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INFLUENCE OF TOWN WASTEWATERS AND AGRICULTURAL ACTIVITIES ON WATER GENOTOXICITY DURING DIFFERENT RIVER LEVEL REGIMES

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ABSTRACT

Water, being of critical importance for the survival of life on the planet, requires our permanent care and attention. There is a growing concern about the genotoxicity of complex environmental mixtures in surface waters, due to the risk of genetic damage and cancer, both in aquatic organisms and humans. The present study focuses on exploring the status of water pollution in areas with combined industrial and agricultural activities in order to estimate the magnitude of toxicity and genotoxicity using the *Allium* test. We collected samples from the Sava and Danube rivers, upstream and downstream of Šabac (Sava River) and Smederevo (Danube River). In both rivers sampling was done in periods of low and high-water levels. Levels of toxicity were low in both rivers. However, the presence of organic pollution, observed as a higher mean length of the roots, was seen in all sample groups compared to negative control. The highest values of genotoxicity at locations upstream of both Šabac and Smederevo were obtained in samples collected in a period of high-water level, but only the upstream sample from the Sava River had reached a significant level of genotoxicity. This can be considered as a consequence of intensive agricultural activities. Our results indicate that communal town and industrial wastewaters influence river water quality more significantly during low level regimes, while high water levels increase the risk of exposure to chemicals used in agriculture.

Keywords: *Allium* test, genotoxicity, Sava River, Danube River, town wastewaters, agriculture pollution

INTRODUCTION

Nowadays, when it comes to water, the term clean water crisis is increasingly used. It is becoming gradually more apparent that the amount of healthy drinking water is decreasing in the face of the constant increase of the human population and furthermore due to the increase in water consumption brought by a rise in standards.

Considering how limited the amount of drinking water is and how important the water is per se, it is of paramount importance to know not only what pollutes the water, but also what the consequences for the consumers of that water and their offspring might be (SCHWARZENBACH *et al*., 2010). In the past, because of its vast surface area and volume, people have treated the aquatic environment as a convenient place for the disposal of biological and technical waste. However, it has now become apparent that the deposition of toxicants into aquatic ecosystems has led to those toxicants accumulation in both sediments and upstream food chains (SONSTEGARD, 1977; STATHAM *et al*., 1976).

Rivers and lakes cover approximately only 2.3% of the global land surface area on Earth (LEHNER and DOLL, 2004). Thus, actual water quality management requires a clear understanding of how and why water quality differs across space, both within and between different river catchments. Freshwaters are exposed to multiple persistent and evolving threats (REID *et al*., 2019). RICCIARDI and RASMUSSEN (1999) estimated that in North America, 123 species of freshwater animals are near extinction and that the extinction rate in the aquatic system is five-fold higher than on land reaching 4% per decade. According to HE *et al*., (2019) freshwater megafauna populations declined by 88% from 1970 to 2012. Such a high rate of species extinction in aquatic ecosystems is likely caused by pollution originating from activities occurring in the terrestrial environment as well as from changes in artificially induced river flows. More than one third of rivers in the United States (BERNHARDT *et al*., 2005) and even more than 45% of rivers in China (VOROSMARTY *et al*., 2010) are judged as polluted.

Water pollutants come from three main sources: human and domestic waste (largely biodegradable organic products), industrial waste (mainly toxic waste that readily accumulates in the food chain) and agricultural chemicals (fertilizers and pesticides) that are used to obtain high yields. Many substances that enter the water and air as by-products of industrial production, agriculture, or fossil fuel combustion are potential mutagens and/or carcinogens. Water pollution problems vary widely across regions of the world. In general, agricultural pollution is the least resolved problem.

Industrial and urban wastewater is generally characterized by the presence of extremely complex mixtures containing numerous inorganic and organic compounds. This complexity makes it very difficult to chemically assess the danger posed by polluted water. In these situations, different variants of the *Allium* test are well suited for assessing the toxicity and potential genotoxicity of different water samples (RANK and NIELSEN, 1993; VUJOŠEVIĆ *et al*., 2001; VUJOŠEVIĆ *et al*., 2008; LEME and MARTIN-MORLAES, 2009; RADIĆ *et al*., 2010).

It can generally be said that the state of waters in Serbia is insufficiently known (ABORGIBA *et al*., 2016; KOSTIĆ *et al*., 2017). Regular bacteriological and chemical control gives insufficient data on real pollution level. The study of pollution of the Sava and Danube Rivers in the Belgrade region, as the area of their confluence, showed how different chemical analyses can produce different assessments of the river quality and lead to potential misinterpretation of pollution levels (ANTONIJEVIĆ *et al*., 2014).

The present study was motivated by the need for a preliminary estimation of the water quality of two rivers, Sava and Danube, upstream and downstream of the towns Šabac and Smederevo during different water levels. The toxic and genotoxic potential of the water samples was assessed using *Allium* anaphase-telophase test.

MATERIALS AND METHODS

Two samplings were done independently on the Sava and Danube rivers, upstream and downstream of Šabac (Sava River) and Smederevo (Danube River). Samples of surface water from both rivers were collected upstream (loc 1) and downstream (loc 2) from the towns and analysed using the *Allium* anaphase-telophase test. Differences in the degree of pollution between loc1 and loc 2 of each river were considered to be a contribution of urban wastewater. On the Sava River loc 1 was at 44.792769° 19.691358° and loc 2 at 44.745365° 19.762664° (Figure 1a). On the Danube River loc 1 was at 44.658590°, 20.869184° and loc 2 at 44.694314° 20.959865° (Figure 1b). Distances between loc 1 and loc 2 were approximately 10 km in both cases. Sampling was done in periods of low (October/November 2017) and high-water levels (March/April 2018). According to the Republic Hydrometeorological Service of Serbia (http://www.hidmet.gov.rs/eng/hidrologija/index.php) water levels during the first sampling were -68 cm at Sava River and 466 cm at Danube River, while at the second sampling levels were 529 cm and 618 cm, respectively.

a

Figure 1. Locations upstream (loc 1) and downstream (loc 2) of towns: (a) Šabac - Sava River and (b) Smederevo – Danube River.

Following modified procedure for *Allium* anaphase-telophase test (RANK and NIELSEN, 1993) commercial onion bulbs of *Allium cepa*, weighing between 2-4 grams, were set in groups of 12 for each test sample and control groups, and left to grow for five days. For each sampling period 4 test samples were set up. Two control groups were used. As a positive control, we used a known mutagen methyl methane sulfonate MMS (SIGMA M-4016) at a concentration of 10 μg/l. Negative control was synthetic water. In test groups sample water was replenished once a day. Two onion bulbs with the poorest growth were eliminated from each group on the third day.

To determine general toxicity, the length of roots in 10 bulbs was measured in each sample. For genotoxicity analysis root tips were heated in 2N HCl solution for 12 minutes at 65°C and macerated. Five root tips (1-2 mm in length) from one onion were placed on each slide, stained with 2% orcein and squashed. Slides were coded and examined blind.

The level of toxicity was measured as the mean root length in tested samples in comparison to the negative control. To assess level of genotoxicity in each sample and controls (positive and negative) the following aberrations, in anaphase and telophase, were scored: bridges, fragments, vagrant chromosomes, multipolarity, and C-mitoses. A Two-by-Two contingency χ^2 statistical test was employed to determine the significance of differences between the analysed groups including controls, separately for each river.

RESULTS AND DISCUSSION

Šabac and Smederevo are considered as important regional industrial centers positioned on the banks of the large rivers (Sava and Danube). Thus, these rivers have been used for communal and industrial municipal effluent discharge and for transport related to production.

During this study, the water level on the Sava River differed for 597 cm between the first (low water level) and the second (high water level) sampling, while that difference was 152 cm on the Danube.

Roots grew better (for 4.3-12.6 %) in all analyzed river samples comparing to the negative controls, thus pointing to the presence of organic pollution in both rivers. Differences in the average root growth between loc 1 (upstream) and loc 2 (downstream) of the towns were not significant, indicating that communal and industrial wastewaters from both towns were not toxic.

However, the level of genotoxicity was dependent on the water level. We obtained the highest values of genotoxicity at locations upstream of both Šabac and Smederevo, in samples collected in a period of high water level (Figure 2), but only the sample from loc 1 on Sava River was significantly different ($X_{(1)}^2=29.51$; $p<0.001$) from aberrant cells found in the negative control (6.97%). Both towns, besides being regional industrial centers, are situated in regions of intensive agricultural activities. A large increase in river water levels leads to flooding of coastal agricultural land. As a consequence, chemicals used in crop production reach the water, raising the level of genotoxicity upstream of towns.

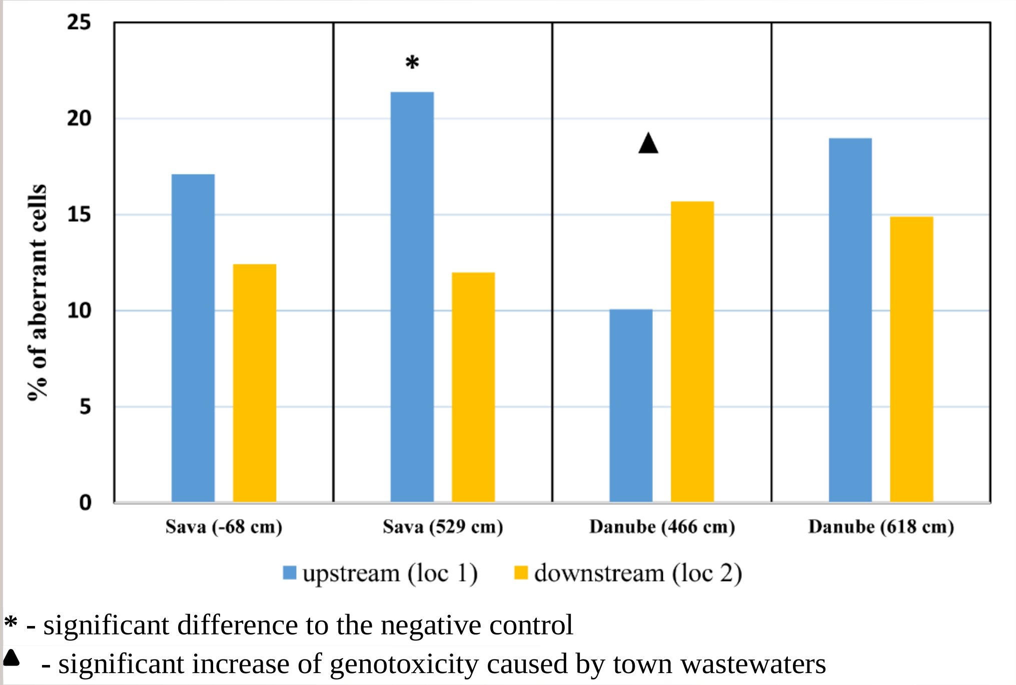

Figure 2. Percentage of aberrant cells in *Allium* anaphase-telophase test for the river water samples collected upstream and downstream of Šabac (Sava River) and Smederevo (Danube River) during low and high water levels (water levels on date of sampling, according to the Republic Hydrometeorological Service of Serbia, are given in brackets).

It is well known that pesticides frequently show genotoxic effects (summarized by LEME and MARTIN-MORALES, 2009). Furthermore, GADEVA and DIMITROV (2008), found that types of chromosomal aberrations are correlated with the concentration of different pesticides, and some of them in concentrations comparable to those used in practice can be designated as potential aneugens. ROSCULETE *et al*. (2018) showed that a higher herbicide concentration increased the percentage of chromosomal aberrations and had a higher mitodepressive effect.

In the period of low water level on the Danube, significant genotoxicity differences were obtained between loc 1 and loc 2 ($X_{(1)}^2$=7.62; p=0.006), indicating that urban wastewaters of Smederevo caused notable pollution. It is important to emphasize that the values at both sites, upstream and downstream, did not differ significantly from the negative control. Similar result was reported by FATIMA and AHMAD (2006); by using the *Allium* test they found increased genotoxicity in the samples of industrial effluence of two cities in India, containing mainly pesticides and heavy metals. Upon applying the same test, GANA *et al*. (2008) obtained increased genotoxicity in the samples from different industrial effluents.

Large rivers have high water flow, which could minimize the effects of chemicals, but local effects could be significant. This means that the potential damage to the environment can be much greater in small rivers. It seems that communal town and industrial wastewaters influence more significantly the river water quality during a low-level regime, while high water levels increase the risk of exposure to chemicals used in agriculture.

CONCLUSION

The increased levels of root growth in all river samples in comparison to negative controls point to the presence of organic pollution in both rivers. Levels of genotoxicity were the highest at points upstream of towns Šabac and Smederevo, in samples collected in periods of high-water levels. This can be a consequence of increased runoff from land characterized by intensive agricultural activities. Levels of river water define which kind of pollution will be the dominant ones. Town communal and industrial wastewaters have a higher impact on river water quality during low level regimes, while high water levels increase the risk of exposure to chemicals used in agriculture.

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