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Ecotoxicological Characterization of Marine Sediment in Kostrena Coastal Area

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A B S T R A C T

Samples of marine sediment were taken on 4 selected sites close to the shipyard industry in Kostrena coastal area. Concentration of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals (Cu, Pb, Zn, Hg, Fe) were analyzed from chemical-analytical and toxicological aspect. For toxicity detection, the bacterial bioluminescence test (Vibrio fisheri) was used. Concentration of total PAHs varied in the range from 697 to 7807 μg/kg dry weight in marine sediments. The concentration of PCBs in sediment was 1.1 mg/kg dry weight. The highest concentrations of heavy metals were found at the station within the shipyard. PAH toxicity was not correlated with the toxic potential of sediments. The obtained results indicate a high degree of environmental risk, especially at stations within the shipyard, with the 54% possibility of toxic effects. Chemical determination of the concentration of conventional pollutants is not sufficient for assessing the quality of the marine environment and it is necessary to use other approaches in order to evaluate the biological impact.

***Key words**: marine sediment, PAHs, PCBs, heavy metals, toxicity test, Vibrio fisheri*

Introduction

By pollution of the marine environment it is considered, directly or indirectly, the introduction of harmful substances or energy into the marine environment resulting in different consequences, such as harm on living resources and marine life and threatening to human health[1–3]. One of the reasons comes from ships and shipyard industries[1]. Based on convenient geographic position Kostrena, in a coastal area near Rijeka, intensive marine traffic and development of many industrial plants let to an increased entry of various toxic chemicals into marine environment[4]. Main purpose of shipyard industry, covered by this study, is ship repair, construction and repair of various facilities of marine technology (off-shore), as well as the construction and repair of platforms for exploration and exploitation of oil and gas.

The aim of this study was to determine the concentrations of heavy metals (copper, lead, zinc, iron and mercury), PAH's and PCB's in marine sediment samples. PAH's in the marine environment can be natural and anthropogenic origin. Although part of the polycyclic aromatic hydrocarbons in the environment comes from natural sources, in the last few decades, human activities such as burning fossil fuels and transportation of oil and petroleum products have contributed significantly to increasing concentrations of polycyclic aromatic hydrocarbons in the marine environment[5]. Pollution from ships can be divided into: those caused by the regular functioning of pollution sources (uncontrolled and irresponsible discharge of ballast water) and those caused by the planned, deliberate disposal (dumping when loading and unloading) and accidental caused by sudden maritime accidents and spills[6]. The presence of heavy metals in marine environment represents a serious problem, because of their marked toxicity, resistance to degradation and biological accumulation[7]. Polychlorinated biphenyls (PCB's) are toxic synthetic organic aromatic compounds

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obtained by chlorination of biphenyl in the presence of a catalyst, and belong to the group of persistent organic pollutants. PCB's in the marine environment originate only by accident in case of an incident, or spills from improperly discarded transformers and capacitors or maybe leaching into the soil and thus PCB's can directly enter in the marine environment[8]. Some PAH's are known to be toxic, but field in investigations concerning correlation between PAH load and toxic potential are controversial[9]. The widely used technique for study toxicity and environmental quality of marine coastal areas is the Microtox® bioassay (Machery-Nagel, Germany).test was developed using bacteria *Photobacterium phosphoreum* (later renamed into *Vibrio fisheri*). It detects pollution by organic chemicals and it is widely used as toxicity marker[10]. Considering the impact of industry and contamination with polycyclic aromatic hydrocarbons PAHs, heavy metals and polychlorinated biphenyls PCB's, it is extremely important to monitor stress level by reaching the marine ecosystem, through constant monitoring of environmental factors near the shipyard industry located in Kostrena coastal area.

Materials and Methods

Study site and sampling collection

Kostrena is situated in North-Western part of the coastal area of the Adriatic Sea, nearby eastern side of the city of Rijeka. Total area of 12.7 km^2, sea area of 4.78 km^2 and it has 12.5 km long coast. Development of the field began in 1896, in the late 1960s the shipyard was moved 3 km to the South to its present location at Martinšćica Bay**,** which has characteristics with an adequate water depth to accommodate vessels with deep draughts. Our study area, with significant influence of shipyard on environment, is located between 45° 18' N; 14° 28' E and 45° 17' N; 14° 29' E, 3 km from the largest Croatian port Rijeka. Marine sediment was collected from 4 sampling sites from inside the shipyard to evaluate any difference in sediment quality. MB (Martinscica Bay), RB (Red buoy), PE (Pecine), and SB (Svezanj Bay) as a control point. The surface layer of sediment (0.5 cm) was collected by SCUBA diving as previously described[11]. The sea depth varied between 24 and 42 m. After sampling the sediments were frozen in polyethylene bags and kept at –20 °C until further processing. Samples were stored and transported to the laboratory using standard procedures[12,13].

Polycyclic organic hydrocarbon (PAH) analyses

For individual PAH determination we used a modification of the method previously described by Alebić Juretić[14] and Bihari[11]. The PAHs were identified and quantified using HPLC module system HP 1050 (Hewlett Packard, Palo Alto, USA) consisting of quaternary pump with rheodine valve injector (HP 7125) and 20 μL sampling loop, variable wavelength UV detector and integrator (HP 3396). PAHs were separated on reverse phase cartridge column HP Li Chrospher 100 RP-18 (5 lm, 250 × 4 mm) with appropriate guard column (Li Chrospher 100 RP-18, 5 lm, 4 × 4 mm). For PAH separation a gradient elution with solvent system methanol-water was used (flow 1.5 mL/min): t = 0 min 80% MeOH + 20% H_2O, t = 5 min 95% MeOH + 5% H_2O and t = 8 min 98% MeOH + 2% H_2O. The absorption was measured at 254 nm.

Heavy metals analyses

Sample preparation for heavy metals analysis, were performed using standard protocols[15]. Heavy metals were quantified using atomic-adsorption spectrometer Perkin Elmer 200 Analyst 600, SAD. Copper, lead and iron were analyzed by flameless technique by standard method (HRNEN ISO 15586:2008), zinc (DIN 38 406 Part 16-1990 Cations-group E), and mercury by AMA 254 (Advanced Mercury Analyzer, LECO).

Polychlorinated biphenyl (PCB) analyses

The procedure used for PCB analyses was under standard protocols and it was previously described by Traven[11]. For PCB analyses approximately 20 g of wet sediment was homogenized with anhydrous Na_2SO_4 and extracted with 100 mL n-hexane for 2 h on a shaker; after that the extract was stored for 12 h at 4 °C. The extract was then filtered through anhydrous Na_2SO_4 and evaporated on the rotary evaporator until dryness. The dry residue was dissolved in 5 mL of petrol-ether saturated with acetonitrile, and transferred to a separating funnel to which 50 mL of acetonitrile saturated with petrol-ether was added. The acetonitrile fraction was separated and evaporated on a rotary evaporator until dryness. The dry residue was dissolved in 5 mL of petrol-ether and purified on a fluorisil column with some alumina on top using 100 mL of n-hexane as the eluent. The extract was evaporated until dryness, dissolved in 2 mL of n-hexane and analysed by GC/MS. The GC/MS system used was GCMS-QP2010 gas chromatograph / mass spectrometer (Shimadzu Corporation, Kyoto, Japan) equipped with AOC-5000 autoinjector (CTC Analytics AG, Twingen, Switzerland).

Microtox® sample handling and toxicity testing

The Microtox® assay is rapid toxicity test that measures the effect of a toxin on light production by a luminescent bacterium, *Vibrio fisheri*. Sediment samples were prepared by standard protocols[16,17]. Toxicity tests were carried out using wet sediments. However, aliquot of each sediment was weighed, dried and reweighed to allow Microtox® toxicity values to be expressed on a dry weight basis. Toxicity of the sediment was determined by measurement of the decrease of the initial bioluminescence of the *Vibrio fisheri* bacteria NRRL B-11177, using Microtox®-test DIN EN ISO 11348-3 Biofix®. Changes in luminescence were measured using Monolight 2010 (Analytical luminescence laboratory) illuminometer. Results were expressed as EC_{50}-pollutant concentration with 500 % decrease in bacterial bioluminescence.

Statistical analysis and mathematical models to calculate the probability of toxicity

Statistical analysis was performed using Statistica 8.1 (StatSoft Inc., Tulsa, USA). The level of statistical significance was calculated by two-tailed Student's t-test and set at $p<0.05$. The probability that a certain concentration of a compound in marine sediments may cause a toxic effect can be calculated using the equations that express the likelihood of toxic effects of each compound as a function of its concentration in marine sediments. The probability of toxicity was measured by mathematical models used by US EPA[18]. Knowing that it is a standardized test[19], it is possible to compare the results from different studies.

Results

PCBs concentration in marine sediment has been detected only on MB site and it was 1.1 mg/kg dry weight. Total PAH concentration in marine sediment ranged from 697 to 7807 μg/kg dry weight. Share of carcinogenic PAH in total PAH was high in all sediment samples, ranging from 40 to 47% (Table 1).

Copper concentrations in marine sediment ranged from 20 to 149 mg/kg dry weight, zinc concentrations ranged from 23 to 391 mg/kg dry weight, lead concentrations ranged from 11 to 141 mg/kg dry weight, mercury concentrations ranged from 0.024 to 0.212 mg/kg dry weight and iron concentrations ranged from 5115 to 20000 mg/kg dry weight.

EC50 values in marine sediment samples ranged from 368 to 4596 μg/kg dry weight. Correlation between total PAH concentration and toxicity of marine sediment samples showed week connection between these two parameters ($r=0.1170$).

Results obtained indicate a high degree of environmental risk, especially at stations within the shipyard (CP), with the 54% possibility of toxic effects from mixture of PAHs and heavy metals.

Discussion

The aim of our investigation was to determinate PAH, PCB and heavy metals concentration in marine sediment within the shipyard located in Kostrena coastal area and also to compare the obtained results with similar investigations obtained in Kvarner Bay in recent history. There are only few studies previously published on presence of PAHs and heavy metals in marine sediment in Kvarner Bay, Adriatic sea. In study conducted by Bihari[9], concentrations of PAHs in Rijeka bay ranged from 213 to 695 μg/kg dry weight. In our investigation about pollution from oil industry[2], also located in Kostrena Bay, concentrations of PAHs were ranged from 58 to 1116 μg/kg dry weight. In study conducted by Traven[11], PAH concentrations in marine sediment were ranged from 113.82 to 11478.65 μg/kg dry weight. Results obtained in these studies are similar but lower than in our study.

In investigation conducted by Baumard[20], total PAH concentrations in marine sediment from area with well developed heavily industry ranged from 1 to 20500.00 μg/kg dry weight, which is much higher than those obtained in our study. According to Guzella and De paolis study[21] of marine sediment in Adriatic coast from Trieste Bay to very southern end of Italian coast, PAH concentrations ranged from 27–527 μg/kg dry weight, which are much lower than those obtained in our investigation.

Regarding published data on PCBs concentration in marine sediments in this region, study conducted by Traven in 2008 reports PCB values from 48–67 ng/g dry weight. Other locations such as Rovinj (NE part of the Adriatic) and Dubrovnik (Southern part of the Adriatic) reported values of 37.5 ng/g dry weight and 68 ng/g dry weight, respectively[22]. The values reported in this study show lower concentrations 1.1 ng/g dry weight. A study

TABLE 1
CONCENTRATION OF PAHs IN MARINE SEDIMENT

μg/kg dry weight	MB	RB	PE	SB
Acenaphten	1085	406	80	17
Phenantrene	763	486	127	53
Anthracen	103	68	17	7
Fluoranthen	1721	1574	368	162
Pirene	1253	1310	301	129
Chrysen+ Benzo(a)anthracene	1285	1256	288	137
Benzo(b)fluoranthene + Benzo(k) fluoranthene	818	670	173	111
Benzo(a)pyrene	502	477	98	52
Indeno(1,2,3cd)pyrene	277	252	38	29
Total PAH	7807	6499	1490	697
Share of carcinogenic PAH				
CARC (μg/kg dry weight)	2882	2655	597	329
CARC/PAH (%)	36	40	40	47

published by Danis[23], which included sediments sampled along and off the Belgian coast reported PCBs values in the range between 0.23 and 21.1 ng/g dry weight, which is similar to the values reported in this study.

With respect to heavy metal pollution, all heavy metals analysed (Pb, Hg, Fe, Cu and Zn) were detected in all samples (Table 2). The highest concentration of heavy metals was found in the sediment collected from the Martinscica Bay, especially with respect to Pb, Hg, Fe and Zn. Copper concentrations in this study are higher than the concentrations that were detected in Cuculić[24] study in the Krka National park, and Baran[25] and Tsakovski[26] in Poland. Salizzato[27] in the Venice channel found higher values of copper concentrations than detected in Kostrena area. The concentrations of zinc, lead and mercury in this study are similar to concentrations that have been detected by Cuculić[24] in the Krka National park. Concentration of iron in Kostrena sediment sampels are in the range of level concentrations obtained in the Venice channel[27] and Poland[25,26].

Ranking stations with total concentration of PAH compounds in marine sediments, would be as follows (from lowest to highest): SB < PE < RC < MB. Ranking the stations by the probability of toxicity for the mixture of PAH determinations, would be (from lowest to highest): SB < PE < MB < RC. The reason for different rankings station may be the fact that not all PAHs are equally toxic, and it is possible that the proportion of toxic aromatics in the mixture were higher although their total concentration was lower. Therefore, an environmental risk that such a mixture of compounds can cause can be higher.

Conclusion

Our results represent negative impact of the shipyard on the marine sediment of Kostrena coastal area. They indicate that individual samples can be toxic, although the concentration of certain conventional pollutants is very low, which suggests that the chemical determination of conventional pollutants is inadequate indicator of the quality of the marine environment and that the absence of compounds that are conventionally determined in environmental samples does not necessarily mean the absence of toxicity. Chemical-analytical and toxicological assessment of the quality of the marine environment should be based on the modern concept of biological effect directed chemical analysis. Based on everything above, luminescent bacteria toxicity test is a simple, reliable and sensitive method for monitoring the toxicity of aquatic environment. Even though, some parameters investigated in this study are low and results of Microtox® toxicity of some sediment samples were high, that does not mean that the final result did not affect any other xenobiotic present in the marine sediment, but was not the subject of this research.

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EKOTOKSIKOLOŠKA KAREKTERIZACIJA MORSKOG SEDIMENTA U OBALNOM PODRUČJU KOSTRENE

S A Ž E T A K

Uzorci morskog sedimenta uzeti su na 4 odabrana mjesta u blizini brodogradilišta u obalnom području Kostrene. Koncentracije policikličkih aromatskih ugljikovodika (PAU), polikloriranih bifenila (PKB) i teških metala (Cu, Pb, Zn, Hg, Fe) su analizirane s kemijsko-analitičkog i toksikološkog gledišta. Za detekciju toksičnosti korišten je test bakterijske bioluminiscencije (*Vibrio fisheri*). Ukupna koncentracija PAU u uzorcima morskog sedimenta varirala je u rasponu od 697 do 7807 μg/kg suhe tvari. Koncentracija PKB u sedimentu je iznosila 1,1 mg/kg suhe tvari. Najviše koncentracije teških metala utvrđene su na mjernoj postaji unutar brodogradilišta. Toksičnost PAU nije korelirala s toksičnim potencijalom sedimenta. Dobiveni rezultati ukazuju na visoku razinu okolišnog rizika, posebice na mjernim postajama unutar brodogradilišta, s 54% mogućnosti toksičnih učinaka. Kemijsko određivanje koncentracije konvencionalnih zagađivala nije dostatno za procjenu kvalitete morskog okružja, pa je neophodno koristiti druge pristupe u procjeni bioloških učinaka.