Science of the Total Environment xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Flooding modifies the genotoxic effects of pollution on a worm, a mussel and two fish species from the Sava River

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HIGHLIGHTS

- Impact of flooding was assessed in organisms belonging to different trophic levels.
- Flooding decreased the amount and discharge rate of urban wastewaters.
- Flooding introduced contaminants from the nearby fly ash disposal field.
- Flooding modified genotoxic response in worm, mussel and two fish species.
- Genotoxic response in situ was different in studied indicator organisms.

ARTICLE INFO

Article history:
Received 14 February 2015
Received in revised form 21 March 2015
Accepted 27 March 2015
Available online xxxx

Keywords:
Floods
Comet assay
Genotoxicity
Branchiura sowerbyi
Unio tumidus
Abramis sp.

ABSTRACT

Extreme hydrological events, such as water scarcity and flooding, can modify the effect of other stressors present in aquatic environment, which could result in the significant changes in the ecosystem functioning. Presence and interaction of various stressors (genotoxic pollutants) in the environment can influence the integrity of DNA molecules in aquatic organisms which can be negatively reflected on the individual, population and community levels. Therefore, in this study we have investigated the impact of flooding, in terms of genotoxicity, on organisms belonging to different trophic levels. The study was carried out on the site situated in the lower stretch of the Sava River which faced devastating effects of severe flooding in May 2014. The flooding occurred during our field experiment and this event provided a unique opportunity to assess its influence to the environment. The in situ effects of this specific situation were monitored by measuring physical, chemical and microbiological parameters of water, and by comparing the level of DNA damage in coelomocytes and haemocytes of freshwater worms *Branchiura sowerbyi*, haemocytes of freshwater mussels *Unio tumidus* and blood cells of freshwater fish *Abramis bjoerkna*/*Abramis sapa*, by means of the comet assay. Our study indicated that the flooding had a significant impact on water quality by decreasing the amount and discharge rate of urban wastewaters but simultaneously introducing contaminants from the nearby fly ash disposal field into river by runoff, which had diverse effects on the level of DNA damage in the studied organisms. This indicates that the assessment of genotoxic pollution in situ is strongly affected by the choice of the bioindicator organism.

1. Introduction

Freshwater ecosystems are under the pressure of multiple stressors, such as organic and inorganic pollutants, geomorphological alterations, land use changes, water abstraction, invasive species and pathogens (Vörösmarty et al., 2010). Extreme hydrological events, such as water scarcity and flooding, can modify the effects of other stressors, which could result in the significant changes in functioning of the ecosystem (Klok and Kraak, 2008; Navarro-Ortega et al., 2015). The year 2014 was quite unusual considering extremely high amount of rainfall which strongly affected the hydrology in terms of water discharge of the Sava River and its tributaries, resulting in extensive floods throughout the Sava River Basin. In our study we were focused on the medium-sized urban settlement (a town of circa 50,000 inhabitants) situated on the banks of the Sava River which suffered devastating effects of the flood. This unfortunate event presented the unique opportunity for studying the effects of multiple stressors on aquatic organisms under the very specific circumstances. This town is one of the main industrial

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http://dx.doi.org/10.1016/j.scitotenv.2015.03.120
0048-9697/

hotspots with the largest thermal power plant in Serbia situated in the town area. The town also affects water quality of the Sava River by two main sources of pollution: a) power plant with related fly ash disposal field situated on the river bank and b) wastewaters from this town are discharged in the Kolubara River (significant right hand tributary). Extremely high water levels in May 2014 caused flooding of the entire town and surrounding villages and subsequently complete evacuation of the population. This resulted in the decrease of amount and discharge rate of urban wastewaters practically eliminating the influence of this source of pollution on water quality of the Sava River.

Multiple stressors (primarily organic and inorganic pollutants) can have an influence on the DNA integrity in aquatic organisms which can be negatively reflected on individual and population levels (Jha, 2008). The single cell gel electrophoresis (SCGE) or comet assay has been accepted as one of the major tools for assessing pollution related genotoxicity in aquatic organisms. Comet assay shows good correlation with micronucleus and erythrocytic nuclear abnormalities tests and detects early signs of the damage in genetic material (Maceda-Veiga et al., 2015). From the reviews of Dhawan et al. (2009) and Frenzilli et al. (2009), it can be seen that a variety of species inhabiting aquatic ecosystems are used as sentinel organisms. However, with such diversity of potential bioindicators, the question arises — does the choice of organism influence the assessment of genotoxicity in situ? Therefore, we have focused on organisms belonging to different trophic levels and exposed to different compartments in aquatic ecosystems (sediment, sediment/water and water).

In freshwater ecosystems, aquatic Oligochaeta (Annelida), oligochaetes or aquatic worms, are one of the principal components of the aquatic macro- and meiofauna. As primary consumers, decomposers and modifiers of the substrate, and as an important link in the food chain, oligochaetes have a very important role in the aquatic ecosystem (Schwank, 1982). Aquatic oligochaetes inhabit a wide range of habitats worldwide, from small streams, to large lowland rivers and lakes, and also, they show a wide range of tolerance to different environmental stressors (nutrient contamination, metal or organic chemical contamination). Limited mobility is an additional reason to choose the oligochaetes as indicator organisms. Within the group of Oligochaeta, tubificids species (representatives of family Tubificidae) are well adapted to high organic pollution in aquatic ecosystems (Rodriguez and Reynoldson, 2011). For our study we have chosen cosmopolitan tubificid species (non-indigenous for Europe, but recently widespread) *Branchiura sowerbyi* (Beddard, 1892) which has been already used in ecotoxicological studies (Naqvi, 1973; Ducrot et al., 2007; Saha and Kaviraj, 2008). As far as we know, this is the first study dealing with assessment of the DNA damage on haemocytes and coelomocytes of *B. sowerbyi* by means of the comet assay.

Mussels have numerous advantages, such as limited mobility, filter feeding, and small diameter of daily movement, which make them favourable bioindicators. In response to environmental stress they show a range of physiological, histological and molecular responses, including abnormal morphology, alterations of antioxidative status, induction of DNA strand breaks, etc. (Pavlica et al., 2001; Bolognesi et al., 2004; Rocher et al., 2006; Coffinet et al., 2008; Klobučar et al., 2008; Binelli et al., 2009; Kolarević et al., 2013). As a representative of freshwater mussels, we have chosen species *Unio tumidus*. In our previous studies we have successfully applied comet assay on haemocytes of this species for the detection of genotoxic potential in situ (Vuković-Gačić et al., 2014) and ex situ (Gačić et al., 2014).

Fish become one of the most suitable models for estimation of the possible risks in the aquatic environment due to their ability to efficiently metabolize and accumulate chemical pollutants (Cavas, 2011). Since fish occupy a top position in the food web, they can act as an amplifier for genotoxic substances present in lower links of the food chain (Boettcher, 2012). In our previous study (Sunjog et al., 2012) we have demonstrated that the comet assay applied on blood cells of *Barbus barbus* can be effective for the detection of genotoxic potential in surface waters. In this study we have chosen two bream species (*Abramis bjoerkna* and *Abramis sapa* as well known by synonym *Blicca bjoerkna*/*Ballerus sapa* as indicated in www.fishbase.org). As far as we know this is the first study dealing with assessment of the DNA damage in the blood of these species.

The major objective of this study was to investigate the influence of extreme flooding to other potential stressors detected in the environment, in terms of genotoxicity. Therefore, we have measured physical, chemical and microbiological parameters of water and compared the level of DNA damage in coelomocytes and haemocytes of freshwater worms (*B. sowerbyi*), haemocytes of freshwater mussels (*U. tumidus*) and blood of freshwater fish (*A. bjoerkna*/*A. sapa*), using the comet assay. Considering the sources of pollution, special attention was drawn to concentrations of metals and metalloids in water, as indicators of power plant related pollution, and microbiological parameters, as indicators of pollution related to urban wastewaters of the town Obrenovac.

2. Materials and methods

2.1. Study area

The study area is presented in Fig. 1. The study was carried out from January to August 2014 on the site (4) situated on the Sava River downstream of the town Obrenovac (2) (circa 50,000 inhabitants). Untreated wastewaters from this town are discharged in the Kolubara River (3), which confluences the Sava River downstream Obrenovac. The largest thermal power plant in Serbia "Nikola Tesla" and fly ash disposal field (1) are situated few km upstream of the sampling site.

2.2. Physical and chemical analyses of water

Physical and chemical parameters (t°C, pH, O_2, conductivity, NH_4^+, NO_2, NO_3^- and PO_4^{3-}) were measured on site using field laboratory (Hanna instruments, WTW — Photolab spectrophotometer) (Table 1). The results of the analyses of the concentrations of heavy metals and metalloids in water were provided by the Agency for Environmental Protection of Serbia measured monthly at routine monitoring site located nearby the selected sampling site (Table 2, Fig. 1). The data regarding water levels were provided by the Republic Hydrometeorological Service of Serbia (Fig. 2).

2.3. Microbiological indicators of faecal pollution

Total coliforms (TC) and *Escherichia coli* (EC) numbers were assessed by the most probable number technique (MPN) performed with Colilert 18 (IDEXX, Ludwigsburg, Germany). Enterococci (EF) concentrations were determined by the MPN with MUD/SF microtiter plates (BIORAD, Vienna, Austria) using the standard method according to ISO 7899-1:1998. Presumptive *Clostridium perfringens* (CP) were isolated by membrane filtration using the standard method according to ISO 14189:2013 (Table 1).

2.4. Specimen collection and sample preparation

Sampling was performed monthly from January to August 2014, with the exception of April due to extremely unfavourable weather conditions.

2.4.1. Worms (*B. sowerbyi*)

The selected species inhabits fine sediment often rich in organic matter. *B. sowerbyi* feeds by taking large amounts of substrate (Wang and Matisoff, 1997), thus modifying physical, chemical and biological characteristics of the upper sediment layers (Matisoff et al., 1999). Selected species is common and abundant (approximately 15%) within investigated stretch of the Sava River (Paunović et al., 2012).

Please cite this article as: Aborgiba, M., et al., Flooding modifies the genotoxic effects of pollution on a worm, a mussel and two fish species from the Sava River, Sci Total Environ (2015), http://dx.doi.org/10.1016/j.scitotenv.2015.03.120