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Determining oxidative and non-oxidative genotoxic effects driven by estuarine sediment contaminants on a human hepatoma cell line

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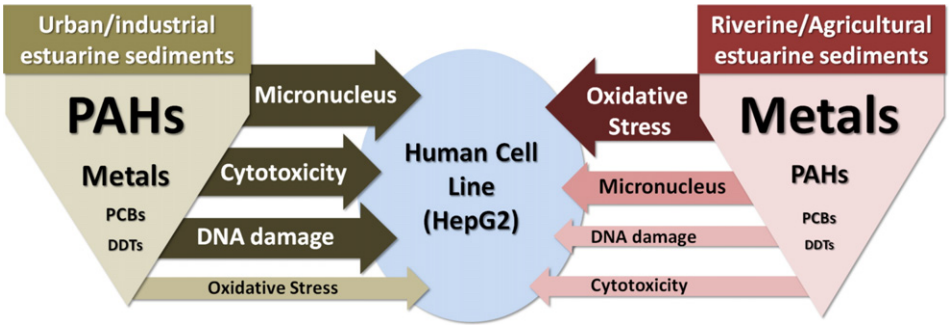
HIGHLIGHTS

- Sediment extracts from impacted estuarine areas induced effects in HepG2 cells.
- Extracts from an industrial area caused higher cytotoxicity and genotoxicity.
- Extracts from the rural area produced higher oxidative DNA damage.
- Cytotoxic and genotoxic effects were consistent with contamination by metals and PAHs.
- In vitro assays with human cells are purposeful to assess environmental hazards.

GRAPHICAL ABSTRACT



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ABSTRACT

Estuarine sediments may be reservoirs of hydrophilic and hydrophobic pollutants, many of which are acknowledged genotoxicants, pro-mutagens and even potential carcinogens for humans. Still, studies aiming at narrowing the gap between ecological and human health risk of sediment-bound contaminant mixtures are scarce. Taking an impacted estuary as a case study (the Sado, SW Portugal), HepG2 (human hepatoma) cells were exposed *in vitro* for 48 h to extracts of sediments collected from two areas (urban/industrial and Triverine/agricultural), both contaminated by distinct mixtures of organic and inorganic toxicants, among which are found priority mutagens such as benzo[a]pyrene. Comparatively to a control test, extracts of sediments from both impacted areas produced deleterious effects in a dose–response manner. However, sediment extracts from the industrial area caused lower replication index plus higher cytotoxicity and genotoxicity (concerning total DNA strand breakage and clastogenesis), with emphasis on micronucleus induction. On the other hand, extracts from the rural area induced the highest oxidative damage to DNA, as revealed by the FPG (formamidopyrimidine-DNA glycosylase) enzyme in the Comet assay. Although the estuary, on its whole, has been classified as moderately contaminated, the results suggest that the sediments from the industrial area are significantly genotoxic and, furthermore, elicit permanent chromosome damage, thus potentially being more mutagenic than those from the rural area. The results are consistent with contamination by pro-mutagens like polycyclic aromatic hydrocarbons (PAHs), potentiated by metals. The sediments from

Abbreviations: BC, Binucleated cell; CBPI, Cytokinesis-block proliferation index; DDT, Dichlorodiphenyltrichloroethane; FPG, Formamidopyrimidine-DNA glycosylase; HCB, Hexachlorobenzene; IARC, International Agency for Research on Cancer; MNBC, Micronucleated binucleated cell; MNi, Micronuclei; NR, Neutral red; OP, Organophosphate pesticide; PAH, Polycyclic aromatic hydrocarbon; PCB, Polychlorinated biphenyl; RI, Replication index; ROS, Reactive oxygen species.

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the agriculture-influenced area likely owe their genotoxic effects to metals and other toxicants, probably pesticides and fertilizers, and able to induce reactive oxygen species without the formation of DNA strand breakage. The findings suggest that the mixtures of contaminants present in the assayed sediments are genotoxic to HepG2 cells, ultimately providing a useful approach to hazard identification and an effective line-of-evidence in the environmental monitoring of anthropogenically-impacted coastal ecosystems.

1. Introduction

Many environmental toxicants are known to induce genotoxicity and mutagenicity, and are potentially carcinogenic, which raises concern regarding their impact on the ecosystem and on human health. For such reason, the characterization of genotoxic effects has become widespread in biomonitoring programs. However, the assessment of environmental contamination remains a difficult task considering the existence of complex mixtures of xenobiotics and their potential interactions, as well as the diversity of toxicant chemistry, availability and mode of action. Altogether, these variables pose a challenge when determining cause–effect relationships and ecological risk. The problem is amplified when determining hazard of aquatic sediment contamination, especially in confined/transition ecosystems, such as estuaries. In fact, sediments from impacted estuaries are long recognized as potential reservoirs of mutagenic substances (reviewed in Chen and White, 2004), including pollutants that have been classified by the International Agency for Research on Cancer (IARC) either as carcinogens, probable carcinogens or possible carcinogens to humans, such as polycyclic aromatic hydrocarbons (PAHs) and organochlorines, including organochlorine pesticides (IARC, 1991, 2012). Moreover, the sediments from those particular areas constitute intricate matrices of organic matter and fine particles that tend to trap and store both hydrophilic and hydrophobic contaminants which can be released back to the biota upon disturbance or, e.g., shifts in the oxic/anoxic status. The complexity of such contaminated matrices thus adds another challenge when addressing toxicity and risk (Chapman and Wang, 2001; Chapman et al., 2002; Chapman, 2007).

Several studies have addressed the toxicity of aquatic sediment-bound genotoxicants by applying a wide variety of tests, *in* and *ex situ*, taking aquatic invertebrates, fish and bacteria as the main target organisms (e.g. Thomas et al., 2002; Chen and White, 2004; Kammann et al., 2004; Boettcher et al., 2010; Costa et al., 2010; Yang et al., 2010). On the other hand, *in vitro* studies with mammalian cell lines, especially human cells, are commonly performed in pharmacological and toxicological research as surrogates for whole-organ or even all-organism approaches. These studies may provide valuable information of the effects and responses of exposure to stressors and therefore, may yield a measure of toxicity and information on the mechanisms behind the detected effects (MacGregor et al., 2001). It must be stressed that estuarine sediment contaminants, including those holding genotoxic and promutagenic properties, may pose a hazard to human populations. Apart from the genotoxicants that tend to adsorb onto particulate matter, sediment-bound toxicants are potentially released to the water column, being also a possible source of water contamination. Humans may be directly exposed to the contaminant mixture through dermal (or other epithelia) contact with the sediments and pore waters *per se*, for instance during catch and/or culture of burrowing organisms (e.g., bivalves) or during recreational activities. However, studies establishing cause–effect relationships between aquatic sediment contamination and human health risk are almost absent. In fact, even studies on the toxic effects of aquatic sediments on human cell lines are rare. Still, Higley et al. (2012) surveyed endocrine disruption in human adrenocortical carcinoma (H295R) cells exposed to sediment contaminant extracts from the Danube River and Shea et al. (2008) exposed human hepatoma (HepG2) cells to metals extracted from sediments from the Great Lakes to determine metallothionein induction. In contrast, *in vitro* assays using fish cell lines (e.g. Woo et al., 2006; Seitz et al., 2008; Rocha et al., 2009; Boettcher et al., 2010; Yang et al., 2010; Šrut et al., 2011), and rodent cell lines (Aouadene et al., 2008) have already been performed. Hence, a bridge between ecological risk and human health risk is still missing regarding the genotoxic/mutagenic effects of aquatic sediment contaminant mixtures.

Although epidemiological studies have been able to associate human exposure to cancer development in a number of occupational and environmental exposures, e.g. tobacco smoke, asbestos, radon, arsenic (Hubaux et al., 2012) those approaches tend to narrow the possibilities of understanding the impact of realistic toxicant mixtures, particularly with respect to mechanisms of toxicity that are crucial to their carcinogenicity (Pope, 2000). In this context, the human HepG2 cell line may be a suitable experimental system to assess the genotoxic effects of mixtures of environmental pollutants and their interactions. This cell line has been widely employed in toxicological studies, mostly because it detains most of the particular biochemical mechanisms of response and biotransformation of liver cells (Knasmüller et al., 2004). This allows the activation, by cytochrome P450 (CYP) monoxygenases, of a wide range of organic pro-mutagens to reactive metabolites which accounts for their genotoxicity through the formation of DNA adducts, and through the production of oxidative radicals as by-products of biotransformation (Henkler et al., 2012).

In the present study, the Sado Estuary, a large estuarine basin (c.a. 180 km^2) located in SW Portugal (Fig. 1), was taken as a case study, for being an area impacted by diffuse sources of pollution of multiple origins. The estuary is biogeographically very heterogeneous and of high ecological and economic importance. A large part of the estuary is classified as natural reserve and holds high value for fisheries and aquaculture. The northern banks include the city of Setúbal, including adjacent urban areas and a heavy-industry park. The latter comprise chemical plants (e.g. for pesticides and fertilizers), a paper mill, a thermoelectrical power plant, shipyards and mineral ore deployment facilities, most of which are potential sources of pollutants that might contaminate the estuary. The area is also of importance for heavy-duty maritime transport, with deep water wharfs being located off Setúbal and its industrial area. The southern banks around the peninsula of Tróia are farthest from pollution hotspots and are primarily important for tourism and leisure. However, the southernmost banks near the river mouth are impacted by extensive agricultural activities. The river itself crosses an important pyrite mining region as well as agriculture areas further upstream and therefore carries metals and other potential toxicants that may be trapped in aquatic sediments.

Recent studies in the Sado Estuary confirmed the occurrence of estuarine sediment contamination capable of inducing adverse effects in organisms, with metals and PAHs being the primary substances of concern (e.g. Caeiro et al., 2005, 2009; Costa et al., 2012). In addition, the genotoxicity of mixtures of sediment-bound contaminants have been analysed through *in vivo* assays with fish (Costa et al., 2008, 2011). Those studies further revealed the high ecotoxicological heterogeneity of this ecosystem, with a clear boundary between urban/industrial and rural/riverine areas (Carreira et al., 2013) but did not address the impact on human health. Therefore the present study aimed primarily at: i) determining the genotoxic effects, at both DNA and chromosomal levels, of sediment-bound contaminants in a human cell line (HepG2); ii) to differentiate the biological effects of areas affected by distinct sets of stressors, namely from industrial and rural origins and iii) to