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Impact of treated wastewater on organismic biosensors at various levels of biological organization

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HIGHLIGHTS

- Bacteria from fish and water have a zoonotic potential and might pose a health risk
- High antimicrobial resistance profiles were determined; particularly to SMX
- The sediment total antibiotic concentrations decreased with distance from the WWTP
- Histological, haematological parameters of fish differed in effluent and downstream
- *Eisenia fetida* is an optimal sentinel organism for environmental pollutants (MXR)

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ABSTRACT

Relating the treated wastewater quality and its impact on organismic biosensors (Prussian carp, *Carassius gibelio* and earthworm, *Eisenia fetida*) was the main objective of the study. The impact on health status of fish living downstream, microbiological contamination and antimicrobial resistance, fish tissue structure, blood biochemistry, oxidative stress, genotoxic effects, as well as multixenobiotic resistance mechanism (MXR) was assessed. Treated wastewater discharged from the WWTP modified the environmental parameters and xenobiotic concentrations of the receiving surface waters. Potential bacterial pathogens from fish and respective waters were found in relatively low numbers, although they comprised aeromonads with a zoonotic potential. High resistance profiles were determined towards the tested antimicrobial compounds, mostly sulfamethoxazole and erythromycin. Histopathology primarily revealed gill lamellar fusion and reduction of interlamellar spaces of effluent fish. A significant increase in plasma values of urea, total proteins, albumins and triglycerides and a significant decrease in the activity of plasma superoxide dismutase were noted in carp from the effluent-receiving canal. Micronucleus test did not reveal significant differences between the examined groups, but a higher frequency of erythrocyte nuclear abnormalities was found in fish sampled from the effluent-receiving canal. Earthworms indicated to the presence of MXR inhibitors in water and sludge samples, thus proving as a sensitive sentinel organism for environmental pollutants. The integrative approach of this study could serve as a guiding principle in conducting evaluations of the aquatic habitat health in complex bio-monitoring studies.

1. Introduction

Safe drinking water and proper sanitation are indispensable factors for sustaining life (Naidoo and Olaniran, 2014), while treated wastewater discharged to a body of water modifies its environmental parameters, both qualitative and quantitative. The discharge of effluent from domestic and industrial sources has detrimental effects on the aquatic ecosystem as this outfall can deposit large amount of organic matter, nutrients and pollutants leading to eutrophication, oxygen deficits and accumulation of pollutants into receiving waterways (Bhatia and Goyal, 2013). Urban wastewater treatment plants (WWTPs) were originally designed to reduce the biological oxygen demand, total suspended solids and nitrogen and phosphorus pollution, while the

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removal of pathogenic microorganisms has received less attention (Lucas et al., 2014). Although the primary and secondary treatments are able to remove up to 99% of fecal indicator bacteria (Servais et al., 2007; Lucas et al., 2014), the quality required to use treated wastewaters might be insufficient to achieve the level required for recreational activities in the receiving water bodies.

Environmental change can increase the vulnerability of aquatic species to toxic chemicals by challenging an organism's capacity to respond or to repair toxic injury or by modifying animal behavior like migration or predation (Couillard et al., 2008). Also, xenobiotics may affect the capacity of aquatic species to adapt to environmental challenges that come with stressors, such as pathogens. Fish are very susceptible to environmental variations, and their physiological status can serve as an early indicator of the specific ecosystem's health (Kaur and Dua, 2014).

A sampling strategy was developed to retrieve representative water, sediment, sludge, and fish samples related to a Croatian WWTP processing municipal, hospital and sugar plant wastewaters. It is a mechanical and chemical–biological facility with activated sludge, encompassing primary and secondary treatments of influents, treating mainly municipal wastewater deriving from a small city of 20,000 residents. Frequently, hospital wastewater is pretreated, but on this location it is connected directly to a municipal sewer and treated at the municipal WWTP. Treatment of such wastewater at the source has advantages of avoiding dilution due to mixing with the urban sewage and avoiding losses into the environment caused by sewer leakage and overflows. The sugar plant, operating at the time of this investigation, is a significant contributor to the wastewater to be treated at the WWTP. Sugarcane industry is among those industries with the largest water demands and, in addition, is an important source of non-toxic organic pollution (Ingaramo et al., 2009).

There is a gap in fundamental understanding of the specific contribution of the WWTP effluent in observed changes in organisms residing in/exposed to effluent-receiving waters and sludge. Also, the effectiveness of the WWTP in eliminating bacteria and pollutants which are not organic (particularly antibiotics), needs to be elucidated. Consequently, the objective of this wide-scale work was to test the hypotheses that 1) the WWTP effluent will induce biological effects on organismic biosensors; 2) resistant bacteria and potential fish and human pathogens will be identified from water and sludge, and antibiotics will be retrieved from sediment. To test our hypotheses, we conducted a series of tests to measure the impact on health status of fish living downstream, microbiological contamination, fish tissue structure, blood biochemistry, oxidative stress, genotoxic effects and multixenobiotic resistance mechanism (MXR): (i) general fish health examination, necropsy and histopathology were performed (hypothesis 1); (ii) rapid phenotypic tests and matrix assisted laser induced desorption ionization connected to the time of flight mass spectrometry (MALDI–TOF MS) were conducted on multiple samples of water, fish, and sludge (hypotheses 1, 2); (iii) fish blood biochemistry parameters and oxidative stress parameters were determined (1); (iv) erythrocytic nuclear abnormalities and micronuclei were enumerated and assessed (1); (v) cellular efflux mechanism mediated by ATP binding cassette (ABC) transporters that bind and actively remove toxic substrates from cells was analyzed post-exposure to raw and treated water and sludge (1); (vi) physical–chemical characteristics and heavy metal contents were determined in water and sludge samples (1);and (vii) antibiotic concentrations were measured in water and sediment (2).

The impact of effluent on fish and earthworms as toxicity biosensors was specifically addressed, especially in the view of active influence of all contributors to the wastewater volume (municipal, sugar industrial, hospital) as it is frequently omitted from investigation of performance of wastewater treatment plants. Such an approach encompasses an integrated overview of the impact of treated wastewater on key environmental and organismal parameters.

2. Materials and methods

2.1. WWTP and description of the sampling sites

The study was carried out in spring 2014, and the samplings of fish, water, sludge and sediment were conducted during the April 23 and 24 (Table 1). The samplings were carried out during the treatment process of a municipal WWTP serving about 20,000 inhabitants, also receiving hospital and sugar plant wastewaters. Treatment includes primary and secondary processes, including settling tanks, grit chambers, activated sludge biological process, aeration tanks, secondary tanks for removing the biomass and other suspended particles. The resultant final treated effluent is discharged into the water canal. This canal further downstream receives additional communal treated water from a biological treatment plant (reed beds) serving a small suburb, widens to enter a County canal (agricultural landuse), which eventually ends up in a Drava river. Therefore, sampling sites for water and sludge are defined as follows: 1: unaffected stream, not related to any industrial nor agricultural waters, considered as a reference site; 2: inflow of raw municipal wastewaters to the WWTP; 3: inflow of sugar plant wastewaters to the WWTP; 4: treated wastewater leaving the WWTP; 5: canal receiving the effluent; 6: canal after the biological treatment plant (reed beds); 7: canal entering the County canal; 8: County canal; 9: County canal downstream before the Drava river; 10: WWTP active sludge; and 11: sludge from the depot (Fig. 1). Water and sludge were collected in sterile glass and polypropylene flasks, refrigerated transported to the lab and immediately analyzed.

2.2. Physical and chemical properties and heavy metal analyses

Physico-chemical properties of water were analyzed according to the international standards as follows: determination of electrical conductivity, pH, suspended solids, dissolved oxygen, permanganate index (COD-Mn), chemical oxygen demand (COD), biochemical oxygen demand after n days (BODn) by dilution and seeding with allylthiourea,

Table 1

Number of samples per sampling location for a) Prussian carp (*Carassius gibelio*) for necropsy, microbiological, blood plasma, genotoxicity, histopathology assessment b) water for physico-chemical, heavy metal, and microbiological analyses, c) sludge for physico-chemical, heavy metal, and microbiological analyses, d) water for analytical chemistry, and e) sediment for analytical chemistry. Samples of water d) were taken at three time-points (morning 8:30, mid-day 14:00, evening 20:30 h) as a one-grab sample, while all other samples were taken in the early morning as a one-grab sample.

Locations:	1/A-1 reference stream	2 inflow of raw municipal water to WWTP	3 inflow of sugar plant waters to WWTP	4 treated water leaving WWTP	5/B-5 canal receiving the effluent	6 canal after the biological treatment plant	7 canal entering the County canal	8 County canal	9/C-9 County canal before the river	10 WWTP active sludge	11 sludge from the depot
a) Prussian carp	8				8				8		
b) Water	1	1	1	1	1	1	1	1	1		
c) Sludge										1	1
d) Water		3		3							
e) Sediment				1	1				1		