



## Urban pollution of sediments: Impact on the physiology and burrowing activity of tubificid worms and consequences on biogeochemical processes



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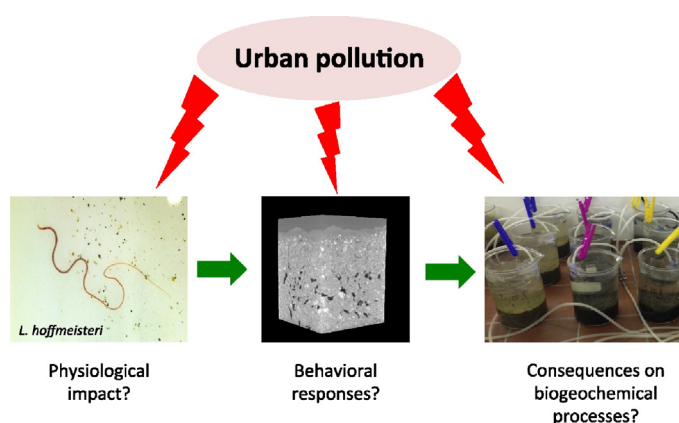
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### HIGHLIGHTS

- We studied the impact of a cocktail of urban pollutants on *L. hoffmeisteri*.
- Tubificid worms were able to withstand the oxidative stress linked to heavy-metals.
- X-ray micro-tomography quantified the burrowing activity of worms in sediments.
- *L. hoffmeisteri* displayed an increased burrowing activity in the stormwater sediments.
- Worms stimulated biogeochemical processes whatever the pollution level.

### GRAPHICAL ABSTRACT



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### ABSTRACT

In urban areas, infiltration basins are designed to manage stormwater runoff from impervious surfaces and allow the settling of associated pollutants. The sedimentary layer deposited at the surface of these structures is highly organic and multicontaminated (mainly heavy metals and hydrocarbons). Only few aquatic species are able to maintain permanent populations in such an extreme environment, including the oligochaete *Limnodrilus hoffmeisteri*. Nevertheless, the impact of urban pollutants on these organisms and the resulting influence on infiltration basin functioning remain poorly studied. Thus, the aim of this study was to determine how polluted sediments could impact the survival, the physiology and the bioturbation activity of *L. hoffmeisteri* and thereby modify biogeochemical processes occurring at the water-sediment interface. To this end, we conducted laboratory incubations of worms, in polluted sediments from infiltration basins or slightly polluted sediments from a stream. Analyses were performed to evaluate physiological state and burrowing activity (X-ray micro-tomography) of worms and their influences on biogeochemical processes (nutrient fluxes, CO<sub>2</sub> and CH<sub>4</sub> degassing rates) during 30-day long experiments.

Our results showed that worms exhibited physiological responses to cope with high pollution levels, including a strong ability to withstand the oxidative stress linked to contamination with heavy metals. We also showed that

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the presence of urban pollutants significantly increased the burrowing activity of *L. hoffmeisteri*, demonstrating the sensitivity and the relevance of such a behavioural response as biomarker of sediment toxicity. In addition, we showed that X-ray micro-tomography was an adequate technique for accurate and non-invasive three-dimensional investigations of biogenic structures formed by bioturbators. The presence of worms induced stimulations of nutrient fluxes and organic matter recycling (between +100% and 200% of CO<sub>2</sub> degassing rate). Nevertheless, these stimulations were comparable within the three sediments, suggesting a low influence of urban contaminants on bioturbation-driven biogeochemical processes under our experimental conditions.

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## 1. Introduction

Urban areas host half of the world's population (up to 80% in most industrialized countries), and this proportion is predicted to increase strongly in the future (United Nations, 2007). One of the major landscape modifications associated with urbanization is an increase in impervious surface coverage (Arnold and Gibbons, 1996). The sealing of urban surfaces alters the natural water cycle by increasing stormwater runoff during rainfall events thereby generating urban flooding and reducing groundwater recharge (Ku et al., 1992; Niemczynowicz, 1999). Stormwater management practices have been developed for 30 years in many countries to limit surface runoff (Marsalek and Chocat, 2002; Hunt et al., 2010). These practices consist in collecting and pouring stormwater into rivers, ponds or infiltration basins (Marsalek and Marsalek, 1997; Barraud et al., 2002). However, urban stormwater is a major source of pollutants like heavy metals or hydrocarbons, which are often associated with suspended sediments (Pitt et al., 1999; Grebel et al., 2013). Consequently, these pollutants may dramatically alter the quality of the sedimentary compartment of ecosystems receiving stormwater from urban areas (riverbeds and infiltration basins). For example, Datry et al. (2003a) estimated that a total of 128 kg of hydrocarbons and 153 kg of heavy metals (Zn, Pb, Cu, Cr, Ni, and Cd) were retained in a small infiltration basin (750 m<sup>2</sup>) draining an urban catchment of 2.5 ha after 30 years of functioning. The pollutant characteristics of these stormwater sediments could strongly affect the biological functioning of water-sediment interfaces that constitute key compartments for organic matter processing and nutrient cycling (e.g., Covich et al., 2004). However, studies concerning the negative aspects of urban pollution on the functioning of water-sediment interfaces remain scarce (but see Nogaro et al., 2007).

Despite high pollutant concentrations, stormwater sediments are often colonised by invertebrate taxa adapted to life in hypoxic and polluted environments such as tubificid worms or chironomid larvae (Datry et al., 2003b; Nogaro et al., 2008). These macro-organisms are known to act as efficient ecosystem engineers through their bioturbation activities: they build and ventilate galleries/burrows in sediments, stimulating microbial activities, nutrient fluxes and organic matter processing at the water-sediment interface (Krantzberg, 1985; Mermillod-Blondin and Lemoine, 2010; Nogaro and Steinman, 2014). Nevertheless, the ability of these organisms to influence ecological processes in sediments can be decreased by the presence of pollutants (Lagauzère et al., 2009a). Indeed, sublethal concentrations of pollutants can significantly affect the burrowing activities of invertebrates in sediments (Keilty et al., 1988; Mulow et al., 2002; Landrum et al., 2004; Lagauzère et al., 2009b). For example, Landrum et al. (2004) measured a significant decrease in sediment reworking activity of the oligochaete *Lumbriculus variegatus* due to sediment pollution by polychlorinated biphenyl (PCB). More recently, Mermillod-Blondin et al. (2013) demonstrated that a pollution of sediments by a polycyclic aromatic hydrocarbon (PAH) inhibits the burrowing activities of tubificid worms and their associated influence on sediment biogeochemistry (aerobic respiration and denitrification). Although these studies highlighted the need to assess the influence of sublethal concentrations of pollutants on bioturbation processes, they remained limited to experiments with one or two pure compounds without considering the

multi-contamination occurring in polluted sediments. This is more precisely the case in sediments from stormwater basins, polluted by a mixture of both metallic and organic compounds.

High levels of pollutants can also affect the physiology of organisms inhabiting infiltration basins (Marmonier et al., 2013). In such a harsh environment, aquatic invertebrates can respond by setting up costly defense mechanisms potentially leading to an increase in both energy reserves (glycogen, triglycerides and/or proteins) utilization rates and oxygen consumption, to fuel these responses. Moreover, recent studies have shown that numerous xenobiotics enhance oxidative stress in aquatic organisms and lead to the implementation of specific defense mechanisms, mainly the increase activity of antioxidant enzymes (see review in Winston, 1991 and Livingstone, 2001).

The aim of this study was to determine how a complex urban pollution of sediments could impact the survival, physiology and bioturbation activities of a worm species considered as a key bioturbator and the consequences of this pollution on biogeochemical processes (nutrient fluxes and organic matter mineralization) occurring at the water-sediment interface. We employed a factorial experimental approach in which the presence of the freshwater tubificid worm *Limnodrilus hoffmeisteri* (0 or 28,000 individuals per m<sup>2</sup>) and sediment treatments (3 sediments: two polluted sediments from stormwater infiltration basins and one stream sediment acting as control) were manipulated in microcosms kept under laboratory conditions. According to Datry et al. (2003b) who did not detect mortality in tubificid worms incubated 25 days in stormwater sediments, we did not expect that urban pollution of tested sediments would influence the survival of tubificid worms, but rather that they would exert a significant influence on their physiology. Thus, the impact of urban pollutants on a selection of physiological traits considered as pertinent metabolic biomarkers (oxygen consumption rate, body levels of glycogen, triglycerides and proteins: Marmonier et al., 2013) was investigated in worms incubated in stormwater and stream sediments. In addition, we tried to determine whether urban pollutants generate oxidative damages (through the level of lipid peroxidation, a major biomarker of oxidative injuries: Livingstone, 2001) and induce an efficient antioxidant response (through the catalytic activity of the major antioxidant enzyme, the enzyme superoxide dismutase: Winston, 1991) in worms incubated in stormwater sediments. The presence of pollutants would also influence the burrowing behaviour of worms as observed with earthworms in soils contaminated by heavy metals (Nahmani et al., 2005). Consequently, a change in burrowing activities would impair the ability of ecosystem engineers - tubificid worms - to influence sediment biogeochemistry, and therefore would affect the functioning of ecosystems receiving stormwater from urban areas. Thus, X-ray computed micro-tomography ( $\mu$ CT) was used in this study to quantify the volume of galleries (so-called biogenic structures) formed by burrowing activities of worms within sediments. This technique allows for accurate and non-destructive observations of internal structures, and is well recognized as an efficient tool to provide three-dimensional images of gallery networks formed by bioturbator organisms in sedimentary environments (Michaud et al., 2003; Dufour et al., 2005; Rosenberg et al., 2007; Mazik et al., 2008; Bouchet et al., 2009). Finally, the influence of worms on biogeochemistry was evaluated by measuring the dynamics of N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>x</sub> (N-NO<sub>3</sub><sup>-</sup> + N-NO<sub>2</sub><sup>-</sup>) and dissolved organic carbon

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