

# INVESTIGATIONS OF THE PRESENCE OF ANTHROPOGENIC MARKER FOR WASTEWATER CONTAMINATION OF THE DANUBE

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## INTRODUCTION AND OBJECTIVES

Caffeine is a purine alkaloid found in more than 60 plant species (coffee seeds, cocoa and teas). It is mostly used in the production of food (80%), medicines (16%), and cosmetic products (4%). It is an integral part of various drinks (coffee, tea, caffeinated soft drinks), certain food products (chocolate) and medicines. Caffeine is found to be a good indicator for human sewage because of its unambiguous anthropogenic origin. The main paths for caffeine to enter wastewater stream are either in urine or when caffeine-containing products are discharged through household pipelines or sewers.

The main goal of this study is to determine the presence of caffeine in the Danube samples as an anthropogenic marker for wastewater contamination of the Danube.



Caffeine

## MATERIALS AND METHODS

Analysis was performed by solid-phase extraction (SPE) followed by reversed phase high performance liquid chromatography (HPLC). The chromatography used a Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, i.d., 5 µm particle size) at 25°C, with a mobile phase of water/THF (0.1 % THF in water, pH 8) – acetonitrile (85:15, v/v). The flow rate was 0.9 mL/min, and detection by DAD at 273 nm. The samples were collected during September 2019 at ten representative locations of the Danube on the territory of Novi Sad, Serbia, and stored in amber bottles at 4 °C until analysis. Maximum Risk Indexes (MaxRIs) for each sampling site were calculated.

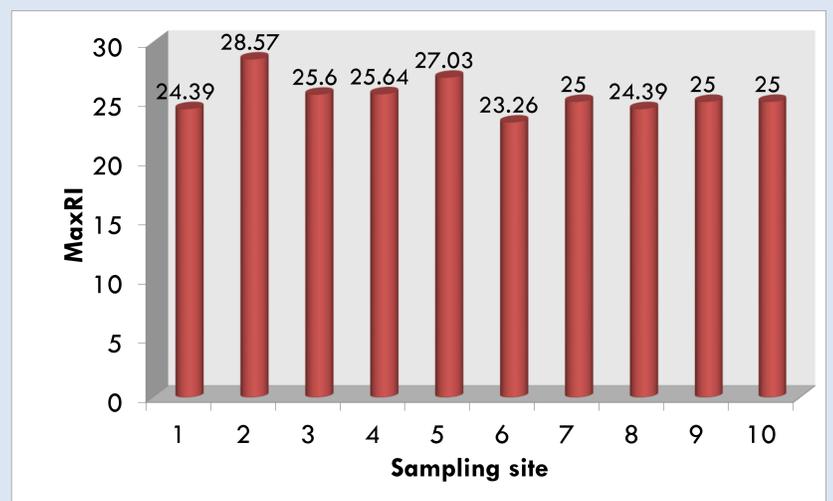
## RESULTS AND DISCUSSIONS

No.	Sampling site	AUC (mAU*s)	Caffeine (ng/L)
1	Ratno ostrvo US	258,43	361,1±0,04
2	Ratno ostrvo DS	189,00	305,94±0,07
3	Beogradski kej US	233,95	341,63±0,08
4	Beogradski kej DS	214,23	325,97±0,04
5	Cepelin US	208,40	321,34±0,06
6	Cepelin DS	277,19	375,97±0,08
7	Štrand US	238,98	345,62±0,04
8	Štrand DS	255,62	358,84±0,05
9	DTD US	243,59	349,29±0,06
10	DTD DS	250,08	354,45±0,05

\*US – upstreams

DS – downstreams

MaxRI – maximum risk index



The levels of potential risk for the river system were divided into three classes: Class I or high risk with  $MaxRI < 10$ ; Class II or sublethal effects on the aquatic organisms with  $10 < MaxRI < 100$ , and Class III or low risk with  $MaxRI > 100$ . Maximum risk indexes (MaxRIs) for resident organisms (fish) in the Danube were calculated for each sampling site and the results showed that all MaxRIs belong to class II ( $10 < MaxRI < 100$ ).

## CONCLUSIONS

The presence of caffeine, which has no natural non-human sources, confirmed the existence of human waste in the Danube. Highest caffeine concentration was detected in the sampling site No.6 (Cepelin DS). The results obtained for MaxRIs indicated that the potential risk for the chronic effects may occur in the aquatic organisms in the long-term period.