Science of the Total Environment xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

Gammarus fossarum as a sensitive tool to reveal residual toxicity of treated wastewater effluents

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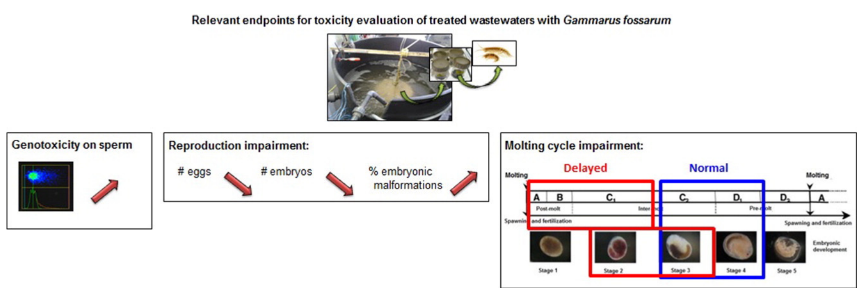
HIGHLIGHTS

- Toxicity assessment of treated wastewaters implemented on encaged gammarids
- Toxicity endpoints informative on long-term effects on organism fitness are chosen.
- Reproduction impairment and sperm genotoxicity appear the most sensitive biomarkers.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 November 2016
Received in revised form 19 January 2017
Accepted 22 January 2017
Available online xxxx

Keywords:
Ozonation
Micropollutant
Genotoxicity
Reprotoxicity

ABSTRACT

Wastewater treatment plants (WWTPs) are one of the main sources of freshwater pollution eventually resulting in adverse effects in aquatic organisms. Treated effluents can contain many micropollutants at concentrations often below the limit of chemical quantification. On a regulatory basis, WWTP effluents have to be non-toxic to the aquatic environment, wherefore not only chemical abatement but also ecotoxicological evaluation through relevant bioassays is required. Standardized bioassays currently used are often not sensitive enough to reveal a residual toxicity in treated effluents. Therefore, attention must be paid to the development of better-adapted approaches implementing more sensitive organisms and relevant endpoints. In this study, the toxicity of two differently treated effluents (activated sludge treated effluents with and without ozonation) towards the ecologically relevant species *Gammarus fossarum* was evaluated. Organism fitness traits such as reproduction and sperm DNA integrity were followed in exposed organisms. In complement, enzymatic biomarkers were measured indicating the presence of neurotoxic compounds (acetylcholinesterase activity), the presence of pathogens likely to increase the toxic effects of chemical compounds (phenol-oxidase activity), and the presence of toxic compounds inducing detoxification mechanisms (glutathione-S-transferase activity). Enzymatic activities were not modified, but significant sub-lethal effects were observed in exposed organisms. In both effluents, females showed a retarded molt cycle, a reduced fecundity and fertility, and >90% of developed embryos exhibited developmental malformations. In addition, a slight but significant genotoxic effect was measured in gammarid sperm. In a whole, no difference in toxicity was found between both effluents. Coupling reproduction impairment and genotoxicity assessment in *Gammarus fossarum* seems to be a valuable and sensitive tool to reveal residual

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http://dx.doi.org/10.1016/j.scitotenv.2017.01.154

toxicity in effluents containing a mixture of micropollutants at very low concentrations. Finally, a direct relationship between the observed toxic responses and the quantified micropollutant concentrations could not be evidenced.

1. Introduction

To date, wastewater treatment plants (WWTPs) continuously discharge micropollutants of concern, which can have biological adverse effects on non-target organisms in receiving ecosystems (Oehlmann et al., 2006; Besse et al., 2013; Morrissey et al., 2015; Petrie et al., 2015). WWTPs were designed and are being constantly improved to reduce efficiently the most part of contaminants, but conventional biological WWTP treatments are not effective to remove some biologically refractory micropollutants (Schwarzenbach et al., 2006; Wick et al., 2009). The Directive 2013/39/EU (European Commission, 2013) supports wastewater treatment plant authorities to develop and use new treatment technologies to reduce the potential hazard of treated effluents towards the aquatic environment. Since effluents should be shown as non-toxic for aquatic flora and fauna before entering receiving waters, standardized toxicity tests using algae, daphnids or fish are required by the European Commission (2000) to evaluate residual toxicity in treated effluents. Effluents can contain many micropollutants at very low concentrations after treatment and it has been shown that standardized bioassays might underestimate effects or might not be sensitive enough to reveal residual toxicity (Kümmerer, 2009; Wigh et al., 2016). Although concentrations of residual compounds are generally in the ng/L range, they still might impair organism fitness under chronic exposure and so the long-term ecological state (Fent et al., 2006; Newman and Clements, 2008). Berger et al. (2016) showed that chemicals were likely to induce effects in the environment at concentrations much lower than those based on laboratory experiments. Moreover, extrapolating single compound toxicity, generally assessed in the laboratory, to field-situations may underestimate the actual impact, because of possible pollutant interactions due to the chemical complexity of effluents and thus of receiving water bodies (Silva et al., 2002). Therefore, special attention must be paid for approaches on sensitive species and bioassays allowing long-term exposure and the measurement of relevant endpoints related to the fitness of organisms.

Gammarids as detritivorous species play an important role in the trophic food chain of the aquatic environment. They decompose organic matter and serve as prey for amphibians, insects, flatworms, other crustaceans such as crabs and crayfish, and fish (MacNeil et al., 2002). Gammarids have been shown as very sensitive to pollution, in particular from wastewaters (Peschke et al., 2014; Schirling et al., 2005; Schneider et al., 2015). They have been used in various studies for toxicity evaluation of river waters and effluents through *in situ* exposure or mesocosm study, by measuring toxicity endpoints such as reproduction, growth impairment and genotoxicity (Bundschuh and Schulz, 2011; Lacaze et al., 2011c; Coulaud et al., 2015). Alterations in reproduction endpoints such as molting cycle, fertility and fecundity may be caused by various pollutants in the effluents and can result in population dynamics impairment (Mazurova et al., 2010; Coulaud et al., 2015; Schneider et al., 2015). Pollutant genotoxicity can lead to organism fitness impairment in particular when affecting gametes. Hence it has been used in many studies as a sensitive sub-lethal endpoint to assess the toxicity of environmental samples (Stalter et al., 2010; Devaux et al., 2011; Lacaze et al., 2011c; Magdeburg et al., 2014). Spermatozoa of *Gammarus fossarum* were found to be the most sensitive cell type for genotoxicity evaluation compared to hemocytes and oocytes (Lacaze et al., 2011a).

Effluents contain a range of pesticides known to block acetylcholinesterase activity (AChE) in chemical synapses leading to neurotoxic effects due to an overactivation of postsynaptic acetylcholine receptors. Measurement of AChE inhibition has been successfully used as a biomarker for the presence of neurotoxic compounds, in particular in gammarids (Fulton and Key, 2001; Xuereb et al., 2009a). The detoxification enzyme glutathione S-transferase activity (GST activity) has been shown to increase with oxidative stress in organisms exposed to a large array of contaminants such as nitro compounds, organophosphates and organochlorines, possibly present in treated wastewaters (Hyne and Maher, 2003; Karaouzas et al., 2011; Turja et al., 2014). Moreover, treated effluents contain various bacteria and parasites that can enhance some adverse effects of pollutants towards organisms. Phenol-oxidase is an important enzyme for arthropod immune defense by triggering encapsulation and melanin deposition to prevent microbial growth (Sugumaran, 2002). A strong synergistic toxic effect was shown in *Enallagma cyathigerum* larvae exposed to the organophosphate insecticide chlorpyrifos (acetylcholinesterase inhibitor) and to the non-pathogenic bacterium *Escherichia coli* (Janssens and Stoks, 2013).

The aim of the present study was to evaluate the residual chronic toxicity of two differently treated WWTP effluents, by measuring endpoints regarding genotoxicity, neurotoxicity, biotransformation, reproduction and immune defense in gammarid *Gammarus fossarum* caged in effluent output.

2. Materials and methods

2.1. Gammarid sampling and acclimatization

The sampling site La Tour du Pin (Isère, France. 45°33′54″N 5°26′40″ E) is located in an unpolluted upstream part of the Bourbre River, where the freshwater amphipod *Gammarus fossarum* is found at a very high population density (Lacaze et al., 2011a). Gammarids (about 8 mm in size) were collected and let to acclimatize for 12 days in the laboratory in a mixture of well and osmosed waters in standardized conditions: temperature of 12 ± 1 °C, pH 7.6 ± 0.2 and a conductivity of 600 ± 50 μS/cm. Oxygen saturation (>90%) was ensured with an aeration pump. Photoperiod was maintained at 16 h light/8 h dark. Gammarids were fed *ad libitum* on black alder leaves (*Alnus glutinosa*) and supplied once a week with freeze-dried *Tubifex* worms.

2.2. Pilot plant description

In situ exposure of gammarids was conducted in a pilot wastewater treatment plant in Bellecombe (Haute-Savoie, France). The capacity of the two pilot treatment lines was 60 L/h with 23 h of hydraulic contact time. The activated sludge biological treatments were operated in order to achieve a full nitrification at minimal sludge age. The concentration of sludge in the aeration tanks was 2–3 g/L. The treatment lines were fed with a mixture of raw urban and hospital wastewater at a ratio of 50% v/v with average total suspended solids (TSS), chemical oxygen demand (COD) and $N\text{-}NH_4$ concentration of 139 mg/L, 614 mg/L and 41 mg/L, respectively. Gammarids were exposed to an effluent treated with conventional activated sludge (CAS) and to an effluent treated with conventional activated sludge combined with the ozonation of the mixed liquor in the recirculation loop ($CASO_{3\ loop}$). The specific transferred ozone dose, expressed per volume of feeding effluent was 9.4 mg of ozone per liter (Fig. 1).

Please cite this article as: Wigh, A., et al., *Gammarus fossarum* as a sensitive tool to reveal residual toxicity of treated wastewater effluents, Sci Total Environ (2017), http://dx.doi.org/10.1016/j.scitotenv.2017.01.154