Available online at www.sciencedirect.com
SCIENCE DIRECT®
Science of the Total Environment 366 (2006) 602 – 611
Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind
www.elsevier.com/locate/scitotenv

PAH content, toxicity and genotoxicity of coastal marine sediments from the Rovinj area, Northern Adriatic, Croatia

Nevenka Bihari *, Maja Fafanđel, Bojan Hamer, Blanka Kralj-Bilen

Institute «Ruđer Bošković», Center for Marine Research, G. Paliaga 5, HR-52 210 Rovinj, Croatia

Received 8 March 2005; received in revised form 28 November 2005; accepted 1 December 2005
Available online 24 January 2006

Abstract

Surface marine sediments collected from 8 sampling sites within the Rovinj coastal area, Northern Adriatic, Croatia, were used for determining priority pollutant polycyclic aromatic hydrocarbons (PAHs) and toxic/genotoxic potential of sediment organic extracts. Total PAH concentrations ranged from 32 μg/kg (protected area) to 13.2 mg/kg dry weight (harbor) and showed clear differences between pristine, urban industrial and harbor areas. PAHs distribution revealed their pyrogenic origin with some biogenic influence in harbor. At all sampling sites sediment extracts showed toxic potential that was consistent with the sediment type. No correlation between toxicity measured by Microtox assay and concentrations of individual or total PAHs was found. Noncytotoxic dose of sediment extracts showed no genotoxic potential in bacterial *umu*-test. DNA damage is positively related to total PAHs at 4 sampling sites (S-1, S-2, S-3, S-6), but the highest DNA damage was not observed at the site with the highest total sediment PAH content (S-5).

Keywords: PAHs; Marine sediments; Toxicity; Genotoxicity; Rovinj area; Northern Adriatic

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous wide spread contaminants arising mainly from incomplete combustion of fossil fuels, organic materials, wood and petroleum. Once formed, PAHs enter marine coastal areas through the spillage of petroleum, industrial discharges, atmospheric fallout and urban runoff (Neff, 1979). Because of their low water solubility and their hydrophobicity PAHs in the marine environment rapidly become associated with organic and inorganic suspended particles (Chiou et al., 1998) and subsequently deposited in sediments. In such a way sediments become a sink for particle-sorbed contaminants and can serve as a reservoir of toxic/genotoxic contaminants that continually threaten the health and viability of marine biota. Sedimentary PAHs tend to accumulate to high concentrations. Levels of PAHs in sediments vary, depending on the proximity of the sites to areas of human activity. Sediment concentration and distribution of PAHs may also fluctuate due to biodegradation of these chemicals, a process which is reliant upon abiotic and biotic factors which are dependent on site characteristics. The analysis of sediment PAH can serve as a useful index of the contamination level and the source of PAH input to the aquatic environments (Sicre et al., 1987; Budzinski et al., 1997; Gui-Peng, 2000). Analysis of composition,

* Corresponding author. Tel.: +385 52 804 715; fax: +385 52 813 496.
E-mail address: bihari@cim.irb.hr (N. Bihari).

0048-9697/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2005.12.001

distribution and source of PAH in sediments of the Adriatic Sea are scarce and mainly focused on Gulf of Trieste (Guzzella and DePaolis, 1994; Notar et al., 2001).

Some PAHs are known to be toxic and increase toxic potency of sediment (Shor et al., 2004). However, the relationship between PAH concentration and sediment toxicity is not consistent. Toxic effect is strongly correlated to PAH concentration in discharges of drill cuttings near North Sea oil platform (Grant and Briggs, 2002) while no significant correlation between toxicity and concentrations of individual or total PAH in Bay of Kavala Aegean Sea, Greece is found (Papadopoulou and Samara, 2002). The widely used technique for studying toxicity is the Microtox® bioassay for indication of organic and metallic contamination. This bioassay is rapid, sensitive method for determining relative toxicity by measuring the reduction in bioluminescence of the bacterium *Vibrio fisheri* after exposure to extracts of contaminated sediments. It has been successfully applied for determination of sediment and water toxicity from different areas including inner canals of the City of Venice (Pavoni et al., 1998), Lake Jamsanvesi, Finland (Hyotylainen and Oikari, 1999), Bay of Kavala Aegean Sea, Greece (Papadopoulou and Samara, 2002), North Sea (Grant and Briggs, 2002) and Po River, Italy (Vigano et al., 2003).

Some PAHs and their metabolits affect DNA that can induce mutations (Hener et al., 1997; Singh et al., 1998) and have carcinogenic properties (IARC, 1987). They are also recognised as a group of strongly progenotoxic marine contaminants. The expression of their genotoxicity results from their biotransformation to reactive metabolites. Evaluation of their genotoxicity can be achieved by measurement of increase in potential genotoxicity and mutagenicity of sediments (Chen and White, 2004) and by measurement of DNA damage in organisms living in PAH contaminated area (Pandrangi et al., 1995). Some investigations have shown that PAHs are the main mutagenic component present in the sediment (LaRocca et al., 1996) and positive correlation between mutagenicity and PAH contamination was reported (Chen and White, 2004). However no correlation of measured mutagenicity and chemical or physical parameters could be established along the German Baltic sea coastline (Zietz et al., 2001). Only sediments containing high concentrations of PAH (>10 mg/kg) were found to be mutagenic (Vondraček et al., 2001) with main mutagenic activity associated with benzo(*a*) pyrene (Marvin et al., 2000).

For the detection of chemical mutagens and carcinogens *umu*-test has been developed (Oda et al., 1985). It is based on the ability of substances to induce SOS response and expression of *umuC* gene in *Salmonella typhimurium* bacterial strain following DNA damage. The *umuC* gene in the bacteria is fused with *lacZ* gene and induction rate of *umuC* is assessed by the determination of β-galactosidase activity. It has been standardised according to DIN and ISO and has been used to detect genotoxicity using a wide range of mutagens (Reifferscheid and Heil, 1996), complex mixtures (Whong et al., 1986), environmental samples (Bihari et al., 1990; Hamer et al., 2000) and waste waters (Dizer et al., 2002).

Genotoxicity of PAH contaminated area could also be measured by determination of DNA damage in marine organisms. Estimates of the relative rates of DNA damage indicate that single strand breaks are the most prevalent type of damage (Bernstein and Bernstein, 1991). DNA strand breaks may be introduced directly by genotoxic compounds, through the interaction with reactive intermediers or oxygen radicals, or as a consequence of excision repair enzymes (Park et al., 1991; Eastman and Barry, 1992; Speit and Hartmann, 1995). DNA strand breaks have been widely measured by different methods and they have been accepted as biomarkers of genotoxicity in marine fish and bivalves (Depledge, 1998; van der Oost et al., 2003) especially for exposure to PAHs. Levels of DNA damage, measured as single strand breaks by Comet assay, in buleheads *Ameriurus nebulosus* were elevated at PAH contaminated sites (Padrangi et al., 1995) and in hornyhead turbot *Pleuronichthys verticalis* increased linearly with increase in concentrations of high molecular weight PAHs (Roy et al., 2003). DNA damage in mussels reflects elevated PAHs concentration in environment (Steinert et al., 1998) and correlates with PAHs content in mussel tissue (Perez-Cadahia et al., 2004).

In this study we combined chemical and biological approaches in analysis of coastal marine sediment of the Rovinj area, Northern Adriatic, Croatia. The aim was to assess the relationship and interdependency among sediment PAHs, sediment toxic/genotoxic potential and DNA damage in mussels. Sediment toxic and genotoxic potential was measured by Microtox® bioassay and *umu*-test, respectively. For the DNA damage determination in mussel *Mytilus galloprovincialis* hemolymph alkaline filter elution technique was performed.

2. Materials and methods

2.1. Sample collection

The surface layer of sediment samples were collected by SCUBA divers from 8 different sampling sites in