Science of the Total Environment 496 (2014) 165–178

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Micro-organic pollutants and biological response of mussels in marinas and ship building/breaking yards in Turkey

O.S. Okay [a,*], B. Karacık [a], A. Güngördü [b], M. Ozmen [b], A. Yılmaz [a], N.C. Koyunbaba [a], S.D. Yakan [a], V. Korkmaz [b], B. Henkelmann [c], K.-W. Schramm [c,d]

[a] Istanbul Technical University, Faculty of Naval Architecture and Ocean Engineering, 34469, Maslak, İstanbul, Turkey
[b] İnönü University, Arts and Sciences Faculty, Department of Biology, Laboratory of Environmental Toxicology, 44069, Malatya, Turkey
[c] Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Ecological Chemistry, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany
[d] TUM, Wissenschaftszentrum Weihenstephan für Ernährung und Landnutzung, Department für Biowissenschaften, Weihenstephaner Steig 23, 85350 Freising, Germany

HIGHLIGHTS

- Highest organic pollutant levels were found in ship/shipbreaking yard stations.
- There is a strong correlation between toxicity test results and pollutant levels.
- Enzyme activities in digestive glands showed significant differences between sites.

ARTICLE INFO

Article history:
Received 18 March 2014
Received in revised form 4 July 2014
Accepted 9 July 2014
Available online xxxx

Editor: Eddy Y. Zeng

Keywords:
Marina
Ship building/breaking yards
Micro-organic pollutants
Sediments
Mussels
Enzyme assays

ABSTRACT

Concentrations of PAHs, PCBs and OCPs in sediments and mussels (caged and/or native) were determined at 16 stations in six major sites of coastal Turkey. The biological effects of pollution were evaluated using sediment toxicity tests and enzyme activity assays. EROD, PROD, GST, AChE, CaE, and GR activities were evaluated using the digestive glands of mussels. The total PAH concentrations in the sediments varied between nd and 79,674 ng g^{-1} dw, while the total OCP concentrations were in the range of nd to 53.7 ng g^{-1} dw. The total PAH concentrations in mussels varied between 22.3 and 37.4 ng g^{-1} ww. The average concentrations of total PCBs in mussels were 2795 pg g^{-1} ww in the shipyard, 797 pg g^{-1} ww in Marina 2 and 53 pg g^{-1} ww in Marina 1 stations. The results of whole-sediment toxicity tests showed a strong correlation between toxicity test results and pollutant concentrations. Selected cytosolic enzyme activities in digestive glands differed significantly depending on localities. These differences in enzyme activities were mainly related to the different pollutant levels of the sampling sites. The micro-organic contaminant profile patterns, toxicity tests and biomarker studies showed that shipyards and shipbreaking yards are the major potential sources of organic pollution in coastal areas.

1. Introduction

Pollution due to activities in marinas, shipyards and shipbreaking yards has become an important problem throughout the world, and the marine environment in these areas has been seriously affected by oil spills, garbage dumping, metal accumulations, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorinated pesticides (OCPs) (Covelli et al., 2001; Storelli et al., 2001).

PAHs, PCBs and OCPs have been recognized as important pollutants in aquatic environments. PAHs are widespread chemicals generated by the combustion of organic matter at high temperatures (pyrolitic origin), by the release of petroleum (petrogenic origin) and by the degradation of organic matter (diagenic origin) (Neff, 1979). As PCBs and OCPs have been synthesized for commercial use, they do not have a background level. PCBs have been used primarily in coatings, inks, flame retardants, paints, heat-transfer systems, and hydraulic fluids (Manz et al., 2001; Breivik et al., 2002). The OCPs in the coastal marine areas is mainly due to runoff from agricultural areas. While the production, use and discharge of PCBs and OCPs have been restricted and/or banned in many countries, because of their lipophilic character, historical use and persistency, they are still detected in sediments and organisms. They are also known to cause toxic effects at the individual level (Ael et al., 2012). The identification of the chemical concentrations and their sources is the main step in either minimizing or reducing the contamination levels in the environment (Breivik et al., 2004). The

* Corresponding author: Tel.: +90 212 285 64 07; fax: +90 212 285 64 54.
E-mail address: oya.okay@itu.edu.tr (O.S. Okay).

http://dx.doi.org/10.1016/j.scitotenv.2014.07.035
0048-9697/

integration of chemical analyses with ecotoxicological techniques may lead to a better assessment of these pollutants and may facilitate the identification of environmental impacts.

Anthropogenic activities in the Turkish coastal regions intensified during the second half of the last century. As a result, there has been a remarkable growth in yacht tourism and in the shipbuilding industry in Turkey to the extent that the shipbuilding and repair industry is considered one of the most promising industrial sectors in Turkey. Heading up the industry is Tuzla, the largest shipbuilding industrial area in Turkey with more than 80 yards (OECD, 2011).

The purpose of this study was to investigate the occurrence, source and distribution of PAHs, PCBs and OCPs in sediments, in caged and, if they exist, in native mussels (*Mytilus galloprovincialis*) at the six main sites of Turkey, all of which have different pollution levels. Meanwhile, biological effects of pollutants were also evaluated by whole sediment toxicity testing and by the application of a suite of biomarkers to the mussels (*Mytilus galloprovincialis*). Using the digestive glands of mussels, enzyme biomarker responses were evaluated for ethoxyresorufin O-deethylase (EROD), pentoxyresorufin O-deethylase (PROD) and glutathione S-transferase (GST) activities as biotransformation systems; acetylcholine esterase (AChE) and carboxylesterase (CaE) activities as toxicity responses; and glutathione reductase (GR) as oxidative stress markers. Multimarker studies in organisms have provided valuable information for monitoring studies (Aarab et al., 2004; Schmidt et al., 2012; Islas-Flores et al., 2013; Costa et al., 2013).

Bivalves, especially the genus *Mytilus* (both native and caged), have been frequently employed as bioindicators in the marine environment (Andral et al., 2004; Schintu et al., 2008; Baussant et al., 2009; Marigomez et al., 2013). As filter feeders, and because of their low enzymatic activity, they have been shown to be one of the most successful model organisms for time-integrated accumulation of pollutants (Zorita et al., 2007; ICES/OSPAR, 2009). A sessile characteristic of the mussels is an advantage for estimating local pollution. Caging studies enable monitoring studies in areas where local mussels are not present.

This study is a first attempt to determine the levels and effects of the aforementioned pollutants in the selected sites.

2. Materials and methods

2.1. Study area

The study area consists of 16 stations in six major sites of coastal Turkey, namely, Saros Bay (SB) (northeastern Aegean Sea), Çanakkale Strait (CS), Tuzla shipyard area (S) (İstanbul, Marmara Sea), Aliağa shipbreaking area (Aegean Sea), Marina 1 (Mediterranean) and Marina 2 (Marmara Sea) (Fig. 1). Saros Bay (three stations) is a relatively clean site with no industrial activity in the surrounding area. The most important economic activities are fisheries and agriculture. The length of the bay is approximately 61 km with a maximum width at the opening to the Aegean Sea of approximately 36 km. Çanakkale Strait (one station) connects the Aegean Sea to the Marmara Sea. In the outlet of the strait, strong surface currents flow from the Marmara Sea to the north, along the eastern coast of Çanakkale (Pazi, 2008). Tuzla shipyard area (one reference and three sampling stations) is the major shipbuilding region and is situated 50 km east of İstanbul. There are 27 yards in the area that engage in new building with the largest yard having an annual maximum construction capacity of 650,000 DWT. The area also provides repair and maintenance services (OECD, 2011). The selected reference station for the shipyard area, a small pier belonging to the İstanbul Technical University, is located in a bay in the Tuzla region. Marina 1, M1, (one reference and three sampling stations) is situated in the Mediterranean Sea. The reference station was in the coastal area of a small village in the same area. The marina has been in service for eleven years and has sea and dry-dock service capacities for approximately 600 and 100 yachts, respectively. The second marina, M2, (three sampling stations) is one of the largest marinas in Turkey and has been in service for 27 years. The transplanted mussels in the reference station were lost during the deployment period, and due to the rocky characteristics, it was not possible to conduct a sediment sampling at this station. M2 has greater capacities than M1, with approximately 1250 and 425 yachts for sea and dry-dock services, respectively. Since 1976, the shipbreaking area (SBY) (one sampling station) in Turkey has been located in an organized industrial zone owned by the state and rented to private investors along the coast of Aliağa, 50 km north of Izmir, on the Aegean coastline of Turkey. There are 29 shipbreaking yards in the area with a total annual capacity of approximately 600,000 LDT (light displacement tonnage) (Neşer et al., 2008).

The surface seawater temperatures at the Marmara and Mediterranean sites during the sampling period ranged between 11 °C and 12 °C and 15 °C to 17 °C, respectively. The surface salinity at the Marmara site was quite low (23 to 24 ppt) compared with that at the Mediterranean site (35 to 39 ppt).

2.2. Sampling and deployment

Mussel deployment/retrieval and sediment sampling were accomplished during March and April of 2012. Approximately 1000 g of surface sediment samples (0–10 cm) were collected by free diving. They were then mixed and stored in prewashed glass bottles. For caging aims, Mediterranean mussels (*Mytilus galloprovincialis*, 4 to 5 cm in length) were collected from relatively clean local areas. The mussels for the Mediterranean (Med) and Marmara (Mar) stations were retrieved from two different locations due to the salinity differences of the marine ecosystems. The locations included a local see bream farm located offshore of Bodrum for the Aegean/Mediterranean stations and a site at the Istanbul Strait for the Marmara Sea stations. Chemical analyses and enzyme assays were applied to one set of mussels before transplantation (BT) in the sites. The rest of the mussels were transported to the laboratory and acclimatized in aquariums for two days prior to deployment. Twelve cages were deployed for 30 days in the water column at a depth of approximately 1 m from the surface and 2 to 3 m above the sediments. Three of the cages were lost (M1-Ref, M2-Ref and SBY) during the exposure period and all transplanted mussels died within the 30 day period at station S3, in the Tuzla shipyard area. Local mussels existed at only two sites — S-Ref and M2A.

Collected samples were immediately transferred to the laboratory. Sediment samples for toxicity testing were stored at 4 °C in dark for no longer than one week prior to testing. The samples for chemical analysis were frozen at −20 °C.

For enzyme assays, 15 to 20 mussel samples were carefully cleaned using sea water, and each mussel was dissected under field conditions. The digestive glands of the mussels were wrapped with aluminum foil and kept in liquid nitrogen. The transferred samples were stored at −80 °C until enzymatic assays were performed.

The details of the sampling, deployment and testing strategies applied the study area are provided in Table 1.

2.3. Sediment toxicity test systems

Whole sediment toxicity tests were conducted using immobilized *Phaeodactylum tricornutum* cells in alginate and Mediterranean mussels. All tests were conducted in a temperature controlled room at 20 ± 2 °C. The seawater (20 to 22 ppt) used in experiments was collected from a relatively clean site in the Istanbul Strait and filtered through activated carbon, GF/C (1.2 μm) and then through 0.2 μm millipore filter paper. The principle of the immobilized algae preparation method has been previously described by Santos et al. (2002). To perform toxicity tests with algal beads, 35 mL of homogenized sediments were placed in 10 cm-diameter glass petri dishes to which 70 mL of filtered seawater was added. Approximately 100 beads were then placed on the sediments, covered with transparent lids and incubated under continuous