

# Polycyclic Aromatic Hydrocarbons and Heavy Metals in Kostrena Coastal Area

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## ABSTRACT

*The aim of this study was to determine pollution by polycyclic aromatic hydrocarbons (PAH) and heavy metals in seawater and sediment in Kostrena coastal area, as well as their toxicity using bioluminescence based tests. Total PAH concentration in seawater ranged 1.7–155.3 ng/L. The share of carcinogenetic PAH was relatively high, ranging 22–48.3%. Nickel concentrations in seawater were beyond detection limits (<0.1 µg/L), vanadium concentrations ranged 0.66–1.96 µg/L, chrome concentrations were beyond detection limits, and copper concentrations were also beyond detection limits or extremely low (up to 0.32 µg/L). EC<sub>50</sub> values in seawater ranged 23.80–90.90 ng/L. Correlation between total PAH concentration and toxicity of seawater showed strong connection between them (r=0.9579). Total PAH concentration in marine sediment ranged 58.02–1116 µg/kg dry weight (d.w.). The share of carcinogenetic PAH was extremely high ranging 10–53%. Nickel concentrations in marine sediment ranged 8–24 mg/kg d.w., vanadium concentrations ranged 24–42 mg/kg d.w., chrome concentrations ranged 11–19 mg/kg d.w., and copper concentrations ranged 7–25 mg/kg d.w. EC<sub>50</sub> values in marine sediment ranged 818–4596 µg/kg d.w. Correlation between total PAH concentration and toxicity of marine sediment showed weak connection between them (r=0.2590). Previous studies of seawater samples from areas of the Adriatic sea under the direct influence of oil industry did not include concentrations of heavy metals, which makes our study the first to present such comprehensive results. Our results point out the need for further evaluations and following of marine environment pollution and its consequences on living organisms and marine ecosystem in whole.*

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

## Introduction

Quality of seawater is one of the public health priorities in Croatia<sup>1</sup>. Continuing oil production and refinement growth which is necessary for economic growth led to misbalance between human environment pollution and quality of life. Based on convenient geographic position of Kostrena, in a coastal area near Rijeka, intensive marine traffic and development of many industrial plants led to an increased entry of various toxic chemicals into marine environment. In its development history, Kostrena shows continuing growth of shipyard, energetic plant, road traffic, and also oil refinement industry, which with their intensive activities led to an increased emission of high quantities of toxic chemicals.

Seawater and marine sediments are under direct influence of municipal and industrial wastewater, which often contains various chemicals, especially polycyclic aromatic hydrocarbons (PAH)<sup>2,3</sup>. After biotransformation in marine organisms, 16 PAH show either acute toxicity or carcinogenicity, being widely disseminated in marine environment and therefore considered as one of the major pollutants.

The presence of heavy metals in marine environment represents a serious problem, because of their marked toxicity, resistance to degradation and biological accumulation<sup>4</sup>. The main source of heavy metals in seawater

and marine sediments are nearby rivers and lakes<sup>5</sup>, as well as chemical industry. Basic ecological characteristics of heavy metals are resistance to degradation and persistency. Once present in marine environment (either as particles or dissolved), heavy metals accumulate in marine sediments, and partially in living organisms<sup>6,7</sup>.

Toxicity tests based on bioluminescence include protozoa, algae, bacteria, crabs, seashells and fishes<sup>8–10</sup>. Bulich in 1979 described a first commercial test – based on luminescent bacteria usage for toxicity determination after short-term exposure (less than 30 minutes) to various pollutants<sup>11</sup>. That test was developed using bacteria *Photobacterium phosphoreum* (later renamed into *Vibrio fischeri*). It detects pollution by organic chemicals, as well as metals, and is widely used as acute toxicity marker<sup>12</sup>.

Because of everything above mentioned, Kostrena is considered an area of high health risk for people, and represents a suitable area for research of this type. Only by systematic research we can better understand pollution dynamics and estimate risk for the ecosystem. The aim of this study was to determine pollution by PAH and heavy metals analyses in seawater and marine sediments and compare results with previous studies, as well as to determine their toxicity using bioluminescence based tests.

## Materials and Methods

### Study area

Kostrena is situated in the Northwestern part of the coastal area of the Adriatic sea, nearby eastern side of the city of Rijeka. With the total area of 12.07 km<sup>2</sup>, sea area of 4.78 km<sup>2</sup>, and 12.5 km long coast, Kostrena is characterized by significant influence of oil industry, power plant and shipyard on environment.

### Sampling

Seawater and marine sediment samples were collected on 4 stations nearby oil industry in Kostrena area. Samples were then transported to laboratory and stored using standard protocols<sup>13,14</sup>.

### PAH in seawater

After being chemically prepared using solid phase extraction (SPE) by standard protocols<sup>15–17</sup>, samples were analyzed by using gas chromatography with mass spectrometry (GC-MS).

### PAH in sediment

After being chemically prepared by standard protocols<sup>18,19</sup>, samples were analyzed by using liquid chromatography (LC) with ultraviolet and visible parts of electromagnetic spectrum (UV-VIS) and fluorescent detector.

### Heavy metals in seawater

Samples prepared by standard protocols<sup>20</sup> were analyzed using Autosampler ASX 510, Cetac, SAD, con-

nected to high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS) to quantify heavy metals in seawater.

### Heavy metals in sediment

After sample preparation by standard protocols<sup>21</sup>, heavy metals were quantified using atomic-adsorption spectrometer Perkin Elmer 200, Analyst 600, SAD. Heavy metals (vanadium, copper and nickel) were analyzed by flameless technique (HRN EN ISO 15586:2003), and chrome by standard method (HRN EN ISO 1223:1998).

### Toxicity tests

Seawater and sediment samples were prepared by standard protocols<sup>22,23</sup>. Toxicity of the sediment was determined by measurement of the decrease of the initial bioluminescence of the *Vibrio fischeri* bacteria NRRL B-11177, using Microtox-test DIN EN ISO 11348-3 Biofix®. Changes in luminescence were measured using Microtox 500 (AZUR Environmental) luminometer. For determination of the seawater toxicity Microtox-test DIN EN ISO 11348-2 Biofix® was used. Changes in luminescence were measured using Monolight 2010 (Analytical Luminescence Laboratory) luminometer. Results were expressed as EC<sub>50</sub> – pollutant concentration with 50% decrease in bacterial bioluminescence.

### Statistics

Statistical analysis was performed using Statistica 8.1 (StatSoft Inc., Tulsa, SAD). The level of statistical significance was set at  $p < 0.05$ .

## Results

Total PAH concentration in seawater ranged from 1.7 ng/L to 155.3 ng/L. The share of carcinogenic PAH in total PAH was relatively high in all seawater samples, ranging 22–48.3%.

Nickel concentrations in seawater were beyond detection limits ( $< 0.1 \mu\text{g/L}$ ) on all stations, vanadium concentrations ranged 0.66–1.96  $\mu\text{g/L}$ , chrome concentrations were beyond detection limits ( $< 0.1 \mu\text{g/L}$ ) on all stations, and copper concentrations were also beyond detection limits or extremely low (up to 0.32  $\mu\text{g/L}$ ).

EC<sub>50</sub> values in seawater samples ranged from 23.80 to 90.90 ng/L. Correlation between total PAH concentration and toxicity of seawater samples showed strong connection between the two parameters ( $r = 0.9579$ ).

Total PAH concentration in marine sediment ranged from 58.02 to 1116  $\mu\text{g/kg}$  dry weight. The share of carcinogenic PAH in total PAH was extremely high in all sediment samples, ranging 10–53%.

Nickel concentrations in marine sediment ranged from 8 to 24 mg/kg dry weight, vanadium concentrations ranged from 24 to 42 mg/kg dry weight, chrome concentrations ranged from 11 to 19 mg/kg dry weight, and copper concentrations ranged from 7 to 25 mg/kg dry weight.

EC<sub>50</sub> values in marine sediment samples ranged from 818 to 4596 µg/kg dry weight. Correlation between total PAH concentration and toxicity of marine sediment samples showed weak connection between the two parameters ( $r=0.2590$ ).

## Discussion and Conclusion

According to Baumard et al. and Gogou et al. PAH concentrations in marine sediment samples are the reflection of their accumulation during longer periods of time, so it was our aim to determine PAH concentrations range in marine sediment samples being under the influence of oil industry pollution<sup>24,25</sup>.

There have been only a few studies previously published on presence of PAH in marine sediment in Adriatic sea. In a recent study by Bihari et al. PAH concentrations in Rijeka bay ranged from 213 to 695 µg/kg dry weight<sup>26</sup>. Magi et al. also in the Adriatic sea, Chioggia coast – Italy, found PAH concentrations in marine sediment ranging from 24.1 to 507.1 µg/kg dry weight<sup>3</sup>. Guzzella and De Paolis measured concentrations ranging from 27 to 527 µg/kg dry weight on the Adriatic coast from Trieste bay to the very southern end of Italian coast<sup>27</sup>. Results obtained in these studies are similar to our results.

Total PAH concentrations in studies of marine sediment from areas with chemical and heavily industry ranged 18.5–4888 µg/kg dry weight; 1–20500 µg/kg dry weight; 720.62–1871.37 µg/kg dry weight and 428–3257 µg/kg dry weight<sup>24,28–30</sup>. These results are much higher than those obtained in our study. According to Salizzato et al. study of marine sediment from Venice channel, total PAH concentrations ranged from 1 to 120 µg/kg dry weight, and are much lower than those obtained in our study<sup>31</sup>.

Salizzato et al. obtained similar results to ours on nickel concentrations in marine sediment of Venice channel, ranging 10–36 mg/kg dry weight<sup>31</sup>. Cuculić et al. found copper concentrations in marine sediment samples of Mljet National Park ranging from 6.8 to 18.9 mg/kg dry weight<sup>32</sup>, which are in accordance to those obtained

in a recent study by Tsakovski et al., while chrome concentrations were similar to our study (20.06 mg/kg dry weight) and vanadium concentration of 15.86 mg/kg dry weight also not being much lower than our results<sup>33</sup>.

Since correlation between total PAH concentration and toxicity of marine sediment samples was weak, while carcinogenic compounds were found in all sediment samples from Kostrena area, it is arguable whether Microtox®-test is in every situation the best marker of ecotoxicity caused by concentration of a single pollutant.

Results of our study are in accordance with those from Bihari et al. who analyzed PAH concentrations in Kvarner bay area that ranged from 0 to 305 ng/L<sup>26</sup>. Fernandes et al. found PAH concentrations ranging from 2 to 687 ng/L in Sein river, which is similar to our results<sup>34</sup>. On the other hand, our results are much lower than those from some other studies conducted on rivers in China, with much higher concentrations ranging from 6960 to 26900 ng/L and from 4228 to 29325 ng/L<sup>17,19</sup>. Such high values suggest intensive human activities, such as agriculture and shipyards.

Previous studies of seawater samples from areas of the Adriatic sea under the direct influence of oil industry did not include concentrations of heavy metals, which makes our study the first to present such comprehensive results.

Based on everything mentioned above, our results point out to the fact that concentration of conventional pollutants alone is not always the best marker of marine environment pollution. Since the number of toxic chemicals is extremely big, it is impossible to resolve that issue by measuring their total concentrations<sup>35</sup>.

This study addresses the issue of marine environment pollution with PAH and heavy metals that originate from oil industry and are being deposited into the seawater by various environmental processes. Because of their resistance to degradation and persistency, accumulation is inevitable. Our results point out the need for further evaluations and following of marine environment pollution and its consequences on living organisms and marine ecosystem in whole.

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## **POLICIKLICKI AROMATSKI UGLJIKOVODICI I TEŠKI METALI U OBALNOM PODRUČJU KOSTRENE**

### **SAŽETAK**

Cilj ove studije je bio utvrditi zagađenje policikličkim aromatskim ugljikovodicima (PAU) u morskoj vodi i sedimentu obalnog područja Kostrene, kao i njihovu toksičnost korištenjem testova baziranih na bioluminiscenciji. Ukupna koncentracija PAU u morskoj vodi imala je raspon 1,7–155,3 ng/L. Udio karcinogenih PAU je bio relativno visok i iznosio je 22–48,3%. Koncentracija nikla u morskoj vodi bila je ispod granica detekcije (<0,1 µg/L), koncentracija vanadija iznosila je 0,66–1,96 µg/L, koncentracija kroma bila je ispod granica detekcije, a koncentracija bakra je bila ispod granica detekcije ili vrlo niska (do 0,32 µg/L). EC<sub>50</sub> vrijednosti iznosile su 23,80–90,90 ng/L. Korelacija između ukupne koncentracije PAU i toksičnosti morske vode ukazivala je na jaku međusobnu povezanost ( $r=0,9579$ ). Ukupna koncentracija PAU u morskom sedimentu iznosila je 58,02–1116 µg/kg suhe težine (s.t.). Udio karcinogenih PAU je bio vrlo visok i iznosio je 10–53%. Koncentracija nikla u sedimentu je iznosila 8–24 mg/kg s.t., koncentracija vanadija iznosila je 24–42 mg/kg s.t., koncentracija kroma iznosila je 11–19 mg/kg s.t., a koncentracija bakra iznosila je 7–25 mg/kg s.t. Korelacija između ukupne koncentracije PAU i toksičnosti morskog sedimenta ukazivala je na slabu međusobnu povezanost ( $r=0,2590$ ). Prethodne studije na uzorcima morske vode iz područja Jadranskog mora pod izravnim utjecajem naftne industrije nisu uključivale koncentracije teških metala, što ovu studiju čini prvom koja iznosi ovako sveobuhvatne rezultate. Naši rezultati ističu potrebu za daljnjim istraživanjem i praćenjem zagađenja morskog okoliša i njegovih posljedica na žive organizme i morski ekosustav u cjelini.