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ICP-AES analysis of metal content in shell of mussel *Mytilus galloprovincialis* from Croatian coastal waters†

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Metal content in samples of shell of mussel *Mytilus galloprovincialis* was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The efficiency of conversion of crude samples into solution by acid digestion in an open plate and in a microwave oven was examined by use of certified reference material of marine sediment and laboratory made standards of calcite and aragonite. Influence of high Ca content matrix on emission intensities of Al, Ba, Cd, Cu, Fe, Mg, Mn, Na, Ni, Pb, Sr and Zn was observed as depression of emission signal for most of the measuring elements, ranging from 0.8% to 8%. Greater values were noted at Ba and Ni emission lines. Enhancement of signals was observed for Na and Mg lines. The determination of As, Sb, Se and Sn was performed by HG/ICP-AES. The greater abundance of Sn was found in samples collected near the Al-processing industry centre. No detectable concentrations of As, Sb, and Se were found in shell samples. Results of ICP-AES metal analysis showed that samples collected near harbours, city waste or sewage outlets, and chemical industry centres indicate the certain level of contamination. It is shown that shell analysis provides useful data in determination of marine environment status.

Keywords: ICP-AES; metal content; shell of mussel *Mytilus galloprovincialis*; total digestion

1. Introduction

In most studies of environmental degradation of coastal areas, metals are recognised as a cause of serious pollution. Special attention is usually paid to heavy metals because of their toxicity and non-degradability in the environment. Bioaccumulation of metals in marine organism tissue was recorded in a number of monitoring projects of the status of the marine environment. Among several types of marine organisms which have been established as biomonitoring species, mussels are shown to be very useful tools because of their ability to accumulate chemical elements and compounds in tissues. Mussels have wide geographical distribution; they are typical sedentary organisms with relatively high

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abundance in the ecosystem; the collection and identification of species could be easily performed; and they have suitable dimensions as a sample for investigation purposes. For this reason, they are commonly used as bio-indicators of changes in the marine environment [1–3].

Mediterranean Blue Mussel (*Mytilus galloprovincialis*) has been widely used to monitor accumulation of metals, especially bivalent metals, and lipophilic organic compounds. They are primarily accumulated in the soft tissue of mussels; therefore, most investigations are focused on bioaccumulation of metals in flesh [4–6]. Some results suggest that *M. galloprovincialis* could be an efficient indicator for lead, zinc and cadmium [7]. Analytical methods applied in most of the published studies are usually based on atomic absorption spectrometry (AAS) with electrothermal atomisation in the case of As, Pb, Cd and Zn determination, or with cold vapour generation in the case of Hg determination. The use of EDXRF and ICP-MS technique in simultaneous determination of heavy metals and metalloids in the mussels' soft tissue are also described in the literature [4–8].

The metal content in shell was rarely studied, despite the fact that it could provide a great amount of useful information in the biomineralisation process. It is already known that the shell of *Mytilus* species is made up of rhombohedra calcite in the outer layer, and orthorhombic aragonite in the inner layer [9]. The next most abundant compounds in the shell are magnesium carbonate which forms a hexagonal structure (isostructural to calcite), and strontium carbonate which forms a rhombic structure (isostructural to aragonite). Different mineralogical structure of shell layers implies incorporation of metals with different ionic radius into a calcite or aragonite layer. For example, Fe, Mn, Cu and Zn ion with a smaller radius than Ca can easily be incorporated into a calcite structure, and Cd, Ba and Pb ion with a greater radius can be incorporated into an aragonite structure. Metal content in the shell is influenced by environmental conditions; metals are accumulated through biomineralisation process, precipitation or adsorption from water [10–12]. After incorporation into mineral structure they are not subjected to reproductive or physiological conditions, and kinetic of their removal is extremely slow comparing to soft tissue [13]. Nevertheless, slow growth and long life of mussels provide a time integrated indication of environmental contamination, which is very useful in the recording of long-term effects at monitoring sites and also could be applied in paleo-chemistry reconstructions [9,14]. Analytical methods in determination of metals in shell are mostly AAS or ICP-MS which involve a previous procedure of total digestion of crude samples. The direct measurement of metals from powdered crust samples uses XRF or laser ablation technique coupled to ICP-MS. The excellent analytical performances of the LA-ICP-MS method provide insight into incorporation of particular metal into calcite or aragonite layer [15].

In this work, shell of *Mytilus galloprovincialis* were analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES). Mussel samples were collected at several locations along the east coast of the Adriatic Sea, especially near the hot-spots of possible anthropogenic impact on the marine environment, i.e. near harbours, city waste outlets, industrial zones, and also near the estuaries of several rivers. The aim of this work was primary to establish an appropriate analytical procedure for the ICP-AES determination of metals in samples consisting of carbonate matrix and to test potential of quantitative determination of metalloids by a hydride generation system coupled to plasma. The efficiency of conversion of crude samples into solution by acid digestion in an open plate and in a microwave oven was examined. Furthermore, the problem of selecting an

adequate reference material in control of analytical procedure was also the matter of the present study. Finally, most of the data published through the Mediterranean Mussel Watch Project described impact of metals on mussels' soft tissue [4–8], and there are only a small number of studies concerning shell analysis [16–18]. Therefore, the obtained concentrations of metals in *M. galloprovincialis* shell presented here as the result of ICP-AES method application could serve as an insight into environmental status of chosen locations on the east coast of the Adriatic Sea.

2. Experimental

2.1 Instrumentation

A *Prodigy High Dispersion ICP* inductively coupled plasma optical emission spectrometer (Teledyne Leeman Labs, Hudson, NH, USA) was used for the metal content determination in all samples. The operating parameters of the instruments and hydride generation device are given in Table 1. Emission lines of selected metals used in this work along with method detection limits (c_L) are given in Table 2. The detection limits were calculated using IUPAC recommendation, i.e. the concentration equivalent to three times standard deviation (3σ) of the signal of the blank solution. The method detection limits were expressed in $\mu\text{g g}^{-1}$ of sample mass.

Table 1. ICP-AES operating conditions.

ICP-AES operating conditions		
Instrument	Prodigy High Dispersive ICP	
Spectrometer	High resolution echelle polychromator	
	Large format programmable array detector (L-PAD)	
RF-Generator	40 MHz 'free-running'	
Output power	1.1 kW	
Argon flow	Coolant: 18 L min ⁻¹	
	Auxiliary: 0.8 L min ⁻¹	
	Nebuliser: 36 psi	
Peristaltic pump	1.0 mL min ⁻¹	
Nebulizer	Pneumatic (glass concentric)	
Spray chamber	Glass cyclonic	
Plasma viewing	Axial	
Replicates for each analysis run	3	
Sample uptake delay	30 s	
Hydride generator	Leeman Labs. Inc.	
	Part No. 130–1070	
	Three channel peristaltic pump	0.9 mL min ⁻¹
	T-connector	
	Reaction coil	
	Output power	1.3 kW
	Plasma viewing	Radial

Table 2. Emission lines and detection limits of elements.

Element	Spectral line λ/nm	$c_L/\mu\text{g g}^{-1}$
Al	308.215	0.62
Ba	455.403	0.02
Cd	214.441	0.10
Cr	206.149	0.65
Co	228.615	0.25
Cu	324.754	0.15
Pb	220.353	1.42
Mn	257.610	0.10
Ni	231.604	1.50
Zn	213.856	0.15
Ca	317.933	1.25
Mg	285.213	0.02
Na	589.592	0.25
Sr	407.771	0.02
K	769.897	3.25
Fe	238.204	0.12
As (HG)*	189.042	0.62
Sb (HG)	217.581	1.00
Se (HG)	203.980	2.50
Sn (HG)	189.991	1.87

*HG = hydride generation.

2.2 Reagents

High-purity deionised water (Milli-Q Element system, Millipore, USA) was used for the preparation of standard solutions and dilution of samples. In the sample digestion procedures, analytical reagent grade chemicals were used: nitric acid (65%), hydrochloric acid (36%) and hydrogen peroxide (30%) from E. Merck, Darmstadt. Single element standard solutions of As, Sb, Se, Sn, Al, Fe, Mn, Cu, Zn, Ca, Mg, Ba, Sr, Na, Ni, Cd and Pb (Plasma Pure, Leeman Labs, Hudson, NH, USA) and multi-element standard ICP-Mehrelement-Standardloesung IV (Merck, Darmstadt, Germany) were used for the preparation of calibration standard solutions and control of plasma line positioning. All calibration standards were prepared by appropriate dilution of stock solutions (1 g L^{-1}) in 2% HNO_3 . The calibration range was adjusted from 1 to 100 mg L^{-1} , depending on the sensitivity of selected element. For the determination of hydride forming elements, a fresh solution of 1% $\text{NaBH}_4/\text{NaOH}$ (m/V) was prepared. Tartaric acid (1%) for the reduction of tin was prepared by dissolution of solid compound in ultrapure water.

2.3 Samples

Samples of *Mytilus galloprovincialis* were obtained from the Centre of Marine Research, Institute Ruđer Bošković, Rovinj, Croatia. Mussels were collected from 18 locations on the east coast of the Adriatic Sea in February 2007. The description of sampling locations is given in Table 3. Before the samples were delivered to the laboratory, the soft tissue was

Table 3. Sampling locations.

No.	Sampling site	Description
1	Zablaće	Harbour, city waste and sewage
2	Srima	Fishing port
3	Jadrija	Non-urban area, yachting area
4	Martinska	Estuary (r. Krka), marina
5	Mala Klačina	Bay, yachting area
6	Sv. Ivan	Agriculture, estuary (r. Neretva)
7	Gruž	Harbour, city waste
8	Omiš	Fishing port, estuary (r. Cetina)
9	Rijeka Dubrovačka	Marina, estuary (r. Dubrovačka)
10	Ploče	Harbour, agriculture, estuary (r. Neretva)
11	Srima2	Fishing port
12	Bajlo	Non-urban area, yachting area
13	Bačvice	Urban area, recreational
14	Jadrija2	Non-urban area, yachting area
15	Vranjic	Industrial waste
16	Martinska2	Estuary (r. Krka)
17	Borik	Non-urban area, yachting area
18	Inavinil	Industrial waste

removed; shells were cleaned, dried and powdered in a grinding mill. Every sample consisted of whole shell of almost equal length (5–6 cm) which is habitually reached in the second year of mussel growth. Powdered shell samples were dried in a drying oven at 105°C before starting analytical procedures.

2.4 Analytical procedures for the sample preparation

The ICP-AES method supposes the use of aqueous sample solution, therefore powdered shell samples were digested by the use of mineral acids. For the digestion in an open vessel system, an analytical weight of 100 mg of shell powder was transferred into a glass beaker and 3 mL of concentrated HCl was added. The solution was heated on an electric plate for 15 minutes until the foaming due to carbon dioxide formation had stopped. After cooling, a volume of 1 mL of concentrated HNO₃ was added into the beaker and the solution was heated for 15 minutes. The procedure was repeated by the addition of the same volume of acids. After complete dissolution of residue, an addition of 1 mL of hydrogen peroxide was performed. The solution was heated continuously until the volume of approximately 1 mL remained in the beaker. The solution was quantitatively transferred into a volumetric flask of 25 mL, and filled up with ultra pure water.

Acid digestion procedure was also performed by the use of a microwave oven (MDS-2000 CEM, from CEM Corporation, CA, USA). For this purpose, an analytical amount of 100 mg of powdered sample was transferred into Teflon sample holders and 6 mL of previously prepared *aqua regia* was added. After 1 hour of digestion in open vessels, the solutions were transferred into the microwave oven. Three cycles of 15 minutes of digestion were applied. The pressure was adjusted starting from 80 psi at the first digestion step, 100 psi at the second and 35 psi at the last digestion step. After cooling of

Teflon vessels, the remaining solutions were filtered and transferred into a volumetric flask of 25 mL and filled up to the mark with ultra pure water.

In order to examine if it was necessary to remove the organic shell matrix, an amount of 1 g of powdered samples were weighted into gravimetric crucibles and placed into a muffle furnace where they were heated at 600°C for 1 hour. After heating, the remaining inorganic compounds were dissolved using the procedure for the open vessel digestion as was described previously. It was obvious that by the applied procedure total dissolution of inorganic residue was not obtained, so the residue was filtered to remove non-dissolved particles and was transferred into a volumetric flask of 25 mL.

The sample preparation was based on two replicates in each of the applied procedures. Procedural blank samples were also prepared by the same procedures.

Control of accuracy for applied analytical procedures was performed by use of certified reference material of marine sediments NRCC MESS-3 and IAEA-433. Certified reference material passed the same procedures as described earlier. Two control samples made in the laboratory were also used; the first was PJSC (*Pecten jacobaeus* Standard Calcite) and the second CESA (*Cerastoderma* Standard Aragonite). These control samples were provided courtesy of the Centre of Marine Research. The samples consist of powdered shells of *Pecten jacobaeus* and *Cerastoderma* made of calcite and aragonite mineral of high purity which had already been confirmed by the XRD method. Those laboratory samples served as a control of matrix interferences. The influence of high calcium content matrix on measured results was tested by addition of a multi-element standard solution of 1 mg L⁻¹, 5 mg L⁻¹ and 10 mg L⁻¹ in solutions of CESA and PJSC sample. Solutions of calcite and aragonite shell standards were prepared by digestion procedures in an open vessel and a microwave oven as described above.

In order to establish the concentration of hydride forming elements As, Sb, Se, and Sn the analytical procedures of sample preparation were based on dissolution of samples in HCl. One aliquot of sample solution for the Sn determination was filled up with tartaric acid, and the other for the As, Sb and Se determination with 10% HCl.

3. Results and discussion

3.1 Verifying of sample preparation procedures

ICP-AES analysis of metal content (Al, Fe, Mn, Cu, Zn, Ca, Mg, Ba, Sr, Na, Ni, Cd and Pb) in reference material of marine sediments (NRCC MESS-3 and IAEA-433) prepared by analytical procedure involved microwave oven digestion, gave the results which matched 90–105% of declared certified values (Table 4). The obtained results were acceptable by knowing that accuracy of ICP-AES method usually acquires 10% depending on applied analytical procedure. Significantly lower metal concentrations (<20% of declared values) were obtained in solutions prepared by open vessel digestion and digestion after heating of samples in a muffle furnace. Since marine sediment materials contain a significant amount of silicates, the crude non-dissolved residue after two-step addition of mineral acids was expected. The reason of noted departure for previously heated samples was insufficient dissolution of oxides formed at high temperature, and loss of volatile compounds. This implies that the marine sediment material was not an appropriate choice for testing of efficiency of open vessel digestion, especially if we keep in mind that mussel samples were dissolved in an open vessel more efficiently than in a microwave oven, i.e. much faster and with lower consumption of acids. The other crucial

Table 4. Element concentrations in certified reference material NRCC MESS-3 and IAEA-433 after microwave digestion.

Element	$\gamma/\text{mg kg}^{-1}$ certified IAEA-433	95% Confidence interval/ mg kg^{-1} IAEA-433	$\gamma/\text{mg kg}^{-1}$ measured IAEA-433	Recovery/%
Sr	302	296–308	300	99.3
Ba	268	241–295	272	101.5
Cd	0.153	0.145–0.161	0.143	93.5
Cu	30.8	30.2–31.4	30.1	97.7
Pb	26.0	25.4–26.6	27.2	104.6
Mn	316	312–320	318	100.6
Ni	39.4	38.7–40.1	37.2	94.4
Zn	101	99–103	104	103
Na	13500	12000–15100	12020	89
Element	$\gamma/\text{g kg}^{-1}$ certified IAEA-433	95% Confidence interval/ g kg^{-1} IAEA-433	$\gamma/\text{g kg}^{-1}$ measured IAEA-433	Recovery/%
Mg	11.5	11.1–12.0	11.2	97.4
Al	78.2	76.8–79.6	77.4	99
Fe	40.8	40.3–41.3	41.0	100.5
Element	$\gamma/\text{mg kg}^{-1}$ certified MESS – 3	95% Confidence interval/ mg kg^{-1} MESS – 3	$\gamma/\text{mg kg}^{-1}$ measured MESS – 3	Recovery/%
Sr	129	118–140	120	93
Cd	0.24	0.25–0.26	0.22	91.7
Cu	33.9	32.3–35.5	32.7	96.5
Pb	21.1	20.4–21.8	22.0	104.3
Mn	324	312–336	329	101.5
Ni	46.9	44.7–49.1	44.1	94
Zn	159	151–167	162	101.9
Element	$\gamma/\%$ certified MESS – 3	95% Confidence interval/% MESS – 3	$\gamma/\%$ measured MESS – 3	Recovery/%
Al	8.59	8.36–8.82	8.22	95.7
Fe	4.34	4.23–4.45	4.51	103.9

problem is the fact that shells are built up of calcium carbonate mineral forms. For this reason, marine sediment reference material with mostly silicate matrix composition could serve only as confirmation of proper instrument parameters, and not as a control of shell samples preparation procedure.

Therefore, the applied analytical procedures were tested by use of PJSC and CESA samples which passed the same digestion protocol. Calcite and aragonite structure of those shells do not incorporate heavy metals which has been already proved by XRD. Possible effects of high content calcium matrix solution was examined after addition of multi-element standard of 1 ppm, 5 ppm and 10 ppm, into a solution prepared by digestion of PJSC and CESA in an open vessel. Searching of the elements abundance at a very low concentration range in calcium matrix is an unpleasant analytical task which

predicts a whole separate study of the calcium interference effects in plasma. It is already known that the high content calcium matrices produce either rising or reducing element emission line intensity in plasma. The nature of calcium effects were described by Thompson and Ramsey who found strong correlation of interferences with excitation and ionisation energy for observed elements [19]. High calcium content matrix effects in plasma were studied recently by modern echelle spectrometers [20,21]. Paama and Peramaki have determined several elements in calcite mortars and established effects of Ca. They found that Ca influenced measured concentrations up to 2% when it was present in concentration range higher than 600 mg L^{-1} , and up to 1% in concentration range of $100\text{--}200 \text{ mg L}^{-1}$ [20]. Stepan *et al.* found that depressive effect of Ca on ionic and atomic lines could be minimised with robust plasma conditions, i.e. higher power and lower carrier gas flow [21]. However, matrix matching or internal standardisation is recommended in the case of metal analysis in high content Ca solutions.

Table 5 shows concentrations and recoveries (%) of measured concentrations after addition of 5 mg L^{-1} of multi-element solution. The addition of 1 mg L^{-1} of multi-element solution showed up to 1% greater departure in emission intensities than addition of 5 mg L^{-1} solution. The addition of 10 mg L^{-1} solution showed less influence on line intensities of selected elements.

Influence of high Ca content on emission intensities is observed as depression of signal and lower measured concentrations for most of the determined elements except Na and Mg. They were in predictably greater abundance in shell standards. Values close to 10% of errors are observed for the Ba and Sr line in both samples. Sensitivity of Ba line on high Ca content was already noted in the literature [21]. Much greater errors occurred in Ni measurement, but it might be explained with the mode of the signal observation. The axial observing position for the Ni determination is less favourable than the radial. Generally, variation in concentrations noted after multi-element standard addition on calcite and aragonite standards do not mutually show significant difference.

Table 5. Element concentrations in CESA and PJSC and recoveries after addition of 5 ppm of multi-element standard solution.

Element	$\gamma/\mu\text{g mL}^{-1}$ measured CESA	Recovery/%	$\gamma/\mu\text{g mL}^{-1}$ measured PJSC	Recovery/%
Al	4.80	96.0	4.74	94.8
Ba	4.48	89.6	4.54	90.8
Cd	4.82	96.4	4.92	98.4
Cu	4.89	97.8	4.87	97.4
Pb	4.78	95.6	4.73	94.6
Mn	4.96	99.2	4.69	93.8
Ni	4.12	82.4	4.06	81.2
Zn	4.82	96.4	4.82	96.4
Na	5.90	118	6.02	120
Mg	5.12	102	5.15	103
Sr	4.59	91.8	4.65	93.0
Fe	4.91	98.2	4.68	93.6

3.2 Metal content in the shell of *M. galloprovincialis*

The measured content of the most abundant elements in shell crust is shown in Figure 1. It was found that the average Ca content of *M. galloprovincialis* samples collected at the east coast of the Adriatic Sea takes 36%. For comparison, its relative Atlantic Blue Mussel (*M. edulis*) contains 39% of Ca in shell [9,15]. Main constituents of *M. galloprovincialis* shell showed generally uniform distribution in collected samples. Slightly higher concentrations of Mg and Sr were observed in samples collected from river estuaries (Samples 4, 6, 9, 10 and 16).

Heavy metal concentrations are shown in Figures 2 and 3. Extreme high concentrations of Fe, Ni and Ba were established in the first sample which originates from sample site Zablacé, where harbour and municipal sewage waste is placed. Aluminium and Ba content were also found in higher extent in Sample 18 where industrial waste influences the marine environment.

Copper presence was confirmed in two samples, the first which was sampled near municipal sewage waste, and Sample 10 which was sampled in the estuary of river Neretva that is known as an area with extensive agriculture. The use of protective compounds for agriculture purposes that contain high content of Cu salts could explain the obtained concentration data. Cd presence was established at several samples. It was registered mainly in shells collected near river estuaries where materials from the rock weathering

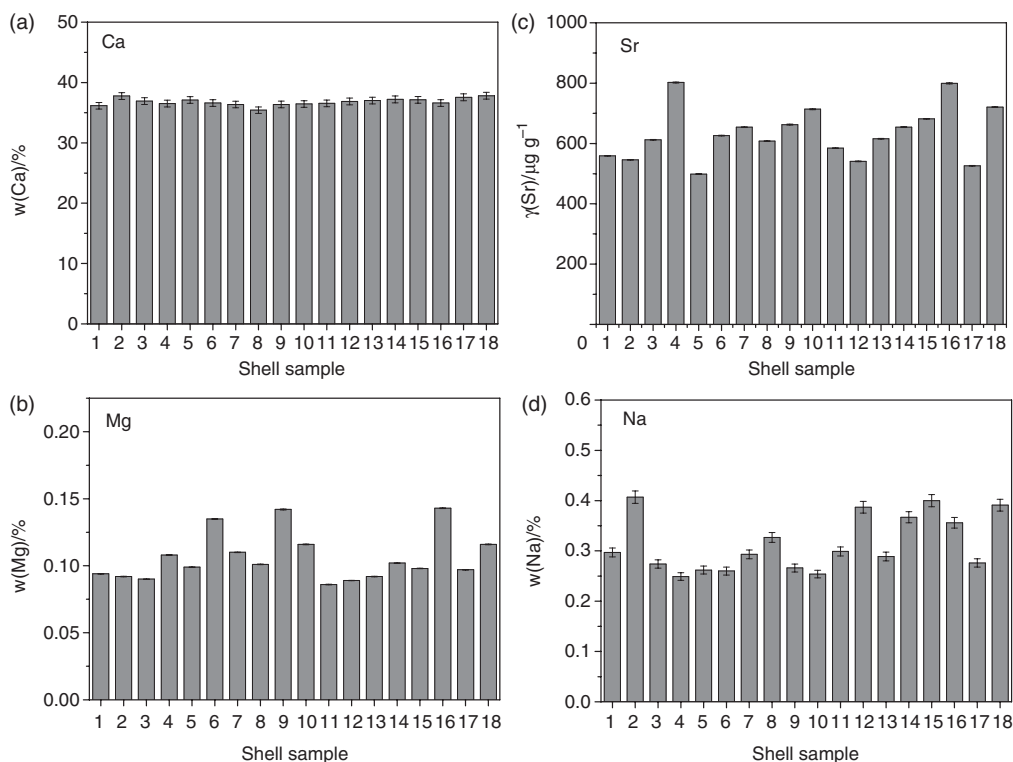


Figure 1. ICP-AES measured content of main constituents of shell. (a) Ca, (b) Mg, (c) Sr and (d) Na.

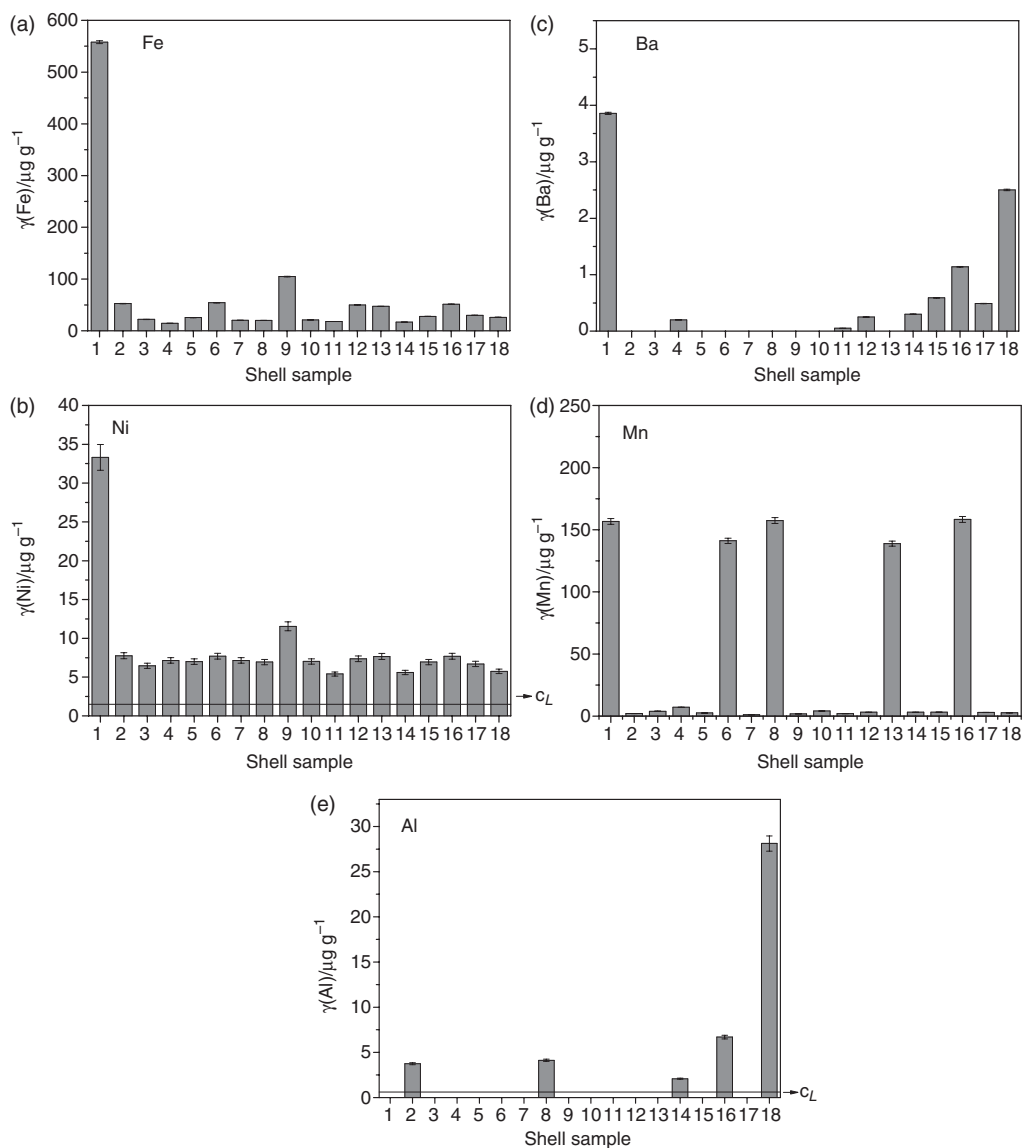


Figure 2. ICP-AES measured content of selected metals in shell. (a) Fe, (b) Ni, (c) Ba, (d) Mn and (e) Al.

process float into the sea (Samples 4, 9, 10, 15, 16). Cadmium concentration is usually increased in estuaries due to the contact of fresh water with seawater. The higher ionic strength of seawater causes the complexation with chloride to form CdCl_2 , the most abundant species of Cd in seawater. Cadmium was also registered in the sample collected near the PVC-industry centre (Sample 18). Concentrations of lead show higher values in samples collected near fishing ports, marinas, harbours and the industrial centre (Samples 3, 4, 14, 15, 17, and 18) where leaded gasoline from ship engines easily reaches the seawater and consequently the seawater organisms.

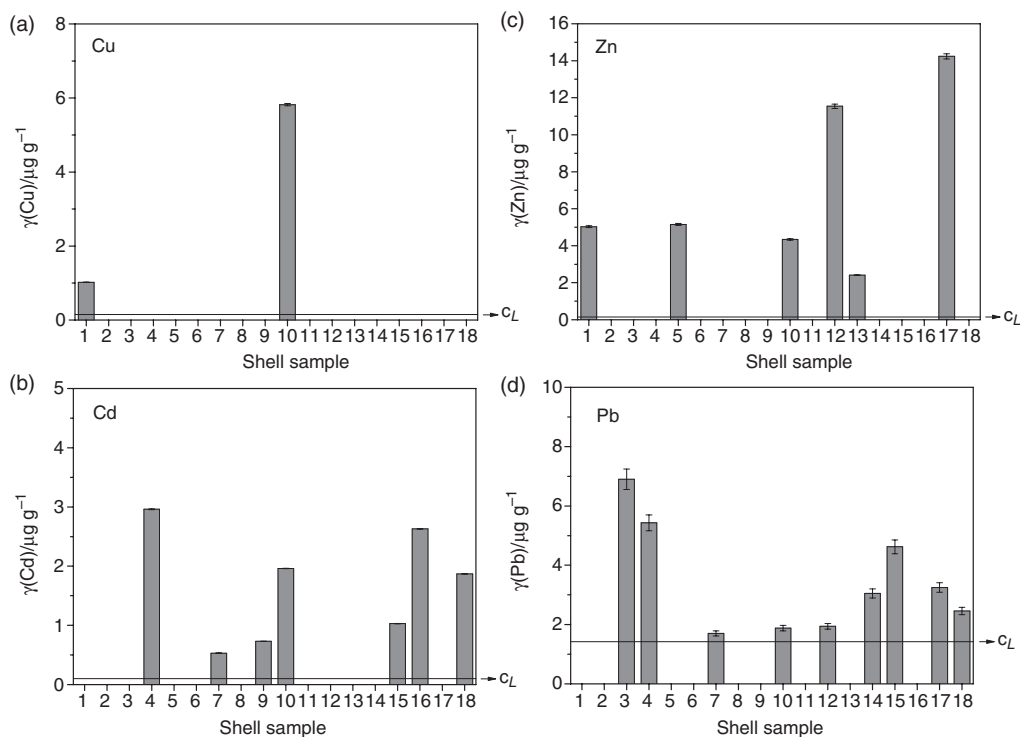


Figure 3. ICP-AES measured content of trace metals in shell. (a) Cu, (b) Cd, (c) Zn and (d) Pb.

3.3 HG/ICP-AES determination of selected metalloids

The determination of As, Sb, Se and Sn by coupling of hydride generator device to ICP spectrometer gave measurable results only for tin. The values of Sn concentration obtained for 16 samples and reference sediment material is shown in Figure 4. The greater abundance of Sn is found in samples collected near the Al-processing industry centre, harbour, and fishing port (Samples 1, 4, 7, 8). No detectable concentrations of As, Sb, and Se were found in shell samples, despite the sensitive measuring technique applied. However, an influence of As on soft tissue was already noted at the east coast of the Adriatic Sea [6,8]. Therefore, the analytical procedure applied here needs some further modifications in order to improve sensitivity of HG/ICP-AES measurements of As, Sb and Se, e.g. addition of reducing agent before and during hydride formation, or applying a different extraction procedure in the sample preparation step.

4. Conclusions

Metal content in samples of shell from the mussel *Mytilus galloprovincialis* was determined by ICP-AES. The efficiency of analytical procedures of sample dissolution which include acid digestion in an open vessel and a microwave oven were examined by use of certified reference material of marine sediments and laboratory prepared samples with matrix similar to shell composition. It was found that sediment material is not an appropriate

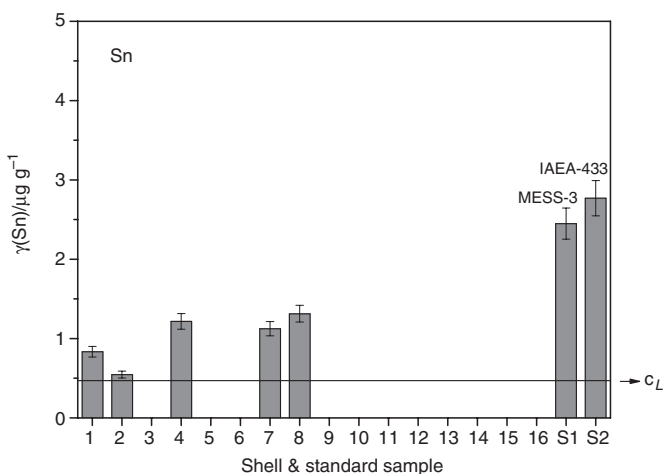


Figure 4. HG/ICP-AES measured Sn content in shell.

control sample of analytical procedures due to incomplete dissolution of silicates. An influence of high content Ca matrices was evaluated by multi-element standard addition on pure calcite (PJSC) and aragonite (CESA) shell samples. The depression of signal less than 10% was noted for most of the observed elements. Among the selected hydride forming elements which were analysed by use of HG/ICP-AES, the Sn content is the only one which was successfully determined. The HG/ICP-AES procedure needs further modifications to improve sensitivity for As, Sb and Se determination. Although, an additional examination of intra-population variability derived from the presented data is limited by lack of samples, generally the results of ICP-AES analysis of metal content showed that mussel samples collected near harbours, city waste or sewage, and chemical industry centres indicate a certain level of contamination. It is also shown that ICP-AES analysis of shell provides reliable data which could be used in determination of current environmental status and also in the estimation of possible anthropogenic influences on the marine ecosystem.

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