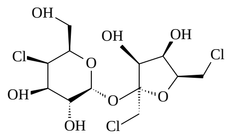
Acesulfame and sucralose are synthetic sugar substitutes widely used in food, beverages and pharmaceutical products. Since these compounds have no caloric value, they are also called non-nutritive sweeteners and are common ingredients in diet products [1]. After ingestion, acesulfame and sucralose are not metabolized in the human body and are excreted unchanged [2]. As a consequence, after the release of municipal wastewater into the environment, these sweeteners are widespread contaminants of natural waters [1,3,4]. Due to high stability in wastewater treatment, they are good indicators of municipal wastewater pollution, with half-life resulting in accumulation in various environmental matrices [3,5]. Some ecotoxicological studies have confirmed their harmful effect, affecting behavior and metabolism of aquatic organisms [6,7]. It was also established that upon prolonged exposure to intense solar irradiation, the photodegradation products of acesulfame are 500 times more toxic to aquatic organisms than the starting compound [8]. Due to the limited data on ecological risks and potential danger to aquatic ecosystem, artificial sweeteners are a newly recognized class of emerging pollutants [1].

In this paper, in order to assess the impact of untreated wastewater discharge on the quality of drinking water sources in Belgrade, the concentrations of artificial sweeteners acesulfame and sucralose in sewage wastewater, receiving river water and corresponding groundwater were investigated. The samples were collected from three sewage canals in Belgrade, along with three samples of receiving water from the Danube and the Sava rivers. Considering the potential mobility of the two sweeteners through the aquifer, two groundwater samples were also taken. All water samples were extracted and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Using the measured concentrations of two sweeteners in river and groundwater, as well as predicted no-effect concentrations (PNEC) for aquatic organisms, ecological risk assessment was performed.

2. Materials and methods

Analytical standard of acesulfame was purchased from Sigma-Aldrich (Buchs, Switzerland), while sucralose was acquired from TCI Europe (Zwijndrecht, Belgium). Methanol was HPLC grade, obtained from Sigma-Aldrich. Chemical structures and physicochemical properties of acesulfame and sucralose are shown in Table 1.

Table 1. Chemical structures and physicochemical properties of the two artificial sweeteners: molecular weight (M_w), octanol-water partitioning coefficient (K_{ow}), and water solubility at 25 ^{o}C (WS)

Artificial sweeteners	*Chemical structure*	M_w (g mol^{-1})	^{a}log K_{ow}	^{a}WS (mg L^{-1})
Acesulfame		163.1	–1.33	9.1×10^5
Sucralose		397.6	–1.00	2.1×10^4

[a] Source: ChemSpider database; http://www.chemspider.com/

Wastewater samples were collected from three sewage canals in Belgrade, two that discharge into the Danube River (samples WW1 and WW2) and one that discharges into the Sava River (sample WW3). Three corresponding receiving water samples from the Danube River (samples RW1 and sample RW2) and the Sava River (sample RW3) were collected downstream from the discharge. Two groundwater samples were collected from the observation well on the bank of the Danube downstream from Belgrade (sample GW1), and the Ranney well constructed on the bank of the Sava in Belgrade (sample GW2). All water samples were filtered through 1–3-μm glass fiber filter (Whatman GmbH, Dassel, Germany) prior to extraction.

The optimized solid-phase extraction (SPE) method [9] was used as sample preparation method to isolate and preconcentrate the sweeteners. The SPE protocol was comprised of the following steps: a water sample (50 mL) with pH adjusted to 3.0 was loaded onto Oasis HLB cartridge (200 mg/6 mL; Waters, Milford, USA). The cartridge packing was then eluted with 10 mL of methanol. The obtained extract was evaporated under nitrogen stream to a volume of 0.5 mL, filtered through 0.45 μm PVDF filter (Roth, Karlsruhe, Germany) into a glass vial and analyzed. The instrumental analysis of the prepared samples was performed using LC–MS/MS method. LC and MS operating parameters for the determination of acesulfame and sucralose were previously optimized [9]. LC analysis was carried out using a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA). Chromatographic separation of analytes was performed 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. As the ionization technique, electrospray ionization (ESI) in negative mode was applied.

In order to assess the ecotoxicological risk to aquatic organisms, risk quotients (RQs) were calculated by dividing the measured environmental concentration (MEC) of acesulfame and sucralose with the corresponding PNEC values for freshwater organisms [4], obtained from the Norman Ecotoxicology Database [10].

3. Results and discussion

LC–MS/MS analysis of wastewater from three sewage canals in Belgrade showed wide presence of acesulfame (up to 7488 ng L^{-1}) and sucralose (up to 4756 ng L^{-1}) in all analyzed samples (Table 2). The highest levels were detected in the sample WW2, which was taken from the largest sewage canal that discharges into the Danube, with 164,653 inhabitants connected to wastewater canal. The recorded concentrations confirm extensive application of these substances in food products in the Republic of Serbia and the metabolic inertness in the human body. However, in other European countries, higher levels of these compounds were found in sewage wastewater (up to 43 μg L^{-1} in Switzerland for acesulfame [5] and up to 60 μg L^{-1} in Spain for sucralose [11]) reflecting much larger population and different consumption patterns of the sweeteners.

Acesulfame and sucralose were also detected in all receiving river water samples (51–76 ng L^{-1} and 68–206 ng L^{-1}, respectively). The highest concentrations were recorded in the Danube River (sample RW2), after major sewage canal (WW2). It is evident that the city of Belgrade is heavily affected by municipal wastewater discharges due to the absence of a wastewater treatment plant (WWTP) and a high population density. In the study of the Danube River in Germany, acesulfame was found to be the dominant sweetener, detected at 730 ng L^{-1}, while sucralose was found at much lower concentrations (20 ng L^{-1}) suggesting lower use in food products [12]. Since acesulfame and sucralose are persistent and show limited removal in WWTPs, these levels indicate the high effluent burden in highly populated areas and different consumption patterns.

The two artificial sweeteners were also detected in both groundwater samples (Table 2), indicating sewage contamination of drinking water sources and a pronounced mobility through aquifer. These results can be explained by their high water solubility and low log K_{ow} values (Table 1). The higher levels of sweeteners recorded in the observation well located on the bank of the Danube (sample GW1) could be explained by the fact that the alluvium of the aquifer is very homogeneous and pollutants are more easily transported to the well, even at greater distances from the river. On the other hand, the alluvium on the bank of the Sava River in Belgrade is characterized by a high heterogeneity of the aquifer, with several semi-permeable layers. For this reason, it takes a long time for pollutants to pass through the aquifer to Ranney well located in the close vicinity to the river course (sample GW2) [13]. The widespread occurrence of acesulfame was also determined in groundwater samples in Switzerland (up to 524 ng L^{-1}) where aquifers are substantially influenced by infiltration of river water contaminated with wastewater effluents [5].

Table 2. Concentrations of detected artificial sweeteners in wastewater (WW), river water (RW) and groundwater (GW) samples

Artificial sweeteners	*Wastewater*			*River water*			*Groundwater*	
	Concentration (ng L–1)							
	WW1	*WW2*	*WW3*	*RW1*	*RW2*	*RW3*	*GW1*	*GW2*
Acesulfame	2786	7488	5383	75	76	51	73	20
Sucralose	1836	4756	4039	68	206	111	25	17

The results of the ecological risk assessment of artificial sweeteners detected in river water and groundwater are presented in Table 3. It was determined that the concentrations of acesulfame and sucralose recorded in the Danube and the Sava rivers, as well as in the groundwater samples do not pose a risk for aquatic organisms (RQ < 0.01). However, there is a possibility of increasing the eco-toxicological risk during the summer period when very high concentrations of these compounds could be recorded at high daily temperatures and lower water levels. Furthermore, the mixture of different water contaminants can pose a complex environmental risk associated with synergic and additive effects [4].

*Table 3. Ecological risk assessment of artificial sweeteners detected in river water (RW) and groundwater (GW) samples with *PNEC values for freshwater organisms from the NORMAN Ecotoxicology Database [10]*

*Artificial sweeteners *PNEC*	*Risk quotient (RQ)*				
	River water			*Groundwater*	
	RW1	*RW2*	*RW3*	*GW1*	*GW2*
Acesulfame *72,4 µg L^{-1}	0.001 (no risk)	0.001 (no risk)	0.0007 (no risk)	0.001 (no risk)	0.0003 (no risk)
Sucralose *29,7 µg L^{-1}	0.003 (no risk)	0.007 (no risk)	0.004 (no risk)	0.0008 (no risk)	0.0006 (no risk)

4. Conclusion

Acesulfame and sucralose were detected in all investigated water samples indicating their high consumption in the Republic of Serbia and continuous contamination of the Danube and the Sava rivers as a result of the discharge of untreated wastewater. Furthermore, these compounds show significant mobility through aquifer, reaching the groundwater, and their presence in groundwater wells indicate sewage contamination of the drinking water sources in Belgrade. The obtained data point to the need of introduction of these substances into routine monitoring programs and necessity of the construction of wastewater treatment plant in Belgrade. In terms of ecotoxicological risk, the results showed that aquatic organisms are not at risk from detected sweeteners. However, artificial sweeteners can become a part of complex mixture of emerging contaminants that jointly can cause harmful effects on the aquatic biota, which requires more detailed ecotoxicological research.

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