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Is the polychaete, *Perinereis rullieri* (Pilato 1974), a reliable indicator of PCB and PAH contaminants in coastal sediments?

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ABSTRACT

A field survey in a transitional environment (lagoon of Venice, Italy) and a laboratory exposure experiment were carried out to validate the use of polychaetes, *Perinereis rullieri*, as indicators of PCB and PAH contaminants in the sediments. Results from the field study showed that PCBs, predominantly the hexa- and hepta-chlorinated biphenyls, were promptly bioaccumulated in the tissues of *P. rullieri*, whereas PAH levels were generally low and fluctuating among seasons.

Organisms experimentally exposed to natural polluted sediments bioaccumulated all the examined PCB congeners, whereas those exposed to the reference sediments were able to reduce them, at least to some extent. A PAH depletion was always observed, although the time variations for the single compounds differed from each other.

The biomarker malondialdehyde (MDA), evaluated both in native and in treated organisms, was helpful as a supporting parameter in elucidating their oxidative stress condition, although depending on numerous natural confounding factors.

1. Introduction

Reliable indications on the integrity of coastal marine ecosystems might be reached by monitoring biological consequences to environmental stressors in species occupying critical trophic positions (Depledge and Fossi, 1994; Galloway, 2006). Effectively, various studies were performed in recent years, attempting to identify the most suitable species, in each environment compartment, to be regarded as consistent surrogates of the overall ecosystem functionality. Considering estuarine sediments, polychaetes, known to play an important role in the functioning of benthic communities (Giangrande et al., 2005), have been recently proposed and used not only as surrogates for marine biodiversity (Olsgard et al., 2003), but also as biomonitor organisms, at least for organic xenobiotics (Ruus et al., 2005; Cornellissen et al., 2006; Magnusson et al., 2006; Durou et al., 2007a,b).

It is well recognised that microorganic pollutant levels recorded within the biota compartment, although dependent on both different types of exposure route and physiological status/age of selected organisms, may be a more reliable indicator of the quality status of the marine ecosystem than those recorded in sediments (Rainbow, 2006; Galassi et al., 2008). In this context, the exploitation of polychaetes is particularly interesting since they may also be useful in evaluating the transfer of contaminants to higher levels in the food web –considering their trophic role as principal preys for several species of bottom-dwelling fish (Ruus et al., 2002, 2005). Polychaetes belonging to the Neredidae family have been already suggested as bioaccumulation indicators within the USEPA official procedures, because of their wide spatial distribution, food web position and relatively long life cycle, generally characterised by a singular reproduction event (USEPA, 1995).

The lagoon of Venice, North-east Italy, is an anthropised coastal ecosystem. Numerous pollutant sources have affected the lagoon of Venice for the last 50 years: the emissions of the petrochemical industries located along its inner border, the sewage and agricultural waste waters drained from the city of Venice and its mainland and the motorboat traffic within it (Guerzoni and Raccanelli, 2004). At the end of the 1970s the pollutant impact reached its highest levels and in the central part of the lagoon both water and sediment pollution were a serious problem, typical of very contaminated harbour areas (Sfriso et al., 1995; Frignani et al., 2001; Secco et al., 2005; Bernardello et al., 2006). Consequently, public environmental agencies have always paid special attention to the assessment of the micropollutants in abiotic and biotic compartments, particularly the most persistent organic ones, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Within this context, the biomarker approach, sometimes adopted together with chemical

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evaluations, has been useful in elucidating possible biological effects, at least in relation to the sentinel organisms investigated, mussels (*Mytilus galloprovincialis*), fish (*Zosterisessor ophiocephalus*) (Livingstone et al., 2000; Lowe et al., 2000; Nasci et al., 2002; Nesto et al., 2007) and, more recently, also the clam *Tapes phylippinarum* (Nasci et al., 2000; Marin et al., 2001; Da Ros and Nesto, 2005).

It is to be highlighted that the lagoon sediments, where almost untreated industrial discharges had settled to the bottom for years, have become themselves a secondary source and a re-cycling compartment, at least for these most persistent pollutants (Frignani et al., 2001).

The main aim of this study was to test whether a relatively little known species of Neredidae polychaetes, *Perinereis rullieri,* is suitable to be used as a biomonitor of organic pollutants, as in the lagoon of Venice the few studies that have dealt with polychaetes to date are mostly inconclusive and present results that are open to doubt (Volpi Ghirardini et al., 1999; Frangipane et al., 2005). *P. rullieri* is a gonochoric species characterised by semelparous reproductive strategy and it lives on mixed substrates formed by rocks, gravel, sand and mud (Prevedelli and Cassai, 2001). In the lagoon these worms can reach a length of 11 cm and reproduce between March and April (Prevedelli and Simonini, 2003). We decided to test this species for its higher distribution area within the lagoon, which hypothetically makes it a more suitable sentinel organism than the most studied *Hediste diversicolor*, mainly distributed along the inner borders of the lagoon. Finally, to preliminarily evaluate the ability of these organisms to cope with environmental stresses, their oxidative status has also been determined. To this end, the malondialdehyde-MDA content has been evaluated as an index of peroxidation of the membrane phospholipids (Gérard-Monnier et al., 1998). Effectively, several studies carried out on marine mussels have demonstrated that lipid peroxidation may be a consequence of chemical injury induced by pollutants (Solé et al., 1996; Lau and Wong, 2003; Box et al., 2007; Gorbi et al., 2008). As for polychaetes, an increase of MDA content in tissues of both *Eurythoe complana* and *H. diversicolor* has been evidenced in a short-exposure experiment to Cu (Nusetti et al., 2001) and in a field study (Moreira et al., 2006), respectively.

2. Materials and methods

2.1. Seasonal study

2.1.1. Sampling sites

Sampling sites were chosen on the basis of both their pollution level (Secco et al., 2005) and the distribution of organisms in the sediments, which needed to be sampled simultaneously. The two sites were sandy beaches located in the central part of the lagoon of Venice. San Giuliano (SG) (45°27.909′N, 12°17.121′E) is an area close to the inner border of the lagoon close to the mainland industrial site of Porto Marghera, which is influenced by both industrial waste and freshwater inputs (Sfriso et al., 1995). The site of Sacca Sessola (SS) (45°24.347′N, 12°19.012′E) is a shallow water area located towards the outer lagoon, surrounded by a net of navigable canals and channels, which are deeply affected by boat traffic and clam fishing (Secco et al., 2005; Sfriso et al., 2005a).

2.1.2. Sample collection and preparation

Polychaetes of similar size (5 ± 1 cm in length) were sampled in April, July, October 2005 and February 2006 at the two sites of SG and SS. Worms were hand-picked at low tide from intertidal sediments and immediately transferred to the laboratory in refrigerated box for subsequent treatments. Polychaetes were sacrificed after being anaesthetised with MS 222 (tricaine methane sulphonate 10%) and then differently processed according to the various analyses. For chemical analyses, a sub-sample of 50 individuals was kept for 3 days in aquaria containing quartz sand and seawater (at the same water temperature of the sampling site and at a salinity of 34 ± 1) to purge sediment particles from their intestines and then stored at -80 °C until subsequent pollutant analyses could be performed. For biochemistry, a whole body of 15 individuals was immediately frozen in liquid nitrogen and stored at -80 °C for MDA determination.

At the same time, sediment samples were also taken from the two areas for the actual chemical characterisation and subsequent estimation of the biota sediment accumulation factor (BSAF). Composite subtidal surface sediment samples (made up with three field replicates, 5–10 cm depth) were seasonally collected from each site using a Van Veen grab, transferred to the laboratory and then sieved (1.5 mm mesh size) to remove larger debris and native animals. The sediments were stored in the dark at 4 °C until analyses.

2.1.3. Chemical analyses of sediments and organisms

For the analysis of PAHs and PCBs in sediments, approximately 2 g of dried sediments were extracted with methylene chloride–acetone (1:1) in a Soxhlet extraction apparatus for 16 h, and the sulphur compounds were removed by soaking the extracts with activated copper powder. As for organisms, 15 individuals were pooled and the resulting tissue homogenates were extracted with n-hexane in a Soxhlet apparatus for 16 h, and the lipid content were quantified by gravimetric determination of the extractable organic material (EOM). Subsequently, both extracts were purified and fractionated by chromatography on an alumina/silica gel column (Fossato et al., 1998).

The concentration of 14 USEPA priority pollutant PAHs (naphthalene (Naph), acenaphtene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Ft), pyrene (Py), benzo(a)anthracene (B[a]A), chrysene (Chy), benzo(b)fluoranthene (B[b]Ft), benzo(k)fluoranthene (B[k]Ft), benzo(a)pyrene B[a]Py, dibenzo(a,h)anthracene (diB[a,h]A), benzo(g,h,i)perylene B[g,h,i]Per) was analysed with high-performance liquid chromatograph (HP 1090, USA) on a reverse-phase column (Supelcosil LC-PAH 250 mm × 2.1 mm 5 μm) with a programmed fluorescence detector. Individual fractions of PCBs were analysed by capillary gas chromatography (Carlo Erba Fractovap 4160, Italy) with electron capture detectors (ECD), in the splitless mode on a 30 m Supelco SE-54 column (0.32 mm ID, 0.3 μm film) (Lowe and Fossato, 2000).

PCB concentrations were determined as total Arochlor (calculated as the sum of a 1:1 1254 and 1260 mixtures) and the sum ($\sum$PCB) of 7 congeners (52, 101, 110, 118, 138, 153, 180). The identification of compounds was deduced from their retention times and quantification was based on peak area measurements as well as comparison with responses of external reference standards. The method detection limits ranged between 0.05 and 0.5 ng/g for PAHs and 0.05 ng/g for PCBs. Blanks were run for the entire procedure and blank sample corrections applied to each set of analysis. Validation of the recovery and accuracy was carried out with an IAEA-417 sediment sample certified reference materials. Overall recovery values ranged from 65% to 102%. Methods were also validated by continuous intercalibration activities (IAEA, 2005, 2007).

For the determination of organic carbon (OC) in the sediments, an aliquot weighed on a silver cup was treated with hydrochloric acid vapours (Hedges and Stern, 1984) before analysis, to completely remove inorganic carbon. Carbon analysis was performed with a Perkin Elmer 2400 CHN elemental analyser, using acetanilide as a standard. The range of the CHN instrument is 1–3600 μg (precision $\pm 0.2\%$).

2.1.4. Biota/sediment accumulation factor (BSAF)

BSAF for PCBs and PAHs was calculated according to the following formula (Ankley et al., 1992): $(C_o/f_1)/(C_s/f_{soc})$, where C_o is the chemical concentration in the organism, f_1 is the lipid fraction of the organism, C_s is the chemical concentration in sediment and f_{soc} is the fraction of organic carbon in the sediment.

2.2. Laboratory experiment

2.2.1. Sediment collection and preparation

The sediment laboratory trials were performed in May 2006. Different from the field study, the sediments needed to be selected only on the basis of their dissimilar pollution level (Secco et al., 2005), irrespective of the presence of polychaets. So, the more polluted one was sampled from a site well-known to be affected by industrial emissions (Tresse, T), and the reference one from a northern pristine area (Palude della Rosa, PR), where polychaets were not found. The sediments were sampled and processed according to the procedures already described in the previous section. Before initiating the experiments, a 3-cm-thick layer of both test sediment was distributed on the bottom of four replicate plastic aquaria ($30 \times 20 \times 13$ cm^3) and covered with about 6–7 cm of filtered seawater (salinity 36). Following a 2-h conditioning period, aeration was provided through plastic devices, suspended 2 cm above the sediment surface. Before organism exposure, one aliquot of sediment from each experimental tank was collected and preserved at -20 °C for individual chemical analyses.

2.2.2. Exposure experiment design

Thirty acclimated individuals were carefully added to each test aquarium, kept unfed at a constant temperature of 20 °C and exposed to static photoperiods of LD 12:12. Half-water was changed twice a week in each aquarium, to avoid increasing