HEAVY METALS CONCENTRATION AND METALLOTHIONEIN CONTENT IN RESIDENT AND CAGED MUSSELS *MYTILUS GALLOPROVINCIALIS* FROM RIJEKA BAY, CROATIA

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SUMMARY

Concentration of lead, cadmium, mercury, arsenic, copper, zinc, chromium, nickel, manganese and iron and metallothionein content were examined in resident and caged mussels (*Mytilus galloprovincialis*) at five sites along Rijeka Bay, a coastal ecosystem highly susceptible to urban and industrial pollution. Higher concentration of metals was found in resident mussels, with the highest level in comparison to control site Lim detected for lead, copper and chromium. Caged mussels from all sites displayed increasing level of metals with respect to control site Lim after one month of exposure. Significantly elevated metallothionein content was found in resident mussels at all sites except Kraljevica shipyard, and in caged mussels at sites Luka Rijeka harbour and urban area Bakar. PCA analysis revealed that mussels resident to urban area of river Rječina mouth, Rijeka harbour and Treći Maj shipyard were clearly separated from Kraljevica shipyard due to different pattern of metal bioaccumulation and metallothionein content. Resident mussels from urban area of Bakar and caged mussels were less influenced by metal load.

KEYWORDS:
Mussels, *Mytilus galloprovincialis*, Metal exposure, Metallothionein content, PCA analysis.

INTRODUCTION

To evaluate the risk for integrity of marine ecosystem, with respect to increasing trends in urbanization and industrialization along the coastal zones, the marine monitoring programs have been widely implemented since the late 70-ties [1]. The health status of coastal areas is assessed by chemical analysis of biota, water and sediment and by monitoring of biological effect of contaminants [2]. Among them, metals are considered as potentially highly toxic and capable to induce a whole range of deleterious processes ultimately decreasing the quality of marine ecosystem.

Bivalve mussels *Mytilus galloprovincialis* are bioindicator organisms frequently used for monitoring the quality of coastal waters [3] and are commonly employed for assessment of heavy metal pollution [4-8]. Exposure of a variety of marine organisms, including mussels, mainly to cadmium, copper and zinc, is associated with induction of metallothioneins, low molecular weight, cysteine rich cytosolic polypeptides involved in homeostasis of essential and detoxification of non-essential metals [9-11]. Metallothionein content determination is employed for assessment of adverse biological effects of metals pollution [12-15].

In this study, heavy metal concentration and metallothionein content were determined in resident and caged mussels from Rijeka Bay, to evaluate the extent of metal pollution and to provide the baseline data for future monitoring of the anthropogenic impact in this economically important region of the north-eastern Adriatic. Rijeka Bay is surrounded by many land-based sources of contamination spread along the coastline related to heavy industries and urban wastewater. Consequently, a wide range of metals are released directly into to the sea, by flows through the coastal waters and rivers, or from distant sources by atmospheric transport as evidenced by recent report on sediment metal concentration [16-17].

MATERIALS AND METHODS

Five sampling sites along Rijeka Bay selected for the study were located in the proximity to discharge points of urban sewage and industrial outlets and within the zone of intensive marine traffic (Figure 1 and Table 1).

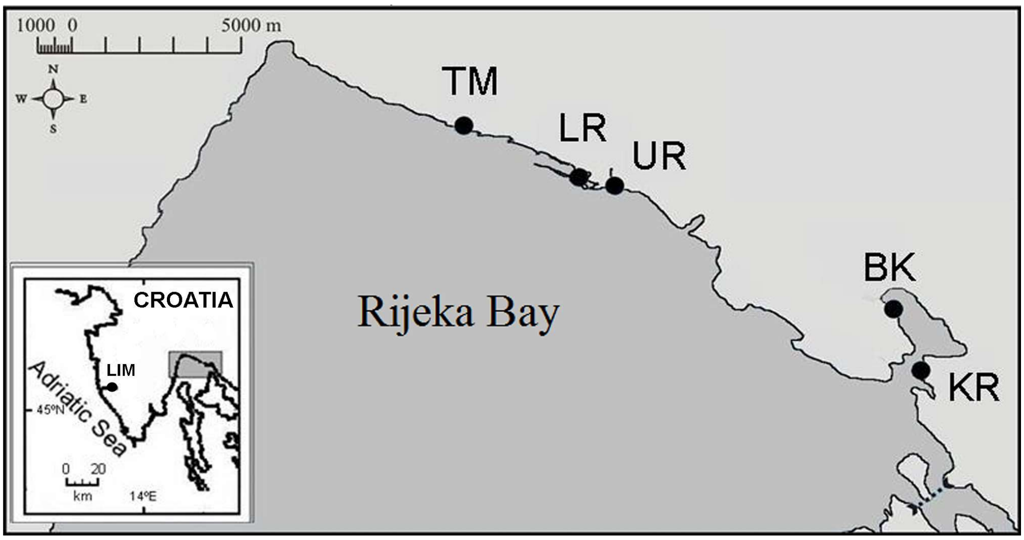

FIGURE 1 - Geographic location of sampling sites. TM - Treći maj; LR - Luka Rijeka; UR - river Rječina mouth; BK - Bakar; KR - Kraljevica; LIM - control site of origin.

TABLE 1 - Sampling site codes, short description and environmental parameters.

Site code	Description	Temp. (°C)	pH	Sal. (‰)	DO (mg/l)
LIM	Mariculture (control site of origin)	19.0	7.99	35.71	8.20
TM	Shipyard	19.5	8.10	35.29	8.33
LR	Harbour, industry, urban runoff	19.0	8.06	27.30	8.34
UR	River mouth, urban runoff	17.5	8.16	16.40	10.85
BK	Urban runoff	19.0	7.74	13.38	10.04
KR	Shipyard, urban runoff	19.0	7.88	27.78	7.67

Abbreviations: Temp, temperature; Sal, salinity; DO, dissolved oxygen

The study was carried out in April 2006, in the period of lowest influence of temperature and reproductive processes on the metal accumulation and metallothionein induction [18-19]. Mediterranean mussels (*Mytilus galloprovincials* Lam.) of shell length 5-6 cm, and weight 15 – 20 g, were purchased from aquaculture farm within the inner part of Lim Bay (site Lim) on the north eastern part of Adriatic Sea. ~~Site Lim was considered as control site.~~ In accordance with previous biomonitoring studies [13], site Lim was considered as control site, since it is far from urban and industrial influence. Mussels were transported in wet and cool plastic containers to selected sites within two hours from collection. Sets of randomly chosen animals were placed in synthetic mesh and immersed close to the mussels resident to sampling site. After one month of exposure, caged mussels as well as mussels resident to investigated sites, were simultaneously collected, transported to the laboratory as described above, and immediately processed. Water temperature, pH, salinity were determined using Mettler Toledo - SevenMulti® meter, whereas dissolved oxygen (DO) was measured using Winkler method (Table 1).

Following collection, the soft tissue of thirty mussels was dissected with a clean scalpel blade, pooled, freeze-dried and homogenized according to AOAC Official Method 937.07 [20]. Aliquots (1g) of mussels' tissue sample were added with 5 ml of 65 % HNO_3 and subsequently digested in a microwave oven (MLS 1200 MEGA, "exhaust module" EM-45/A, Milestone, Italy) according to standardized procedure (HRN EN 14084). The digested samples were then diluted 5 times (v/v) with Mili-Q water. Lead, cadmium, arsenic, copper, chromium, nickel, manganese and iron were analysed by atomic absorption spectrophotometry (Perkin Elmer, Analyst 600) in a graphite oven with autosampler (Perkin Elmer AS 800). Zinc was analyzed using air acetylene flame atomic absorption spectrophotometer (Perkin Elmer, AS 200) and mercury was analyzed with cold-vapour flow injection Mercury System (Perkin Elmer, FIAS

400) supplemented with Autosampler (Perkin Elmer, AS 90). Metallothionein content was determined using spectrophotometric method based on the estimation of the sulfhydryl residue content [21]. Five pools were prepared from three digestive glands (fifteen mussels in total per site) and homogenized in three volumes of ice-cold 20 mM Tris–HCl buffer, pH 8.6, containing 0.5 M sucrose, 0.006 mM leupeptin, 0.5 mM PMSF and 0.01% β-mercaptoethanol using Teflon Potter homogenizer. The homogenate was centrifuged at 15000*g* for 30 min (+4ºC) and the pellet discarded. Aliquots of 1 ml of supernatant were added with 1.05 ml of cold ethanol (−20°C) and 80 µl chloroform, and centrifuged at 6000*g* for 10 min. Metallothioneins present in the supernatant were collected by precipitation with three volumes of cold ethanol (−20°C), kept at −20°C for 1 h and centrifuged at 6000*g* for 10 min. The pellets were then dissolved with 87% ethanol and 1% chloroform in 0.5 M sucrose homogenizing buffer and dried. The metallothionein content was evaluated by colorimetric method using Ellman's reagent (5.5 dithiobis 2 nitrobenzoic acid, DTNB) [22]. Pellets were re-suspended in 300 µl of solution containing 5 mM Tris–HCl buffer with 1 mM EDTA at room temperature. Aliquots of 4.2 ml containing 0.2 M Na-phosphate buffer pH 8.0 and 0.43 mM DTNB were added to the suspension of pellets. Following 30 minutes of incubation and final centrifugation of samples at 6000*g* for 10 min, the absorbance of the supernatant was measured in triplicates at 412 nm. Metallothionein content was calculated using reduced glutathione (GSH) as reference standard [21] and expressed as micrograms of metallothionein per gram of wet weight tissue.

Statistical differences in metallothionein content between sampling sites were established by Wilcoxon test. Mean values of the metallothionein content and chemical data were used for the correlation analysis using Spearman's test. A value of $p<0.05$ was considered significant. Principal component analysis (PCA) was applied using data on metal concentrations and metallothionein content in the tissues of resident and caged mussels from all sites, and control site of origin. The data used for PCA analysis were logarithmically transformed.

RESULTS AND DISCUSSION

Concentrations of ten metals (lead, cadmium, mercury, arsenic, copper, zinc, chromium, nickel, manganese and iron) in resident and caged mussels from all sites and from control site Lim are given in Figure 2. Generally, both resident and caged mussels displayed elevated concentration of metals with respect to mussels from control site Lim. Differences in the accumulation pattern of metals were observed between resident and caged mussels.

Lead, copper and chromium generally displayed the most notable increase both in resident and caged mussels in comparison to control site Lim.

The concentrations of lead found in the tissues of resident mussels were near or above 6 mg/kg d.w, with exception of urban site UR. As for the caged mussels, the highest concentration was found in LR harbour (4.3 mg/kg d.w.). Levels of lead above 5 mg/kg d.w. were recently found within Rijeka Bay by other authors [23-24] indicating an ongoing trend in the input of this metal possibly due to nearby intensive anthropogenic activities. Contamination of sediment with lead recently found within the zone investigated in this study was also strongly related to anthropogenic input, mainly in relation to port activities and oil refinery [16-17]. In general, lead concentrations reported in this study fall within the range of values previously found in polluted hot spots along the eastern Adriatic coast [5-6,24] and some Mediterranean areas [15]. However, they were lower than those recently reported sites under urban and industrial impact along the south eastern Adriatic coastal zone [25].

Resident mussels displayed concentration of copper between 1.3 and 8.2 mg/kg d.w. with exception of KR shipyard where notably higher level (22 mg/kg d.w.) was detected. The observed accumulation of copper ~~which~~ could be associated with the influx of copper-based antifouling paints. Similarly high copper concentrations were previously observed along the eastern Adriatic coast in the vicinity of harbours and urbanised areas [5-6] although in highly polluted environment even higher levels were occasionally found [6,15]. With exception of KR shipyard, the values reported herein for copper concentrations in the tissues of mussels are in the range of those recorded in the south eastern Adriatic coast and the Mediterranean [15,25-26] within coastal zones characterised by different level of anthropogenic impact.

Chromium concentration ranged from 0.59 to 1.61 mg/kg d.w. and from 0.41 to 0.78 mg/kg d.w. in resident and caged mussels, respectively. ~~with t~~The lowest level was found at urban site BK, and highest level at urban site UR, for both populations of mussels. ~~Although g~~Generally higher level of chromium in comparison to control site Lim was found particularly in the tissues of resident mussels. Nevertheless, it should be taken into account that the concentrations found at the investigated sites did not exceed the range of values determined along the eastern Adriatic coast irrespective on the pollution impact [5-6,27].

The concentrations of mercury, iron, cadmium, zinc and nickel in the tissues of both resident and caged mussels at all sites generally displayed lower degree of accumulation in comparison to control site Lim.

Values for mercury concentration varied between 0.12 and 0.79 mg/kg d.w. Values close to or above 0.25 mg/kg d.w. that were detected in resident mussels from LR harbour, TM and KR shipyards and caged mussels from LR harbour and KR shipyard corresponded to those recently detected in the close vicinity to a former chlor-alkali plant [28]. Besides, mercury concentration reported for mussels resident to KR shipyard exceeded those previously reported for highly polluted harbours of Barcelona and Genoa [15] and for the region within Spanish coast known for mercury exploitation and production [4]. Probable contamination of mussels from LR harbour and TM shipyard is in accordance with particularly high mercury enrichment of sediments found at these locations [16-17]. However, even higher concentrations of mercury in the tissues of mussels resident to polluted marine environment were detected in the south eastern part of the Adriatic coast [25,29].

The concentrations of cadmium ranged from 0.63 to 1.75 mg/kg d.w. Values above 1.2 mg/kg d.w. that were found in the tissues of resident mussels at almost all locations could be attributed to cadmium pollution [6]. Furthermore, nearly as high level of cadmium was found in the tissues of caged mussels from TM and KR shipyard. In agreement with our results, concentration of cadmium above 1.5 mg/kg d.w. were recently found at site LR harbour and TM shipyard [23] suggesting relatively steady input of this metal over the last few years. The cadmium concentrations reported herein were lower than those detected in mussels from south eastern Adriatic coast [29]. On the other hand, values for cadmium were above those recorded in Barcelona harbour [15] and some sites within urbanized and industrialized areas of Spanish coast [4].

Zinc concentrations were in the range between 117.1 and 270.6 mg/kg d.w. with maximum values found in resident mussels from sites TM shipyard and LR harbour. These values ~~that~~ were slightly above ~~higher than~~ the typical level established for this metal in other monitoring studies of eastern Adriatic coast [5-6,27] and Mediterranean coastal regions [30].

The range of iron concentrations reported in this study (116.15-418.8 mg/kg d.w.) corresponds to those detected along the eastern Adriatic coast [27]. In particular, the maximum values that were found in the tissues of resident mussels from urban site UR and caged mussels from LR harbour were also previously observed at sites in the vicinity to heavy industries and discharge of untreated wastewater [25,27].

Nickel concentrations varied from 0.47 to 2.78 mg/kg d.w. with the highest values found in resident mussels from LR harbour, urban area UR, TM shipyard and caged mussels from KR shipyard (above 2 mg/kg d.w.). These values were either in agreement [27] or far below those recorded at sites highly influenced by anthropogenic activity along the eastern Adriatic coast [25,29].

Finally, the lowest degree of accumulation with respect to control site Lim was found for arsenic and manganese.

With exception of mussels caged at LR harbour, the concentration of arsenic was above 24 mg/kg d.w. both in resident and caged populations. In addition, arsenic level in the tissues of control mussels from Lim was nearly 27 mg/kg d.w. These values were relatively high when compared to the data reported for unpolluted to highly polluted locations of the eastern Adriatic coast [24,29,31].

Manganese concentrations ranged between 4.4 and 11.2 mg/kg d.w. with the highest values (above 8 mg/kg d.w.) found in resident mussels at sites LR harbour and TM shipyard. However, these maximums couldn't be indicative of manganese pollution considering wide range of values for this metal reported previously for both unpolluted and highly polluted sites along eastern Adriatic coast [6,29].

Resident mussels exhibited lower concentrations of metals than mussels caged at the same site for copper at urban area UR, LR harbour and TM shipyard, cadmium at TM shipyard, arsenic at KR shipyard, nickel at urban site BK and KR shipyard, manganese at urban site UR and iron at LR harbour (Figure 2). This result could indicate adaptation of resident mussels to long-term exposure to above metals at those sites [32-33]. However, more detailed research of temporal trends in metal accumulation is needed to support this hypothesis.

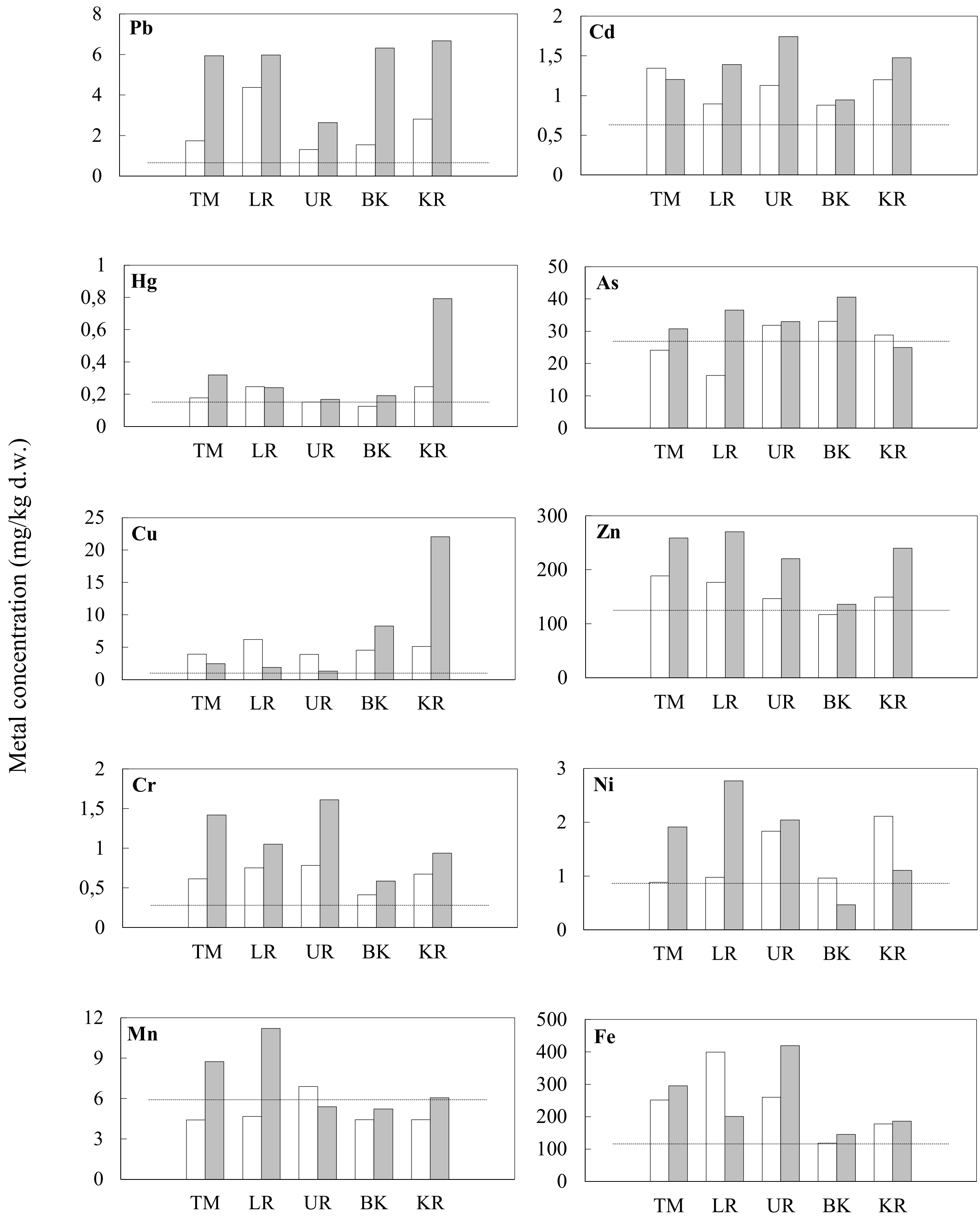

FIGURE 2 - Concentration of metals (mg/kg d.w., dry weight) in the soft tissues of caged (□) and resident (■) mussels from Rijeka Bay. The dotted horizontal line indicates concentration of the respective metal in control mussels from site Lim.

A strong and significant ($p<0.05$) positive correlation between Hg and Cu ($R = 0.83$), lead and copper ($R = 0.94$) and zinc and manganese ($R = 0.83$) was revealed from correlation matrix of metals in the tissues of the resident mussels (Table 2). Caged mussels displayed significant ($p<0.05$) correlations between lead and copper ($R = 0.94$) and mercury and zinc ($R = 0.83$) and iron and chromium ($R = 0.89$). Significant positive correlation between copper and lead and mercury and zinc was also reported previously [6] suggesting similar accumulation and elimination process for these metals.

Metallothionein content in the digestive gland of resident and caged mussels is presented in Table 3. In general, the metallothionein content of both resident and caged mussels was higher with respect to control site. Significant increase was detected in the tissues of resident mussels from all sites except in KR shipyard and caged mussels from urban area BK and LR harbour. The increased metallothionein content in the digestive gland of mussels indicates the biological response to metal exposure. In agreement, elevated metallothionein content in relation to metal exposure was reported in other biomonitoring studies [34-36]. Positive correlation between metallothionein content in the tissues of resident mussels and metal concentration was found for zinc ($R = 0.83$, $p<0.05$) (Table 2). As for the caged mussels, significant correlation was detected only for copper ($R = 0.89$; $p<0.05$). The induction of metallothionein with increasing zinc and copper concentrations in the tissues of mussels was found previously [37-38] in line with the role of this protein in the metabolism as well as detoxification of the excess amount of these essential metals. Elevated metallothionein content could be also attributed to cadmium accumulation in the tissues of mussels. Cadmium is a toxic non-essential metal most often associated with metallothionein induction in mussels [7,18]. However, the clear relationship of each metal and induction of metallothionein is difficult to establish since complex interactions between different metals and other pollutants normally occur in the realistic environmental conditions [39-41]. In addition, the specificities of metal sequestration mechanisms, scavenging of metallothionein-metal complexes and metallothionein turnover are still unknown for most toxic metals [41].

Metallothioneins could be also induced under oxidative stress conditions generated by pro-oxidant activity of organic compounds [42]. As for common marine contaminants such as PAHs, higher concentrations were detected at the sites investigated in the current study (personal communication). However, the clear relationship of PAH's and metallothionein content in the tissues of mussels remains to be clarified. In fact, benzo(a)pyrene (BaP), one of the most toxic of the class of PAHs, failed to induce metallothionein synthesis in mussels *M. galloprovinciallis* [43] and clams *Ruditapes phillipinarium* [44]. However, in the latter study it was implied that BaP metabolites increase the toxicity of metals since the induction of metallothioneins was less pronounced upon exposure to mixture of cadmium and BaP, in comparison to cadmium exposure only.

It is well known that seasonal fluctuations of metal concentrations in the tissues of mussels related to abiotic and biotic factors could have significant influence on metallothionein induction in the tissues of bivalves [18-19,23,36,42]. This study was conducted in spring, to avoid the influence of winter and summer temperature extremes typical for Northern Adriatic, on metallothioneins induction, as suggested previously [19]. ~~the period of the lowest influence of spawning and nutritional factors on metallothionein variation~~ ~~This is also the~~ ~~period when~~ ~~As for the environmental factors,~~ In addition, large fluctuations were recorded in the salinity level between sampling sites. Previous studies revealed considerable negative correlation of salinity and metallothionein level in the digestive gland of mussels [18,45]. Thus, it should be taken in consideration that significantly higher metallothionein content recorded at urbanised sites BK and UR could be also linked to lower salinity (13.38 and 16.40 ‰, respectively), rather than to metal bioavailability alone.

Finally, gradual accumulation of nutrients during the pre-spawning season in summer and autumn could introduce significant variation of metal concentrations and metallothionein content in the digestive gland due to "biological dilution" effect [18]. Moreover, it has been previously reported that mussels display the maximum content of metallothionein in digestive gland in late winter and spring [18,36]. This could explain the elevated values of metallothionein obtained in the current study (above 150 µg/g w.w.) in comparison to other field studies along the eastern Adriatic coast [18,46].

Metallothionein ~~concentrations~~ content in control mussels at site Lim and resident mussels from KR shipyard characterized by substantial input of copper, a known inducer of this protein [7], were nearly equal. Previous laboratory experiments showed that despite significant accumulation of copper in the tissues of mussels no induction of metallothionein was detected suggesting that basal level of this proteins could be sufficient for copper detoxification [47]. On the other hand, no metallothionein increase at KR shipyard could also indicate that the critical level of copper in the tissues could be reached and exceeded due to exposure to relatively high copper concentration, and mussels were not able to cope anymore with metal stress by *de novo* synthesis of metallothionein [48-50]. However, the underlying regulation mechanisms of these molecular events are currently not well understood.

In this study, the response to metal exposure was analysed in resident and caged mussels. Generally higher concentration of metals and metallothionein content was detected in resident mussels at most of the sampling sites. This could be a result of cumulative effect of contaminants as already observed when mussels resident to investigated area were employed in coastal monitoring programmes [51-53]. Caged mussels were exposed for one month, before metal accumulation in the tissues could reach maximum level. In fact, maximum load of metals in the tissues of mussels and adaptation to environmental conditions by reaching steady state equilibrium between metal uptake and excretion is typically observed after three months of exposure in marine environment [32]. However, despite relatively short time of exposure, the increase of metallothionein content at sites BK and LR was observed indicating a rapid response to stress conditions. Thus, it seems that application of caged mussels from the same origin, as an alternative approach used ~~not only~~ to minimise the effect of innate factors (sex, age, reproductive stage), ~~but also~~ and the influence of adaptation to local conditions on biomarker response [51-54] could be applicable for examination of response following short-term exposure to metal stress. Furthermore, our results support the simultaneous use of resident and caged mussels to provide better picture of metal bioavailability in marine environment, as previously suggested [32].

TABLE 2 - Spearman correlation coefficients between MT content and Pb. Cd. Hg. As. Cu. Zn. Cr. Ni. Mn and Fe concentrations in resident and caged mussels. Correlations significant at p<0.05 are indicated by asterisk (*)

	Pb	Cd	Hg	As	Cu	Zn	Cr	Ni	Mn	Fe
resident mussels										
MT	0.09	0.37	0.31	0.54	0.03	***0.83**	0.71	0.77	0.54	0.71
Pb		0.26	0.77	0.09	***0.94**	0.37	-0.09	-0.14	0.09	-0.09
Cd			0.37	-0.09	0.20	0.49	0.77	0.66	0.14	0.77
Hg				-0.26	***0.83**	0.71	0.26	0.20	0.54	0.26
As					-0.03	0.09	0.14	0.09	-0.26	0.14
Cu						0.31	-0.03	-0.26	0.03	-0.03
Zn							0.60	0.77	***0.83**	0.60
Cr								0.77	0.26	1.00
Ni									0.66	0.77
Mn										0.26
caged mussels										
MT	0.77	-0.09	0.37	-0.26	***0.89**	0.14	-0.03	0.14	-0.43	0.20
Pb		0.49	0.83	-0.49	***0.94**	0.66	0.43	0.49	-0.54	0.60
Cd			0.54	-0.20	0.26	0.77	0.49	0.43	-0.60	0.49
Hg				-0.71	0.66	***0.83**	0.60	0.49	-0.26	0.71
As					-0.26	-0.77	-0.09	0.26	0.20	-0.43
Cu						0.37	0.37	0.54	-0.43	0.49
Zn							0.43	0.14	-0.49	0.66
Cr								0.77	0.26	***0.89**
Ni									0.03	0.49
Mn										0.09

TABLE 3 - Metallothionein content (µg/g WW) in the digestive gland of resident and caged mussels from five sites along Rijeka Bay (TM, LR, UR, BK, KR) and control site (LIM). Data are expressed as mean ± S.D (N=5). Significant difference (p< 0.05) with respect to control site LIM is indicated by asterisk (*).

Sampling sites	Resident	Caged
LIM	153 ± 1.3	153 ± 1.3
TM	206 ± 7.9*	158 ± 11.2
LR	219 ± 7.3*	198 ± 18.6*
UR	193 ± 9.2*	147 ± 15.0
BK	184 ± 17.2*	172 ± 6.7*
KR	161 ± 20.3	161 ± 3.7

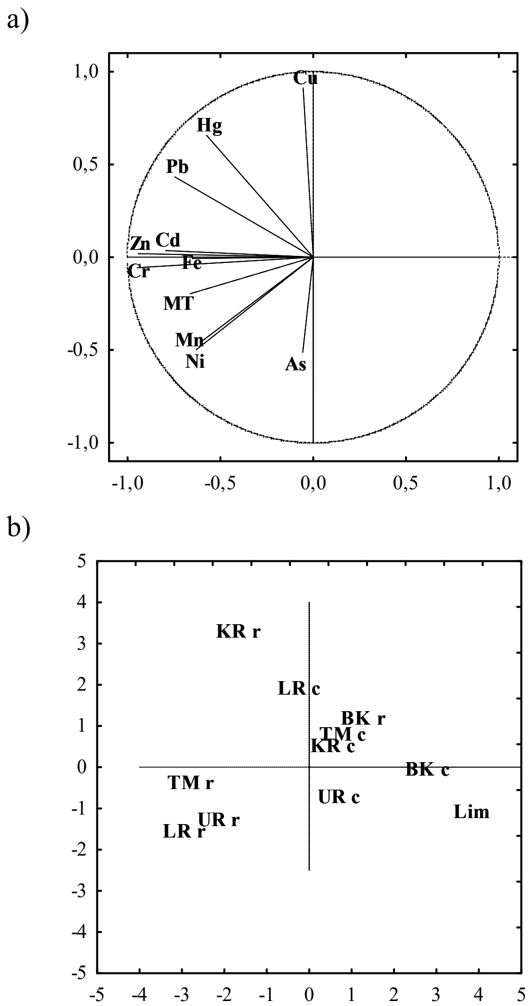

FIGURE 3 - PCA analysis based on metal concentration and MT content in the tissues of resident (r) and caged (c) mussels from sites LR, TM, UR, BK and KR and control site Lim. a) Plot of loadings b) Plot of sample scores.

In order to synthesize the results and illustrate the differences between resident and caged mussels, the Principal Component Analysis was conducted on the metal concentration and metallothionein content for all sampling sites and control (Figure 3a, 3b).

The first and the second principal components explain 65% of the total variability. Resident mussels from LR harbour, urban area UR, and TM shipyard were distinctly separated in the negative part of first component that was characterized by high loadings of MT, Cd, Zn and Cr (Table 4).

TABLE 4 - PCA results: Table of correlations between variables and principal components (PC's). Values in bold represent correlations that are statistically significant (p<0.05).

	PC 1	PC 2	PC 3
MT	**-0.66**	-0.20	0.19
Pb	**-0.75**	0.43	0.38
Cd	**-0.80**	0.04	-0.13
Hg	-0.57	**0.66**	0.19
As	-0.06	-0.51	**0.70**
Cu	-0.05	**0.91**	0.21
Zn	**-0.94**	0.02	-0.03
Cr	**-0.95**	-0.06	-0.14
Ni	**-0.63**	-0.50	-0.18
Mn	**-0.59**	-0.45	0.42
Fe	-0.65	-0.01	**-0.68**

Resident mussels from LR harbour and most notably KR shipyard were positioned in the positive part of PC2, which takes into account the high level of Cu and Hg. In comparison to mussels from above sites, resident mussels from urban site BK and caged mussels from KR and TM shipyards and urban area UR were less influenced by metal input.

Finally, caged mussels from urban area BK were positioned closer to control site Lim indicating lower anthropogenic impact. Multivariate analysis of the overall data clearly visualized differences in the distribution of resident and caged mussels in the PCA ordination plot, in relation to metal bioaccumulation level and metallothionein induction over short and long term exposure period.

CONCLUSION

The comparison of the overall results provided within the frame of this study with the most recent data available in the literature, revealed that metal concentrations found in mussels from Rijeka Bay are consistent with the range of values typically found at sites with different level of anthropogenic activity along the eastern Adriatic coast. Lead, cadmium, mercury and arsenic concentration in Rijeka Bay displayed levels that were comparable to those previously determined within coastal areas in the close vicinity to heavy industries and/or urban wastewater discharge. With exception of concentrations of copper at site KR shipyard, iron at urban site UR and LR harbour and zinc at TM shipyard and LR harbour, the values for other metals analysed in this study, correspond to those previously determined for coastal zones of eastern Adriatic characterised by low or moderate anthropogenic influence. Elevated metallothionein content in resident and caged mussels indicates the physiological adaptation to metal exposure in polluted environment.

A site-specific pattern of metal accumulation and metallothionein content, as well as differences between resident and caged mussels within the studied area were detected. The present study shows that the combined use of resident and caged mussels for detection of metal accumulation and metallothionein induction could improve our knowledge of the impact of metal contamination in marine environment. This study also provided valuable baseline data to support future risk assessment efforts in the coastal area of Rijeka Bay challenged by anthropogenic pressure.

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