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Assessment of the toxicity and genotoxicity of Vistula river water at selected points in Warsaw using *Allium* test

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Abstract: *Assessment of the toxicity and genotoxicity of Vistula river water at selected points in Warsaw using Allium test.* The toxicity and genotoxicity of Vistula river water were evaluated at six selected points in Warsaw downstream from Gruba Kaśka using the *Allium* test. The research was conducted in 2011, which was before the municipal sewage-treatment system in Warsaw had been completed. No toxicity or genotoxicity was detected at the city water-intake facility 1-GK (Gruba Kaśka). Downstream of this point, toxicity and genotoxicity increased until 3-MP (Most Poniatowskiego) was reached, before disappearing at 4-MG (Most Grota-Roweckiego) in the north of the city. The highest toxicity and genotoxicity was observed at point 5-KB (Kolektor Burakowski), which was discharging untreated municipal sewage directly into the Vistula in 2011. No significant toxicity or genotoxicity of the Vistula river waters was observed below point 6-OC (Czajka Treatment Plant). The research should be repeated in 2013 to evaluate the effect of the investments in ecological infrastructure on the condition of the Vistula.

Key words: toxicity, genotoxicity, *Allium*, aberrations, wastewater

INTRODUCTION

Before 2012, Warsaw was one of the few European capitals which had not yet solved the problem of municipal sewage. At that time, approximately 40% of raw sewage was being discharged – untreated – directly into the Vistula. While the Warsaw treatment plants were efficient enough to deal with the overall burden of the city's wastewaters, there remained the problem of transporting them from the central and northern parts of the city, across the Vistula, to the Czajka Treatment Plant (one of the most modern in Europe). By the end of 2012 the problem had been solved, so that the capital is no longer polluting the Vistula with untreated sewage. However, in autumn 2011 the transport system had still not been completed and, what is more, it was necessary for technological purposes to release a proportion of domestic sewage via rainfall canalization. The research, which involved the evaluation of the toxicity and genotoxicity of river water at six selected points downstream of Gruba Kaśka, was conducted in September 2011. Because the area in question, Dolina Środkowej Wisły had been included in the Natura 2000 network (code PLB140004), it was extremely important to finish it before completion of the municipal sewage-treatment system. It is planned to undertake the second part of this research, which will

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involve assessing the effect of municipal ecological investment initiatives on the state of the Vistula waters, in 2013.

Plant bioindicators, with sensitivity similar to mammal-cell tests [Fiskesjo, 1985], are recommended by numerous scientists as very efficient and economical tools for testing toxicity and genotoxicity of environmental samples [Grant, 1999; Ma, 1999; Leme and Marin-Morales, 2009]. *Vicia faba* and *Allium cepa* root-tip assays are used quite often for liquid samples, such as wastewaters, leachates and surface waters [Ma et al., 1995; Smaka-Kincl et al., 1996; Evseeva et al., 2003; Feretti et al., 2012]. It has been found that sensitivity to genotoxic factors – especially in liquid samples – is higher in the *Allium* test [Ma et al., 1995; Obidoska, 2007; Asita and Matobole, 2010]. What is more, it can be used for both toxicity and genotoxicity testing. For these reasons the method was selected for this research.

MATERIAL AND METHODS

Samples were taken from the Vistula river in September 2011 from six points downstream of 1-GK (Gruba Kaśka, city water-intake facility). These were: 2-PS (Pomnik Sapera, outlet from the Południe Treatment Plant), 3-MP (Most Poniatowskiego, rainfall-collector outlet), 4-MG (Most Grota-Roweckiego), 5-KB (Kolektor Burakowski, outlet for untreated municipal sewage), and 6-OC (Oczyszczalnia Czajka, downstream from outlet of the Czajka Treatment Plant). Tap water was used as the control sample.

The *Allium cepa* test was performed according to the procedure modified by Fiskesjo [1985] with two onion cultivars 'Rumba' and 'Centro'. Following two-day incubation, three roots from each onion were taken for slide preparation (genotoxicity test) and the rest was left for a further four days. The lengths of the roots were then measured (root elongation toxicity test). The root tips were stained in aceto-orcein with 1N HCl (9:1) and squashed under a cover glass for the genotoxicity assessment. The observations were carried out under a light microscope using ×480 magnification. The mitotic index (MI) was expressed as a percentage of dividing cells per 1,000 scored cells (mitotic and interphase), while the percentage of anaphases and telophases with chromosomal aberrations (AAT), such as bridges, fragments and vagrant chromosomes, was calculated in 200 anaphase and telophase cells in three replications. AAT was also calculated in the series of dilutions of the most genotoxic water sample from point 5-KB (Kolektor Burakowski), which was diluted to solutions of 50, 25, 12.5, 6 and 3%. The results were subjected to a F-test for the analysis of variance (ANOVA) and subsequently to Duncan's test to assign the means to homogenous groups.

RESULTS AND DISCUSSION

The results of root elongation toxicity tests were consistent for both applied cultivars of *Allium cepa* (Fig. 1). Water toxicity was not observed at point 1-GK (city water-intake facility, Gruba Kaśka). Further downstream, in the centre of

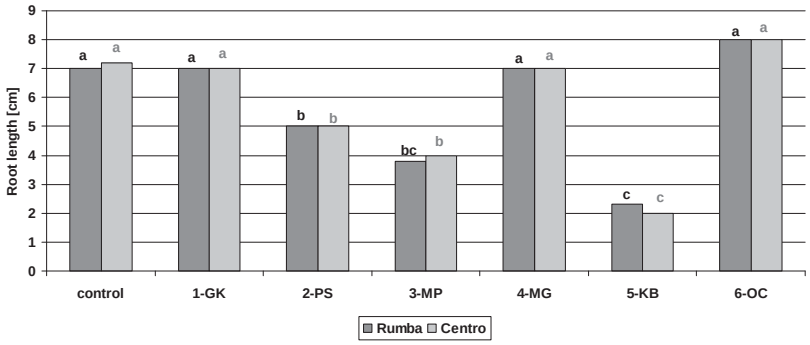

FIGURE 1. The toxicity of Vistula river water at six sample points downstream from point 1-GK Gruba Kaśka assessed by *Allium* root elongation test (means with the same letter for one cultivar do not differ significantly)

Warsaw, at points 2-PS (Pomnik Sapera) and 3-MP (Most Poniatowskiego), significant inhibition of root elongation in comparison with the control was observed, which indicated toxic substances appearing in the river waters passing the outlet from the Południe Treatment Plant and the rainfall-collector outlet which, in September 2011, was discharging domestic sewage. According to Thornton et al. [2001], cadmium, chromium, copper, mercury, nickel, lead, zinc, and organic pollutants may be all present in sewage and rainfall. The toxic effect of the river water was not present in the sample taken at the next point, 4-MG (Most Grota-Roweckiego), in the north part of the city. It is possible that the reasons were physical, chemical and biological processes (self-purification and dilution of pollutants) which normally occur in the water environment [Jarosiewicz, 2007]. Most toxins, however, could have been subjected to the processes of deposition and accumulation in sediments [Rank and Nielsen, 1998; Thornton et al., 2001]. The highest water toxicity (test effect – approximately 70%) appeared at point 5-KB (Kolektor Burakowski), which was releasing untreated municipal sewage straight into the river in 2011. Surprisingly, this effect had disappeared within a relatively short distance and was not increased by the outlet of the Czajka Treatment Plant. In sampling point 6-OC, which was below this outlet, the root elongation test did not detect toxicity. This may have been partly because of the quality of wastewater treatment at the Czajka facility, which is one of the most modern in Europe.

The genotoxicity of the river waters followed a similar pattern (Fig. 2): it increased as far as point 3-MP, disappeared at point 4-MG, recorded its highest result at point 5-KB and declined again at point 6-OC. The genotoxic effect at points 3-MP and 5-KB was much more significant for the 'Centro' cultivar. The most genotoxic sample, from point 5-KB, definitely lost its effect in the concentrations of 12.5% for 'Centro' and of 25% for 'Rumba' (Fig. 3), which would appear to be less sensitive to genotoxic agents. The results for these two onion cultivars were coherent, but some differences

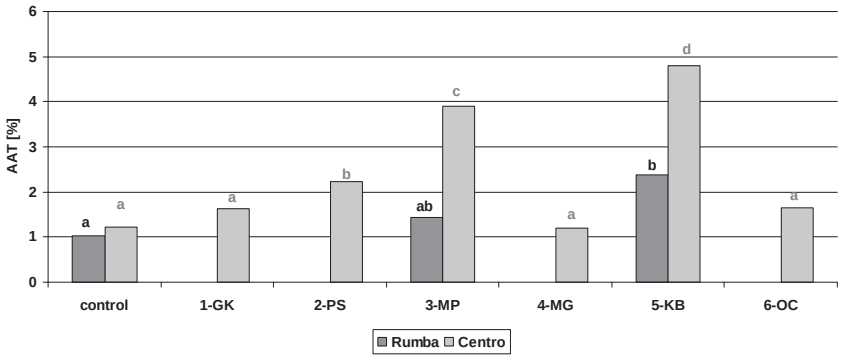

FIGURE 2. The genotoxicity of Vistula river water at six sample points downstream from point 1-GK Gruba Kaśka evaluated by the percentage of chromosomal aberrations in the ana-telophase of *Allium* root meristematic cells (means with the same letter for one cultivar do not differ significantly)

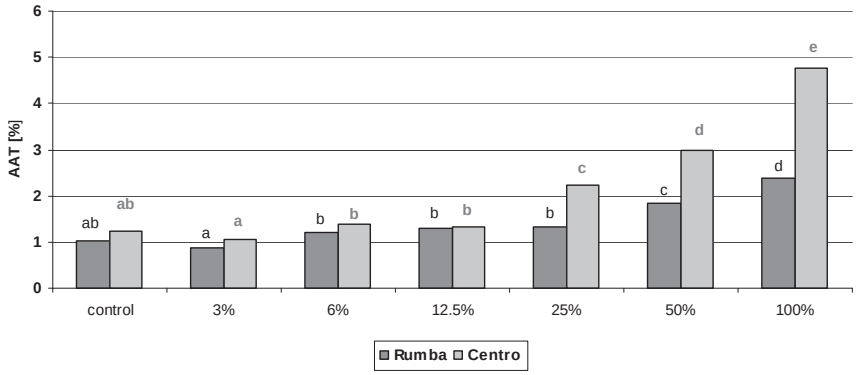

FIGURE 3. The genotoxicity of the dilution series of water sample from point 5-KB Kolektor Burakowski evaluated by the percentage of chromosomal aberrations in the ana-telophase of *Allium* root meristematic cells (means with the same letter for one cultivar do not differ significantly)

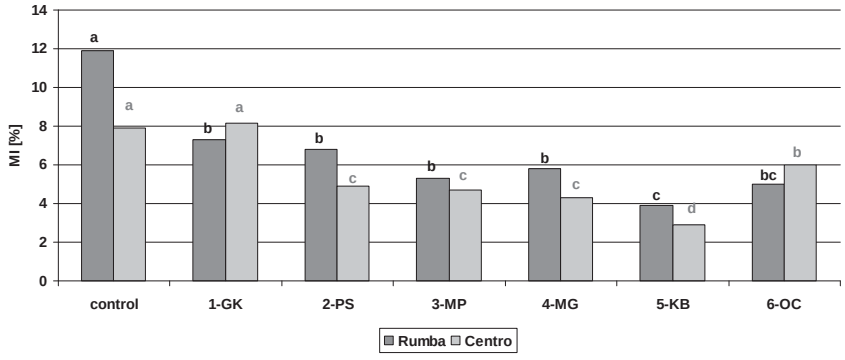

FIGURE 4. The mito-depressive effect of Vistula river waters at six sample points downstream from point 1-GK Gruba Kaśka evaluated by the mitotic index of *Allium* root meristematic cells (means with the same letter for one cultivar do not differ significantly)

in sensitivity to genotoxic agents were noted, as they had also been in the cases of *Pelargonium* [Cecchi et al., 2006] and of *Vicia* [Samborska-Ciania, 1993]. It is therefore important to select the same cultivar of a test plant for any comparison experiments. Samples of water from all six points expressed mitodepressive activity (Fig. 4) in the *Allium* meristematic roots – especially the water from point 5-KB. In the studies concerning Drava river in Croatia, the results for root elongation and mitotic indices at points located above and below the sewage treatment plant were similar, although the percentage of anaphase and telophase aberrations was much higher than in this study [Smaka-Kincl et al., 1996].

CONCLUSIONS

1. In 2011 in Warsaw the toxicity and genotoxicity of the Vistula river waters followed a certain downstream pattern:
   - no toxicity or genotoxicity was detected at 1-GK (city water-intake facility, Gruba Kaśka),
   - below 1-GK, toxicity and genotoxicity increased downstream as far as 3-MP (Most Poniatowskiego) before disappearing, due to dilution and the self-purification processes, at 4-MG (Most Grota-Roweckiego) in the north part of the city,
   - the highest toxicity and genotoxicity was observed at 5-KB (Kolektor Burakowski), which in 2011 was releasing untreated municipal sewage directly into the river waters,
   - no significant toxicity or genotoxicity of the river waters was observed below point 6-OC (Czajka Treatment Plant).
2. It would seem that the Vistula possesses a high potential for water self-purification. However, the majority of toxins may have accumulated in the sediments. If so, they pose a potential risk to the river's wildlife.
3. With the Kolektor Burakowski raw-sewage outlet having been removed, the research should be repeated in 2013 to evaluate the effect of the city's ecological investment initiatives on the state of the Vistula waters.

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Streszczenie: *Ocena toksyczności i genotoksyczności wód Wisły w wybranych punktach w Warszawie za pomocą testu Allium.* Oceniono toksyczność i genotoksyczność wód Wisły w sześciu wybranych punktach Warszawy usytuowanych w dół rzeki od Grubej Kaśki za pomocą testu *Allium*. Badania przeprowadzono w 2011 roku przed ukończeniem warszawskiego systemu oczyszczania ścieków. Nie stwierdzono toksyczności czy genotoksyczności wód w punkcie poboru wody dla miasta 1-GK Gruba Kaśka. Poniżej tego punktu toksyczność i genotoksyczność wzrastała w dół rzeki do punktu 3-MP Most Poniatowskiego, a następnie zanikała w punkcie 4-MG Most Grota-Roweckiego w północnej części miasta. Najwyższy poziom toksyczności i genotoksyczności notowano w punkcie 5-KB Kolektor Burakowski, który odprowadzał w 2011 roku nieoczyszczone ścieki miejskie prosto do Wisły. Poniżej punktu 6-OC Oczyszczalnia Czajka nie obserwowano toksyczności ani genotoksyczności wód Wisły. Badania zostaną ponownie przeprowadzone w 2013 roku, aby ocenić wpływ inwestycji proekologicznych miasta na stan wód Wisły.