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A comprehensive study of the toxicity of natural multi-contaminated sediments: New insights brought by the use of a combined approach using the medaka embryo-larval assay and physico-chemical analyses

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ABSTRACT

Sediment compartment is a long term sink for pollutants and a secondary source of contamination for aquatic species. The abiotic factors controlling the bioavailability and thus the toxicity of complex mixtures of pollutants accumulated in sediments are poorly documented. To highlight the different factors influencing sediment toxicity, we identified and analyzed the physico-chemical properties, micro-pollutant contents, and toxicity level of six contrasted sediments in the Lot-Garonne continuum. Sediment toxicity was evaluated using the recently described Japanese medaka (*Oryzias latipes*) embryo-larval assay with direct exposure to whole sediment (MELAc). Multiple toxicity endpoints including embryotoxicity, developmental defects and DNA damage were analyzed in exposed embryos. Chemical analyses revealed significant variations in the nature and contamination profile of sediments, mainly impacted by metallic trace elements and, unexpectedly, polycyclic aromatic hydrocarbons. Exposure to sediments induced different toxic impacts on medaka early life stages when compared with the reference site. Principal component analysis showed that the toxic responses following exposure to sediments from the Lot River and its tributary were associated with micro-pollutant contamination: biometric measurements, hatching success, genotoxicity, craniofacial deformities and yolk sac malabsorption were specifically correlated to metallic and organic contaminants. Conversely, the main biological responses following exposure to the Garonne River sediments were more likely related to their physico-chemical properties than to their contamination level. Time to hatch, cardiovascular injuries and spinal deformities were correlated to organic matter content, fine particles and dissolved oxygen levels. These results emphasize the necessity of combining physico-chemical analysis of sediment with toxicity assessment to accurately evaluate the environmental risks associated with sediment contamination.

1. Introduction

As the final receptacle of pollutants from various origins (industrial, urban and agricultural) and their ability to accumulate substances such as persistent organic pollutants (POPs) and metallic trace elements (MTE), sediments are considered as both a sink and a long-term source of contamination for aquatic organisms (Burton Jr., 1991). Such contamination could threaten not only benthic organisms living on direct contact to sediment but also the entire aquatic ecosystem and finally human health via bioaccumulation, trophic transfer and/or drinking water (Fent, 2004). The evaluation of sediment toxicity is thus of major concern in environmental risk assessment strategies.

Abbreviations: Bdx, Bordeaux station; Bo, Bouillac station; DBT, dibenzothiophene; dpf, days post-fertilization; dph, days post-hatching; dw, dry weight; ELS, early life stages; ERS, egg rearing solution; GC-ECD, gas chromatography coupled to electron capture detection; GC-MS, gas chromatography coupled to mass spectrometry; Jo, Joanis station; LR, La Réole station; LT, Le Temple station; MELAc, Medaka Embryo-Larval Assay in sediment-contact; MPhe, methylphenanthrenes; Mrc, Marcenac station; MTE, metallic trace elements; PAHs, polycyclic aromatic hydrocarbons; PBDEs, polybrominated diphenyl ethers; PCA, principal component analysis; PCBs, polychlorobiphenyls; PEC, probable effect concentration; POC, particulate organic carbon; POPs, persistent organic pollutants; qPEC, probable effect concentration quotients; SD, standard deviation; SI, supporting information; TEC, threshold effect concentration

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While integrated procedures combining chemical analyses and biological responses are widely recommended (Ahlf et al., 2002), there is still no system of universal toxicity bioassay(s) capable of predicting the potential hazard of contaminated sediments. Because this particular matrix is very complex to analyze, several test phases have been used for sediment toxicity testing as, for instance, extractable pollutants and pore water (Burton Jr., 1991). However, whole sediment exposure is generally considered the most realistic and integrative method to mimic contamination of organisms *in situ* as the uptake route (including sediment-contact and aqueous phase exposure) is very similar to that found in environmental conditions, taking sediment characteristics and physico-chemical properties governing pollutant bioavailability into account (Hollert et al., 2003; Kosmehl et al., 2006).

In conventional ecotoxicity testing strategies, fish represent an indispensable component of integrated toxicity assessments because of their high sensitivity to contaminants, their critical role in the aquatic food chain and their socio-economic importance (Lammer et al., 2009). In the light of European regulations on the protection of animals used for scientific purpose (EC, 2010), fish early life stages (ELS) bioassays have gained interest in environmental risk assessments and (eco)toxicological evaluations. Indeed, their higher sensitivity to a wide range of chemicals when compared with adult or juvenile stages (Hutchinson et al., 1998), their ecological relevance (e.g. recruitment, population wellness) (Burton Jr., 1991; Cao et al., 2009) and the possibility they offer to perform small-scale, high-throughput analyses with an excellent correlation to conventional *in vivo* testing (Lammer et al., 2009) make fish ELS a promising tool to replace traditional acute fish tests (Braunbeck et al., 2005). As a result, fish ELS-based bioassays found their way into the laboratories not only to test chemical (eco) toxicity, but also to assess the hazard of environmental samples such as sediments (Hallare et al., 2005; Hollert et al., 2003; Kosmehl et al., 2008, 2006). These studies investigated embryotoxic, teratogenic and genotoxic potencies of sediments in zebrafish ELS demonstrating the suitability of such whole sediment-contact embryo-larval assays for natural sediment testing as it enables to evaluate the impacts of the sole bioavailable fraction of particle-bound pollutants from complex environmental matrices containing a mixture of a multitude of chemicals. Japanese medaka embryos offer similar practical advantages to zebrafish embryos for developmental toxicity testing, but its longer development time in the egg provides the possibility to extend the duration of embryonic exposure to 9–10 days at 26 °C (as opposed to 48–72 h for zebrafish), which can mimic a more chronic exposure to contaminants (Barjhoux et al., 2012).

The Medaka Embryo-Larval Assay in sediment-contact (MELAc) was developed in our laboratory. It consisted in an incubation of Japanese medaka (*Oryzias latipes*) embryos at the surface of sediment for the whole duration of the embryonic phase. As recommended, several non-invasive lethal and sublethal endpoints of embryotoxicity and teratogenicity are analyzed during the bioassay. Additional genotoxicity assessment is also performed on 2-days post-hatching (dph) larvae using the Comet assay. This procedure proved its applicability and its relevance for hydrophobic substances, MTE and sediment organic extract toxicity testing (Barjhoux et al., 2014, 2012; Cachot et al., 2007; Vicquelin et al., 2011). In the present study, we propose an application of the MELAc to the evaluation of whole raw sediment (i.e. with no prior extraction procedures) from a multi-contaminated environment: the Lot-Garonne continuum (South West of France).

This area is highly impacted by historical polymetallic pollution, first highlighted in the early 1970s by the National Observation Network, and characterized by very high Cd bioaccumulation in bivalves collected downstream, in the Gironde estuary, revealing it was in fact the most contaminated along the European coastline. The main source of Cd was identified in the upper part of the Lot River (Latouche, 1992), in a small Lot tributary (the Riou-Mort River) draining the waste area of a now-abandoned factory previously specializing in zinc ore treatment, which had been active for over a century in the Decazeville industrial basin. Although mining activities stopped for several decades and remediation procedures are in progress, the Lot-Garonne-Gironde fluvial-estuarine system remains clearly impacted by MTE such as Cd, Pb, Cu and Zn (Audry et al., 2004). These MTE are mainly transported in the particulate phase along the Lot-Garonne-Gironde continuum and constitute the major metallic inputs in the estuary (Audry et al., 2004). Sediments within the Lot-Garonne system represent a real storage compartment for MTE that can be remobilized in the water column during natural (flood, storm) or anthropogenic (dredging, dam flush) events and salinity gradient rising in the Gironde estuary (Audry et al., 2010). While the effects of the polymetallic contamination of the water column have been investigated in several aquatic organisms such as diatoms, bivalves and fish (e.g. Arini et al., 2012, 2011; Orieux et al., 2011), to our knowledge there is no study directly investigating the toxicity of sediments from this area.

The present study aimed to assess the embryotoxicity, teratogenicity and genotoxicity of six multi-contaminated sediments from the Lot-Garonne system using the MELAc. In parallel, a comprehensive characterization of sediments was carried out including MTE and POP contaminant levels and physico-chemical properties. Correlation and principal component analyses were conducted to investigate the possible interrelationships between physico-chemical and contamination parameters between themselves as well as with biological responses. Finally, a discussion on the toxic potential of the studied sediments and the relevance of the considered biomarkers is proposed.

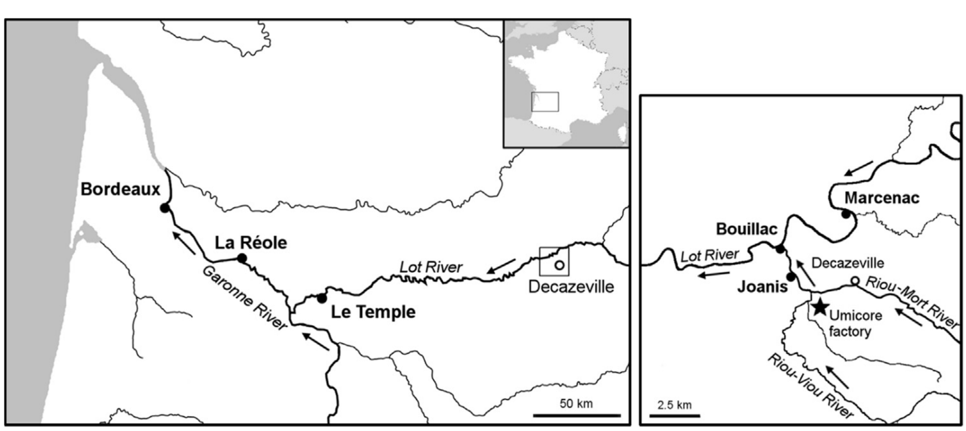

Fig. 1. Location of the study area and the six sampling stations (solid black dots) along the Lot-Garonne system.