EVALUATION OF BIOMARKERS RESPONSE AT THE SE GULF OF TRIESTE (SLOVENIA) AND BOKA KOTORSKA (MONTENEGRO)

Andreja Ramšak [1]*, Bojana Tkalcic [2] and Zoran Kljajic [3]
[1] Marine biology station, National institute of biology - ramsak@mbss.org
[2] 2Predstruge 66, 1312 Videm-Dobrepolje, Slovenia
[3] 3Institut za biologiju mora, Dobrota bb., p.fah 69, 85330 Kotor, Montenegro

Abstract
In order to assess spatial distribution and temporal trends of pollution, response of different biomarkers of exposure were measured at three stations in Slovenian coastal waters included in long term biomonitoring MED POL programme and at two stations in Montenegro. Biomarkers response showed no differences between stations with different ecological status. Keywords: biomonitoring, Adriatic Sea, *Mytillus galloprovincialis*
Keywords: Adriatic Sea, Mollusca, Monitoring

Introduction
A suite of biomarkers were used to measure effects of pollutants at northern and southern Adriatic, two regions with different pollution loadings and ecological characteristics. Both examined areas are still under high anthropogenic pressures with emphasizing urban and industrial wastewaters, tourism and agriculture. Both examined coastal area is highly pressured by building and reconstruction, small shipyards and maritime transportation. The coastal sea is intensively exploited by fishery and aquaculture. Chemical monitoring of sediments and sea water was mostly focused to persistent aromatic hydrocarbons and heavy metals. Distribution of certain heavy metals showed most pronounced difference between areas; stations in northern Adriatic being more loaded with mercury (1) and southern stations are more loaded with lead. The stations received different pollution loadings mostly from point sources. Ecological status of sampling stations was yearly evaluated in northern stations according to guidelines WFD (2).

Results and discussion
Biomarkers responses were measured in mussels (*Mytillus galloprovincialis)* collected from their natural beds at three sites along Slovenian coast and at two sites at Boka Kotorska Bay. Reference sites were Bay of Strunjan (SLO) and Bay of Kotor (MNE). Metallothionein concentration (MT) and micronuclei frequency (MN) was analysed and evaluated according to the recommended methodology (3), acetylcholinesterase activity (AChE) was determined using Ellman colorimetric method (4) using the whole viscera in crude homogenate and acetycholin as substrate. The statistical differences among samples from different sites were tested by the analysis of variance (ANOVA Tukey-post-hock HSD test).

Table 1. Response of biomarkers from sampling sites at northern and southern Adriatic Sea.

	Slovenia			Montenegro	
Biomarker	Bay of Koper	Bay of Strunjan	Bay of Piran	Kotor	Bijela
Ecological status after WFD	Moderate	Very good	Good	/	/
Condition index	0,12	0,14	0,12	0,09	0,09
Micronuclei frequency (‰)	5±1	3±1	3±1	3±1	4±1
Metalothioneins (µg/g w.w.)	135±16	144±13	117±7	48±5	39±5
Acetylcholin esterase activity (nmol/min mg)	8,34±1	10,7±3,2	9,46±1,4	9,45±5	12,63±1,2

Differences in circulation regime and ecological characteristics between sampling stations at northern part (SE Gulf of Trieste) with stations at southern Adriatic (closed Bay of Kotor and Bay of Tivat) make comparison and analysis of biomarkers very valuable and interesting in terms of pollutants distribution and their effect to mussels. Adoption of pollutans such as heavy metals in caged mussels at northern and southern stations revealed almost equal concentrations of Hg, Pb, Ni, but twice higher amount of Cr in Bay of Koper comparing with other stations (project Mytiad 2008). Slightly higher amount of PAH were determined in mussels from Bay of Kotor during the same experiment comparing with northern reference site Bay of Strunjan. Ten times higher content of organochlorine pesticides was found in mussels from reference site in Bay of Kotor comparing with reference site at Bay of Strunjan. Frequencies of MN were followed in the period of one year at northern stations. Higher frequency was detected during the May at the same station in Bay of Koper and in caged mussels exposed in Slovenian marinas with poor circulation and higher level of pollutants. Station in Bay of Tivat (Bijela) was exposed to higher concentrations of Cu, Pb, Zn; especially Cu can act as genotoxic agents. Condition index should be used in biomonitoring to adjust the metal concentration in mussels and to eliminate the effect of trophic differences between stations. Spatial distribution was evident in condition index among stations which corresponds the trophic conditions at sampling sites. Most pronounced difference between northern and southern stations was in MT concentrations. Three times higher MT concentration was found at northern stations comparing with southern stations in wild mussels collected and analysed at the same time. The level of MT concentration at northern stations was in the same range through ten years period (5). The level of Cd in mussels is up to 1,0 mg kg^{-1} and Hg 0,3 mg kg^{-1} with trend to decreased in last years as established through monitoring programme (6). Substantial difference in heavy metals pollution among northern and southern stations was in concentration of some metals. Station Bijela (Bay of Tivat) have higher levels of some heavy metals (arsenic and lead) with low binding capacity to MT. On the other side, the concentration of total Hg was determined to be ten times higher in northern Adriatic than in other parts (1). The higher level of MT in northern stations could be a consequence of high binding affinity of Hg to MT, even though its low induction ability. The MT concentration showed decrease from northern to southern Adriatic. Results on MT concentrations obtained in mussels from eastern coast of Istria and Kvarner (Croatia) were between 50- 100 µg/g w.w. (7). Activity of AChE was measured in whole mussel tissue and was in the same range at northern and southern stations as measured in September 2009. Lower activity of AChE was found in May at stations in Bay of Strunjan and Bay of Piran.

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