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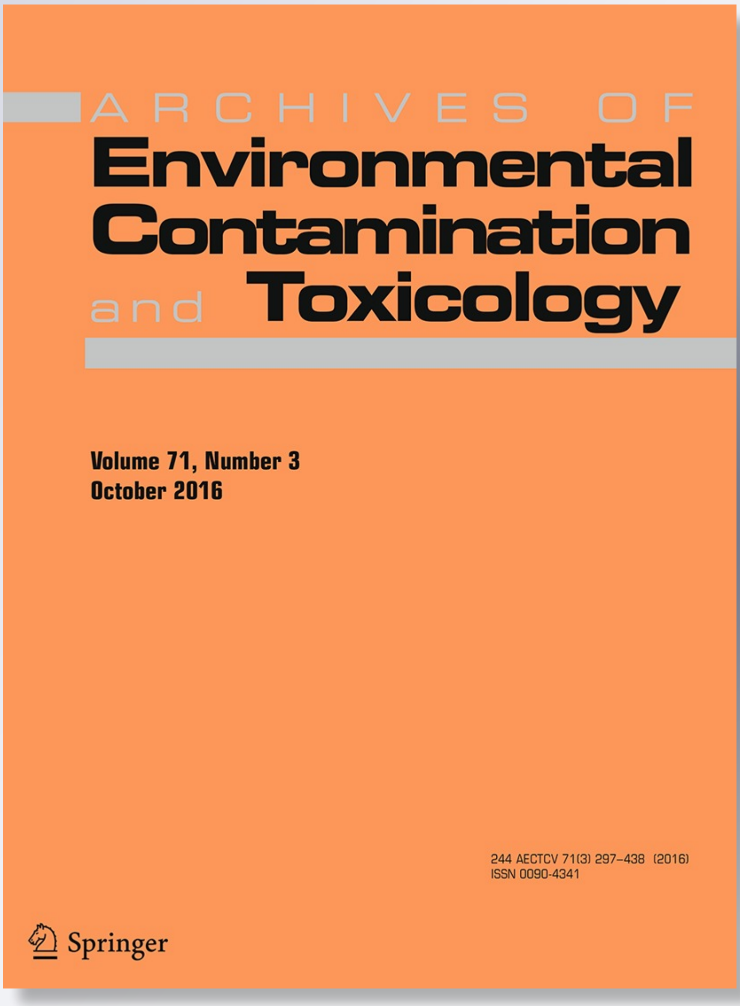
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Trace Metals in Noah's Ark Shells (*Arca noae* Linnaeus, 1758): Impact of Tourist Season and Human Health Risk

Dušica Ivanković[1] · Marijana Erk[1] · Ivan Župan[2] · Jelena Čulin[3] · Zrinka Dragun[1] · Niko Bačić[1] · Ana-Marija Cindrić[1]

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Abstract Commercially important bivalve Noah's Ark shell (*Arca noae* Linnaeus, 1758) represents a high-quality seafood product, but the data on levels of metal contaminants that could pose a human health risk and also on some essential elements that are important for health protection are lacking. This study examined the concentrations of Cd, Pb, Cr, Ni, Cu, Co, and Zn in the soft tissue of *A. noae* from harvesting area in the central Adriatic Sea, to survey whether heavy metals are within the acceptable limits for public health and whether tourism could have an impact on them. The concentrations of analysed metals varied for Cd: 0.15–0.74, Pb: 0.06–0.26, Cr: 0.11–0.34, Ni: 0.09–0.22, Cu: 0.65–1.95, Co: 0.04–0.09, and Zn: 18.3–74.7 mg/kg wet weight. These levels were lower than the permissible limits for safe consummation of seafood, and only for Cd, some precautions should be taken into account if older shellfish were consumed. Increase of Cd, Cr, and Cu in shell tissue was observed during the tourist season at the site closest to the marine traffic routes, indicating that metal levels in shellfish tissue should be monitored especially carefully during the peak tourist season to prevent eventual toxic effects due to increased intake of metals, specifically of Cd.

Noah's Ark shell (*Arca noae* Linnaeus, 1758), a bivalve species of the family Arcidae, represents one of the important shellfish species that are commercially exploited throughout the entire Mediterranean region (Župan et al. 2012), even though the harvested amounts are relatively small due to the specific way of collecting. Between 1991 and 2000, increased demand and capture of various species of the family Arcidae took place on a global scale (FAO 2014). Along the eastern Adriatic coast, *A. noae* represents one of the commercially important species besides Mediterranean scallop (*Pecten jacobaeus*), warty venus (*Venus verrucosa*), and clam (*Ruditapes decussatus*) that are harvested entirely from natural populations (Župan et al. 2012).

As a consequence of the increasing awareness that consumption of bivalves has the same beneficial effects on health and well-being as fish that are high in omega-3s fatty acids, marine bivalves became commercially important species. Data on the biochemical and fatty acid composition of Noah's Ark shell tissue indicated it would be an excellent source of n-3 fatty acids, especially eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acid (Dupčić Radić et al. 2014), and as such it represents a high quality seafood product. Enhanced activities connected with tourism along the Croatian coast affected the increase in the seafood market and intensified the harvesting of natural populations of *A. noae*. Specifically, the increase in consumption of *A. noae* occurs in the peak tourist season (peak tourist months on the Adriatic coast in Croatia are from June until August). Due to this fact, this species of shellfish become interesting for commercial exploitation in the future, and recently, the first preliminary studies on the Noah's Ark shell potential for introduction into integrated aquaculture were performed (Župan et al. 2014).

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Generally speaking, bivalves that are basically sedentary are suitable indicator species of coastal pollution, because their body contaminant burdens normally can be attributed to local pollution sources or locally polluted water and sediment. They give time-integrated information on the bioavailability of chemicals in the water column and sediments (Regoli 1998), and they are known to accumulate high levels of metals in their tissues and yet survive in contaminated environments (Goldberg et al. 1978; Cantillo 1998). *A. noae* lives attached by solid byssus on rocks on all types of bottom that contain hard substrate and occurs either as solitary individual or in clumps. It filters the seawater in order to perform respiration and feeding, and consequently it efficiently accumulates nutrients, but also various toxins from the environment. As a slow-growing organism, *A. noae* can obtain length between 70 and 90 mm and live up to 16 years (Peharda et al. 2002, 2003), but in unexploited populations the largest specimens with 120.3 mm in length and 24 years old were observed as well (Puljas et al. 2015). Therefore, *A. noae* could be a very efficient accumulator of various contaminants, including heavy metals, and it could be potentially a good bioindicator of a long-term metal contamination in the coastal marine ecosystems.

The presence of metals in the environment is a result of their natural origin and of human activities, and such anthropogenic pollution in particular affects coastal ecosystems. Main sources of anthropogenic impact in coastal areas are the residence in the coastal zone, fisheries and aquaculture, shipping, tourism, and land-use practices (agriculture, industrial development). Unlike other bivalve species, for which there are a lot of data on concentrations of metals (Eisler 2010), studies on metal concentrations in soft tissue of Noah's Ark shells are generally lacking. According to our knowledge, only two short reports on concentrations of several trace metals in this shellfish have been published (Ozretić et al. 1990; Cuculić et al. 2010).

The current study area includes one of the most important natural habitats of *A. noae* in central Adriatic, the area of the Pašman channel (PC; Peharda Uljević 2008). This area also represents the main harvesting area for Noah's Ark shells in the central Adriatic. However, this sea region is under considerable anthropogenic pressure of municipal and industrial wastewaters, and organisms living there could be exposed to contaminants originating from different sources. Additionally, an increase in tourism activities, especially in nautical tourism during the peak tourist season could significantly contribute to the potential contamination in general (Favro and Gržetić 2008), but also in this specific harvesting area. Tourist season is characterized by a temporary increase in number of local inhabitants, as well as intensification of marine traffic, which can lead to increased water contamination due to more abundant municipal wastewaters during summer months, as well as due to leaching of specific metals, such as Cr, Cu, and Zn, from antifouling paints and chromate coated parts of boats.

Noah's Ark shells, like other shellfish that are harvested from natural populations, very often are not subjected to any systematic monitoring. Consequently, consumption of such uncontrolled seafood could pose a significant risk to human health (Bogdanović et al. 2014). Until now, there were no comprehensive studies on metal composition and safety issues regarding potential metal toxicity due to consumption of Noah's Ark shellfish. Therefore, the present study can provide baseline data for future assessment of the quality of these shellfish as a food.

Accordingly, the purpose of this study was to provide data about the metal levels in *A. noae* that could pose a human health risk. In particular, this study was designed to determine the concentrations of Cd, Pb, Cr, Ni, Cu, Co, and Zn in the whole soft tissue of Noah's Ark shells sampled from harvesting areas with different levels of potential anthropogenic impacts to evaluate the public health risk associated with the consumption of these shellfish as a valuable seafood item. These specific metals were chosen due to their possible toxicity (Cd, Pb, and Ni; EC 2006; European Parliament and the Council of the European Union, EPCEU 2013), their frequent occurrence in marine environment as a consequence of leaching from boat parts and antifouling paints (Cr, Cu, and Zn; Mihelčić et al. 2010; Singh and Turner 2009; Turner 2010; Ytreberg et al. 2010), as well as due to their physiological importance for marine organisms (Cu, Zn, and Co). The levels of metals were evaluated in relation to the maximum limits prescribed by international regulations, and the information was provided on the amount of Noah's Ark shellfish that can be safely consumed. In addition, the potential impact of nautical tourism on the level of heavy metals in *A. noae* was evaluated by comparing the metal concentrations in two different periods concerning the intensity of tourist activities, the off-season (period from March to May), and the peak tourist season (period from June to August).

Materials and Methods

Study Area and Sampling

The study area is located in the central Adriatic Sea in Croatia, where three sampling sites were selected, as shown in Fig. 1. Two sampling sites were selected in the PC, the harvesting area of *A. noae*, which is spreading in the northwest–southeast direction between the coastline and the island Pašman. Sampling site PC1 (at the depth of 12–15 m) was located in the proximity of the coast,

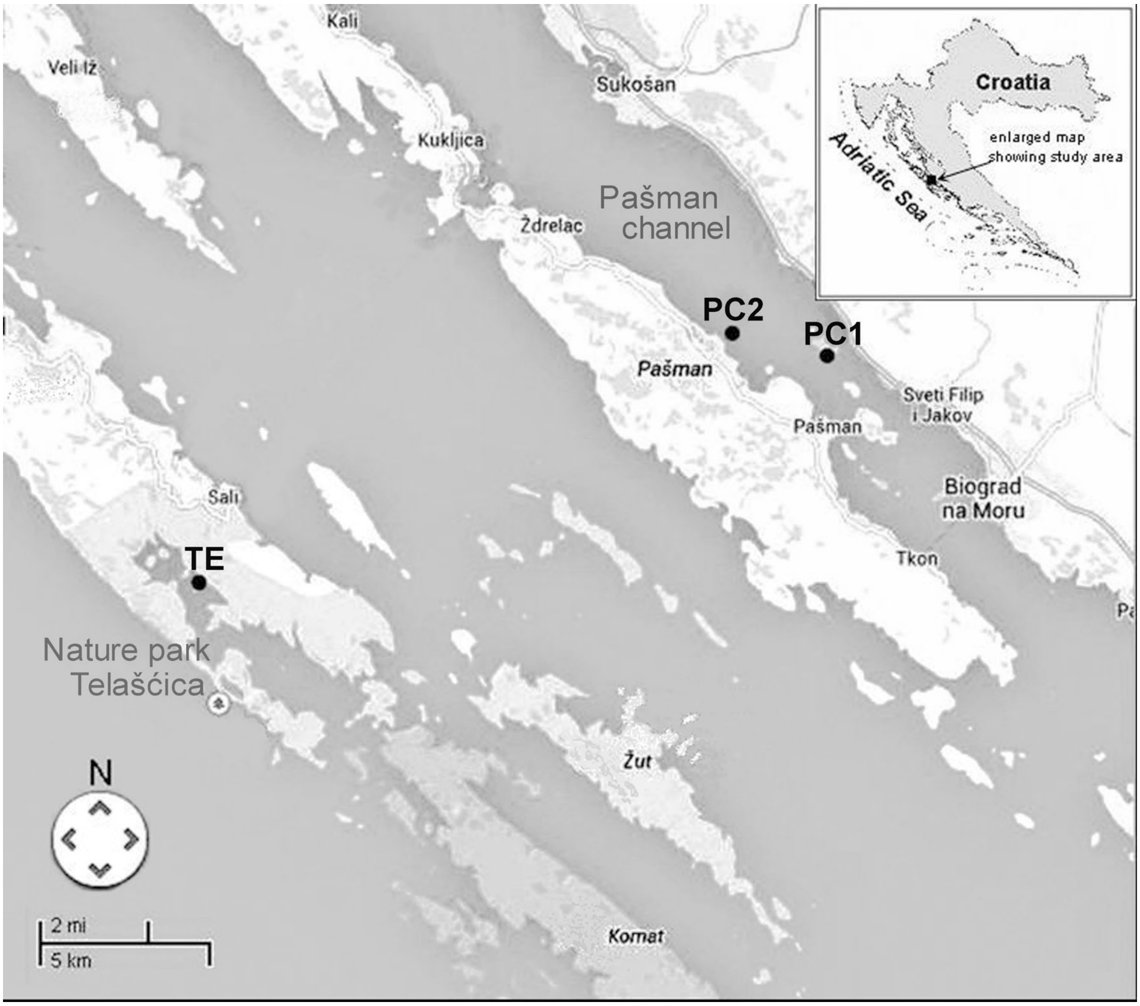

Fig. 1 Study areas in the central Adriatic Sea in Croatia with indicated sampling sites (PC1 and PC2 sampling sites in the Pašman channel harvesting area and TE sampling site in the Telašćica Nature Park)

whereas site PC2 (at the depth of 5–7 m) was located close to the island Pašman. The expected anthropogenic impact on PC1 and PC2 sampling sites originated from several sources: (1) municipal wastewaters of the town Biograd (hospital and marina) in the southeast, which are released into the marine water without prior treatment, (2) municipal and industrial wastewaters of the City of Zadar (international harbour—passengers and transport of chemicals) in the northwest, which are released into the marine water after the treatment in the wastewater treatment plant by undersea outlet, which is placed at the depth of 40 m and distance of approximately 400 m from the shore, (3) the sewerage of Pašman Island settlements, which is released directly into the marine water, without any pretreatment, (4) the vicinity of major marine transportation routes. Third sampling site was selected in the Nature Park Telašćica (TE; at the depth of 10–14 m) located on the Island Dugi Otok and represented the site of expectedly lower anthropogenic impact.

The Noah's Ark shells from natural populations were sampled in the off-season (once per month in March, April, and May 2013) and in the peak tourist season (once per month in June, July, and August 2013) by SCUBA diving and transported in dark and cool containers to the laboratory within 6 h, where they were immediately washed with seawater and the whole soft tissue was dissected using stainless steel equipment. The shellfish were not depurated prior to dissection, although some researchers recommend this procedure prior to metal analyses. However, it has been shown that effect of depuration depends on the bivalve species, as well as on the metal itself, consequently resulting in the decrease of some metals in the bivalve tissues as opposed to increase of the others (Anacleto et al. 2015). An additional reason why the depuration was not applied in this study refers to the fact that in Croatia, for now, *A. noae* shellfish are placed on the market directly, without prior depuration. Our purpose was to evaluate actual metal content in the shellfish tissue to which the final consumers are exposed. Therefore, nine individuals per station per sampling were randomly selected, and their whole soft tissue was combined, without prior depuration, in three composite samples for the heavy metal analysis. Each composite sample was composed of whole soft tissues of three individuals. The length (TE: 8.4 ± 0.4 cm, PC1: 7.6 ± 0.6 cm, PC2: 8.2 ± 0.4 cm; mean ± SD; $n = 54$) and the total mass (TE: 61.0 ± 5.5 g, PC1: 41.4 ± 7.1 g, PC2: 61.0 ± 9.0 g; mean ± SD; $n = 54$) of the individuals were recorded during the study.

The sediment sampling was performed by SCUBA diving within the same area where *A. noae* specimens were collected. To ensure that the sample is fully representative of the substrate, according to the recommendations by Loring and Rantala (1992), five sampling spots were chosen in the diameter of 10 m. On each spot, 0.5 kg of surface and near surface sediment was taken using plastic spatula and stored into plastic bag taking care to avoid the leakage of fine-grained sediment from the spatula and bag. In this way, one composite sample of approximately 2.5 kg of the surface sediment was sampled at each of three selected sites on one occasion, in March 2013. Samples were transported to the laboratory at 4 °C and then stored at −20 °C until analysis. The purpose of sediment sampling was to perform preliminary characterization of shellfish habitat.

Two seawater samples (one in March and another in June 2013) were sampled by SCUBA diving in acid-cleaned perfluoroalkoxy polymer bottles (volume 1 L) from the same depth as the shells with the goal to obtain general insight in dissolved concentrations of studied metals in the seawater in each season. Seawater samples were transported to the laboratory at 4 °C within 48 h, and immediately upon arrival filtered through 0.45-µm pore filter, acidified, UV digested for at least 24 h (250 W Hg-lamp), and stored in polyethylene containers (4 °C) until analysed (Omanović et al. 2006).

Metal Analyses in Whole Soft Tissues of *A. noae*, Water and Sediment

Wet digestions of ark shell tissues were performed in duplicate by weighing approximately 100 mg of homogenized freeze-dried (the average water content in soft tissue was ~80 %) whole soft tissues of *A. noae* with a mixture of 5 mL HNO_3 (65 % Suprapur, Merck) and 1 mL H_2O_2 (30 % Suprapur, Merck) in a tightly capped Teflon vials by heating at 80 °C for 3 h (mild digestion using single-step simulation of hot-plate method). Digested samples were quantitatively transferred into the volumetric flasks of 50 mL, filled to the mark with deionised water (Milli-Q, 18.2 MΩ cm), and stored in polyethylene bottles until analysed. Procedural blank samples also were prepared and analysed.

Sediment samples were defrosted, homogenized by mixing, and dried in the laboratory to constant weight at 105 °C, put through 1-mm sieve, and the resulting <1 mm fraction ground to dust in agate mortar. Wet digestion of sediments was performed by weighing approximately 100 mg of samples using a mixture of 4 mL HNO_3 (65 % Suprapur, Merck), 1 mL HCl (30 % Suprapur, Merck) and 1 mL HF (40 % Suprapur, Merck) in the first step, and 6 mL 4 % H_3BO_4 (Sigma) in the second step in a microwave digestion system (Multiwave 3000, Anton Paar) (EPA 2007).

Analyses of metals in digested Noah's Ark shell tissues and sediments were performed using high-resolution inductively coupled plasma mass spectrometer (HR ICP-MS, Element 2, Thermo Finnigan) using an autosampler (ASX 510, Cetac Technologies) and sample introduction kit consisting of SeaSpray nebulizer and cyclonic spray chamber Twister. Prior to measurements indium (Fluka) was added to all samples as an internal standard (1 µg/L). Measurements of ^{111}Cd and ^{208}Pb were operated in low-resolution mode, whereas ^{52}Cr, ^{60}Ni, ^{63}Cu, ^{59}Co, and ^{66}Zn were measured in medium resolution mode. External calibration was performed using standards prepared in 2 % HNO_3 (Suprapur, Merck) by appropriate dilutions of 100 mg/L multielement stock standard solution (Analytika).

Quality of analytical performance of the laboratories of Division for Marine and Environmental Research was confirmed by successful participation in laboratory performance evaluation studies conducted by international bodies UNEP global environmental monitoring system and International Atomic Energy Agency (IAEA), which gave reliability of the results obtained in this study. Standard reference material IAEA-452 (scallop tissue, IAEA reference materials) and certified reference material MESS-3 (marine sediment, National Research Council of Canada) for trace elements were analysed for quality control check for the determination of metals in the Noah's Ark shell tissues and for the determination of metals in the sediments, respectively (Table 1). In this study, the range of good agreement with the reference material was set at ±30 % for the soft tissue of Noah's Ark shell. All measured metal concentrations were within this range except Zn concentrations. Therefore, Zn concentrations were recalculated using the respective correction factor.

In the seawater, the concentrations of total dissolved Cd, Ni, Cu, Co, and Zn were determined by means of standard

Table 1 Quality control for metal determination in shellfish tissue based on IAEA-452 (trace elements in scallop tissue, standard reference material, distributed by International Atomic Energy Agency reference materials, certified values and the standard deviations are presented), for metal determination in sediments based on MESS-3 (marine sediment reference materials for trace metals and other constituents, certified reference material, distributed by National Research Council of Canada; expanded uncertainty in the certified value is reported) and for metal determination in seawater based on NASS-5 (seawater reference material for trace metals, certified reference material, distributed by National Research Council of Canada; the uncertainties represent 95 % confidence limits for an individual subsample)

	Metal concentrations (mg/kg d.w.)						
	Cd	Pb	Cr	Ni	Cu	Co	Zn
IAEA-452 (scallop tissue)							
Reference values	29.6 ± 3.7	0.371 ± 0.014[a]	–	–	10.8 ± 1.3	1.62 ± 0.20	166 ± 21
Measured values	25.3 ± 0.8	0.356 ± 0.028[a]	–	–	8.94 ± 0.34	1.62 ± 0.06	113 ± 5
MESS-3 (marine sediment)							
Reference values	0.24 ± 0.01	21.1 ± 0.7	105 ± 4	46.9 ± 2.2	33.9 ± 1.6	14.4 ± 2.0	159 ± 8
Measured values	0.24	24.4	97.4	44.0	23.1	12.5	136
NASS-5 (seawater)							
Reference values	0.023 ± 0.003	0.008 ± 0.005	–	0.253 ± 0.028	0.297 ± 0.046	0.011 ± 0.003	0.102 ± 0.039
Measured values	0.020 ± 0.001	0.013 ± 0.003	–	0.273 ± 0.041	0.284 ± 0.029	0.014 ± 0.002	0.128 ± 0.005

Measured values for IAEA-452 are presented as means and SD ($n = 6$) and for NASS-5 measured values and 95 % confidence intervals are presented

[a] Pb is not declared in IAEA-452, and therefore Pb was measured in SRM 1566a (oyster tissue, standard reference material, distributed by US Department of Commerce, National Bureau of Standards, Gaithersburg; the uncertainty is obtained from 95 % prediction interval). Measured value is presented as mean and SD ($n = 6$)

addition method using differential pulse anodic stripping voltammetry as described by Omanović et al. (2006) and Cukrov et al. (2008). Standard addition plot was constructed with five points (sample and four additions of respective metal standard—Cd, Ni, Cu, Co, and Zn). A duplicate measurement at each metal standard concentration was performed to enhance the quality of analysis. A certified seawater reference material for trace metals: NASS-5 (NRC CNRC) was analysed for quality control check (Table 1). All determined metal concentrations were within the certified ranges.

Data Analysis

Data analysis was performed using the Statistica 8 (StatSoft, Inc.) software package. Independent sample t test was used to evaluate differences between two studied periods for a particular location. In order to take into account the possible influence of the differences in shellfish size from different sampling sites on the comparison of tissue metal contents at various sampling locations in each period, an analysis of covariance (ANCOVA) using a shell length as a covariate was used. If the ANCOVA was significant ($p < 0.05$), then specific differences between sampling locations were tested using the LSD post hoc test of multiple comparisons. The significance level was set at $p < 0.05$.

Results and Discussion

Accumulation of Heavy Metals in Soft Tissue of Noah's Ark Shells

Heavy metal concentrations (mg/kg of tissue dry weight, d.w.) determined in the whole soft tissue of Noah's Ark shells in the off-season and the peak tourist season period at the studied sites are shown in Table 2. Significant differences between sites in the tissue concentrations of Pb and Cr in both periods and Cd and Zn in the off-season period were observed. There were no significant differences between sites in the tissue content of Ni, Cu, and Co in any period.

In both periods, Cd and Cr concentrations were the highest in the soft tissues of shells from the TE location, followed by PC2 and PC1 locations, but statistically significant only for Cr in both periods and for Cd in off-season (Table 2). That was unexpected because TE was considered to be under the lowest anthropogenic influence because of its location in protected area (the Nature Park). However, somewhat increased Ni and Cu concentrations in sediment also were found at TE location, whereas Cr concentration was the highest at PC2, followed by TE location (Table 3). It could probably be related to the boating activities and leaching from the chromate coated parts of boats and yachts, as it has previously been

Table 2 Concentrations of heavy metals (mean ± SD of nine composite samples per station per season) in the soft tissue of Noah's Ark shell (*A. noae*) collected at three locations from the eastern Adriatic in the off-season (March–May) and the peak tourist season (June–August)

Sampling sites	Metal concentration (mg/kg d.w.)						
	Cd	Pb	Cr	Ni	Cu	Co	Zn
Off-season period							
TE	2.01 ± 0.65^{a}	0.49 ± 0.08^{a}	1.15 ± 0.13^{a}	0.73 ± 0.11	4.47 ± 0.47	0.30 ± 0.05	154 ± 43^{a}
PC1	0.93 ± 0.19^{b}	0.41 ± 0.07^{a}	0.58 ± 0.09^{b}	0.59 ± 0.10	4.32 ± 0.29	0.24 ± 0.04	153 ± 49^{a}
PC2	1.60 ± 0.55^{a}	0.64 ± 0.24^{b}	0.92 ± 0.15^{c}	0.67 ± 0.19	4.45 ± 0.50	0.28 ± 0.07	212 ± 45^{b}
Min–max range	0.68–3.23	0.34–1.20	0.47–1.4	0.47–1.10	3.42–5.51	0.20–0.45	91–299
Peak tourist season period							
TE	1.91 ± 0.68	0.50 ± 0.15^{a}	1.06 ± 0.30^{a}	0.86 ± 0.21	4.44 ± 0.90	0.30 ± 0.07	194 ± 25
PC1	1.22 ± 0.36*	0.49 ± 0.14^{a}	0.74 ± 0.19^{b}*	0.66 ± 0.17	5.25 ± 0.72*	0.29 ± 0.07	147 ± 27
PC2	1.59 ± 0.55	0.65 ± 0.10^{b}	1.07 ± 0.32^{a}	0.75 ± 0.13	4.50 ± 0.83	0.29 ± 0.06	185 ± 76
Min–max range	0.90–3.67	0.31–0.81	0.54–1.70	0.45–1.14	3.08–6.82	0.20–0.42	100–358
A. noae from							
Mljet: open sea[1]	1.52	0.69	–	–	2.85	–	89.7
Mljet: Veliko jezero[1]	1.57	0.59	–	–	13.23	–	167.6
Mljet: Malo jezero[1]	2.84	0.69	–	–	5.78	–	133.8
Susak[2]	3.35 ± 1.30	0.55 ± 0.60	–	–	–	–	–
MCL (mussels)[3]	3.7	3.2	2.5	3.4	10	n.v.	200

Asterisk indicates significant differences between the two investigated periods for particular location (*t* test, $p < 0.05$), and different letters indicate significant differences between locations in each period (ANCOVA and LSD post hoc test, $p < 0.05$). Period min–max ranges and metal contamination levels (MCLs) for mussels, which are indicative of contamination, also are presented

n.v. No value

[1] Cuculić et al. (2010) (Island Mljet is located in the southern Adriatic Sea; for comparison purposes metal concentrations were multiplied by 5 to obtain dry weight values)

[2] Ozretić et al. (1990) (Island Susak is located in the northern Adriatic Sea; for comparison purposes metal concentrations were multiplied by 5 to obtain dry weight values)

[3] Cantillo (1998)

Table 3 Heavy metal concentrations in surface sediment samples from the studied sites (mean ± SD of two analytical replicates), in different relatively unpolluted Adriatic sediments and the interim marine sediment quality guidelines (ISQGs)

Sampling sites	Metal concentrations (mg/kg d.w.)						
	Cd	Pb	Cr	Ni	Cu	Co	Zn
TE	0.03 ± 0.01	10.51 ± 0.52	36.10 ± 9.71	7.23 ± 0.44	2.31 ± 0.02	0.92 ± 0.06	5.16 ± 0.93
PC1	0.05 ± 0.01	10.12 ± 0.80	12.93 ± 4.54	4.10 ± 0.35	1.54 ± 0.16	1.02 ± 0.12	21.65 ± 17.22
PC2	0.08 ± 0.04	8.99 ± 0.40	63.83 ± 3.78	5.35 ± 0.50	1.88 ± 0.24	0.96 ± 0.06	8.87 ± 7.39
Telašćica Bay, central Adriatic, Croatia[a]	–	<0.6–13	60–208	14.5–87.2	6.6–21	–	20–83.7
Northern Adriatic[b]	<1	7–51	40–129	19–86	4.1–33.4	4–12	29–167
ISQG[c]	0.7	30.2	52.3	15.9	18.7	n.v.	124

n.v. No value

[a] Mihelčić et al. (2010)

[b] Dolenec et al. (1998)

[c] Canadian Council of Ministers of the Environment, CCME (2002)

observed by Mihelčić et al. (2010) at the anchorage points in TE aquatorium, which is visited by nearly hundred thousand tourists during the year (e.g., in 2014 31,900 individual visitors accessed the park on nautical vessels and 67,954 visitors arrived by organised tourist boats from Zadar and Biograd, Telašćica 2015). Still, based on our preliminary data, Cd and Cr concentrations in sediment (Table 3), as well as Cd in water (Table 4), were not the highest at TE location, and therefore differences in the content of tissue Cd and Cr between sites (Table 2) probably should not be attributed to different levels of environmental exposure. Various abiotic and biotic factors also can affect the metal content in shellfish tissue (Phillips and Rainbow 1994; Ivanković et al. 2005). In shellfish, some metals, such as Ag and Cd, show an increase in concentration with age (Cain and Luoma 1990; Raspor et al. 2004).However, this weight (age)-dependant increase in the concentration of metals will be present only if shellfish growth is slow relative to the rate of accumulation of a metal (Phillips 1976).

It is known that *A. noae* is a slow growing species requiring 3–7 years to reach a commercial length of 50 mm (Peharda et al. 2002, 2003). Peharda et al. (2002) found that the length of 7 cm corresponds to the age of 16 years. In our work, the length of Noah's Ark shells ranged mostly between 7 and 8.5 cm, indicating that the relatively old populations of shellfish were sampled. In addition, significant differences between localities in the length and weight of the shellfish were observed. Considering that longer (>8 cm) and heavier (~60 g), and thus older individuals were sampled at TE and PC2 locations than at the location PC1 (in average 7.6 cm and 41 g), higher concentrations of Cd, which have a tendency to accumulate in shellfish tissue, could be expected in soft tissue of the shells from these two sites. Indeed, our results have confirmed this anticipation, because higher Cd concentrations were found at TE site. When comparison between sites was made using shell size as a covariate, the differences ceased to be significant in peak-tourist season (Table 2), confirming shellfish size as probably important predictive factor for Cd accumulation.

Regarding the concentrations of Pb in both periods and Zn in off-season period, significantly higher values (25–40 %) were measured in soft tissue of Noah's Ark shells from PC2 location compared with the other two locations (Table 2). Same as in the case of Cd and Cr, it could not be associated to variations in metal exposure (Tables 3, 4).

Our finding that metal concentrations in shellfish soft tissue could not be associated to metal exposure from the water and sediment is not surprising, considering that at all three studied locations concentrations of metals in sediments were in the range of unpolluted eastern Adriatic coast sediments, or even lower. That was determined by comparison of preliminary data on heavy metal concentrations in sediments obtained in this study with previously published metal concentrations in sediments of the Adriatic Sea that were considered as relatively unpolluted (Table 3; Dolenec et al. 1998; Mihelčić et al. 2010). Furthermore, when comparing preliminary data on metal concentrations in seawater from this study with those from the recommended marine water-quality criteria for the protection of aquatic life and human health (US EPA 2009), i.e., with the maximum contaminant level (MCL) values (Table 4), it

Table 4 Measured concentrations of trace metals (±95 % confidence interval) for seawater samples in investigated locations in March and June

Sampling sites	Metal concentration (µg/L)				
	Cd	Ni	Cu	Co	Zn
March					
TE	0.0088 ± 0.0007	0.350 ± 0.039	0.213 ± 0.009	0.017 ± 0.003	0.358 ± 0.019
PC1	0.0067 ± 0.0008	0.364 ± 0.019	0.206 ± 0.021	0.023 ± 0.005	0.645 ± 0.054
PC2	0.0078 ± 0.0005	0.369 ± 0.016	0.216 ± 0.018	0.023 ± 0.005	0.473 ± 0.021
June					
TE	0.0113 ± 0.0009	0.409 ± 0.040	0.629 ± 0.063	0.013 ± 0.004	1.769 ± 0.085
PC1	0.0130 ± 0.0014	0.432 ± 0.023	0.410 ± 0.031	0.030 ± 0.002	1.364 ± 0.114
PC2	0.0101 ± 0.0007	0.385 ± 0.017	0.510 ± 0.063	0.018 ± 0.001	0.695 ± 0.048
Mljet Island, Adriatic Sea (open sea)[a]	0.0075 ± 0.0002	–	0.250 ± 0.077	–	0.188 ± 0.095
Krka Estuary, Adriatic Sea (average dissolved metal concentration in bottom-seawater layer for winter/summer period)[b]	0.0091/0.0090	0.376/0.458	0.254/0.362	0.018/0.030	0.458/0.562
MCL (µg/L) for human health for consumption: water + organism/organism only[c]	n.v.	610/4600	1300/n.v.	n.v.	7400/26,000

MCL maximum contaminant level, *n.v.* no value

[a] Cuculić et al. (2009)

[b] Cindrić et al. (2015)

[c] US EPA (2009)

was obvious that in both samplings measured metal concentrations were well below concentrations at which adverse effects could be expected to humans as the final consumers of the seafood.

Regarding two studied seasons, significantly higher values in the peak tourist season compared to the off-season period were observed only for Cd, Cr and Cu on PC1 location (Table 2). Because PC1 site is located near the coast and in the vicinity of major marine transportation routes, the intensification of marine traffic during the tourist season could contribute to increased exposure to metals in shellfish from this location, which can be partly corroborated by preliminary water analyses performed in two seasons (Table 4).

Comparison with metal concentrations in the surface waters from the open sea in the eastern Adriatic, which were considered as unpolluted seawaters (Cuculić et al. 2009), showed that seawater at all three studied sampling sites in March could be considered as unpolluted. At all locations, an increase in the concentrations of metals in seawater in June compared to March was observed for both Cd (1.3–2 times) and Cu (2–3 times), whereas Cr, due to analytical difficulties, was not measured. It was an indication of possible anthropogenic impact due to increased tourist activities during the summer, especially due to nautical tourism. The largest increase of Cd in June was observed precisely in site PC1, whereas Cu had the highest values at site TE. Relative to other sampling sites, location of PC1 site is the closest to the coast and the possible land based sources of Cd (e.g., municipal wastewaters, runoff from agricultural land and roads), which are more abundant during tourist season due to temporarily increased number of inhabitants and possibly could have caused the increase in Cd concentration measured in June. Copper increase in June could be explained, at least in part, by its release from Cu-containing antifouling paints. Increased levels of Cu in water and sediments, as a result of the use of antifouling paints, have previously been reported (Singh and Turner 2009; Turner 2010; Ytreberg et al. 2010).

Additionally, metal concentrations in shellfish soft tissues obtained in this work were generally in the same concentration ranges as those reported for the soft tissue of *A. noae* from relatively unpolluted areas in the Island Mljet in the southern Adriatic Sea (Cuculić et al. 2010) and the Island Susak in the northern Adriatic Sea (Ozretić et al. 1990) (Table 2). Furthermore, because there are no available data on the MCLs that could be an indicative of contamination for Noah's Ark shellfish, tissue concentrations of metals obtained in this study were compared with the MCL values defined for mussels (Cantillo 1998; Table 2). At all locations, the average concentrations of metals in Noah's Ark shell tissue were below MCL values for mussels, and only Cd and Zn had the maximal values that were equal to or slightly higher than the MCLs for mussels.

Estimation of Metal Intake and Impact on Human Health Related to Consumption of Noah's Ark Shellfish

Consumption safety of Noah's Ark shell tissue was assessed by metal content comparison with maximum permissible levels (ML) of the elements in shellfish from the consumption guidelines set by the European Food Safety Authority (EFSA; EC 2006) and the United States Food and Drug Administration (USFDA 2001) (Table 5). The average concentrations of toxic elements Cd, Pb, Cr, and Ni obtained in this study were below maximum permissible levels of these elements in shellfish set by the above authorities (Table 5). Except for Cd, where the maximal observed values were only slightly lower than the maximal permitted level established by EFSA (EC 2006), maximal values of the remaining elements were much lower (6, 38, and 364 times for Pb, Cr, and Ni, respectively) than the levels permitted by USFDA (2001).

Furthermore, the amount of shellfish tissue (in kg) that a 70-kg adult needs to consume to exceed the tolerable weekly intake (TWI) of Cd (EFSA 2010) was calculated, as well as to exceed the benchmark dose associated with a 10 % incidence of toxic effects ($BMDL_{10}$) from Pb (EFSA 2011) and to exceed the provisional TWI (PTWI) of Cu and Zn (JECFA 2004), and Cd appears to be the main element of concern. The average ingestion of approximately 0.6 kg (0.2–1.2 kg, depending on the sampling site) of *A. noae* per week would result in exceeding the TWI threshold of Cd (Table 5). However, it should be noted that the size of the commercial Noah's Ark shellfish available on the market is usually smaller than of the individuals sampled in this study (Peharda et al. 2002, 2003) and therefore, the potential human exposure to high concentrations of Cd from the consumption of such younger shellfish would be much lower.

Lead also is like Cd the chemical element of great concern from a public health point of view (Tong et al. 2000). In 2010, the JECFA concluded that the PTWI of 0.025 $mg/kg_{body\ weight}$ per week for Pb could no longer be considered health protective and withdrew it (JECFA 2010). EFSA also withdrew PTWI for Pb in its opinion of March 2010 (EFSA 2010), and in 2011 it gave new recommendations for Pb in the form of benchmark doses associated with a 10 % incidence of toxic effects from Pb ($BMDL_{10}$) (EFSA 2011) (Table 5). In our study, the calculated average amount of Noah's Ark shell tissue that should be consumed daily to exceed the permitted benchmark dose (EFSA 2011) was 0.4 kg (0.2–0.7 kg, depending on the sampling site) for effects on chronic kidney disease

Table 5 Comparison of metal concentrations expressed in mg/kg wet weight (w.w.) from this study with maximum level (ML) of the elements in shellfish from different consumption guidelines set by EFSA (European Food Safety Authority), USFDA (United States Food and Drug Administration), and JECFA (Joint FAO/WHO Expert Committee on Food Additives)

	Metal concentration (mg/kg w.w.)						
	Cd	Pb	Cr	Ni	Cu	Co	Zn
Mean ± SD	0.32 ± 0.12	0.11 ± 0.03	0.19 ± 0.06	0.15 ± 0.03	0.95 ± 0.16	0.06 ± 0.01	36.3 ± 10.7
Min–max range	0.15–0.74	0.06–0.26	0.11–0.34	0.09–0.22	0.65–1.95	0.04–0.09	18.3–74.7
ML in shellfish (mg/kg w.w.)							
EFSA[a]	1.0	1.5	–	–	–	–	–
USFDA[b]	4.0	1.7	13	80	–	–	–
TWI (μg/kg b.w./week)[c]	2.5	–	–	–	–	–	–
$BMDL_{10}$ (μg/kg b.w./day)[d]		BSP 1.5 KD 0.63					
PTWI (mg/kg b.w./week)[e]	–	–	–	–	3.5	–	7.0

Tolerable weekly intake (TWI) of cadmium, benchmark dose associated with a 10 % incidence of toxic effects from lead ($BMDL_{10}$) and provisional tolerable weekly intake (PTWI) for copper and zinc also are presented. Assessment was made based on all collected samples at three sampling sites during six sampling campaigns, or in total 54 independent pooled samples

[a] EC (2006)

[b] USFDA (2001)

[c] EFSA (2010)

[d] EFSA (2011)

[e] JECFA (2004)

(KD). The amount of shell tissue consumed that could cause the effects on systolic blood pressure (BSP) was approximately 2.5 times higher compared to the amount required for KD toxic effects and amounted to approximately 1 kg (0.4–1.8 kg, depending on the sampling site). Although there are no reliable data on catch and consumption of Noah's Ark shellfish in this harvesting region (Peharda Uljević 2008), given that this is a daily consumption of 0.4–1.0 kg of shell flesh, the potential risk of Pb toxicity due to consumption of Noah's Ark shellfish is extremely low, at least for moderate shellfish consumers.

For Cu and Zn, extremely large amounts of ark shell flesh would need to be consumed each week to exceed the PTWI standard values (Table 5), specifically 258 kg (126–377 kg, depending on the sampling site) for Cu and 13.5 kg (6.6–26.8 kg, depending on the sampling site) for Zn.

Although Ni may serve as a cofactor of metalloenzymes, no clear biological function in humans has been identified (Trumbo et al. 2001). According to the currently accepted guidelines, to protect human consumers, edible portions of molluscs should not exceed 80.0 mg Ni/kg wet weight (w.w.) (USFDA 2001; Sankar et al. 2006). From Table 5, it is evident that Ni concentrations in Noah's Ark shellfish from this study were considerably lower than this limit. The Institute of Medicine, Food, and Nutrition Board (2001) has set the level of 1 mg/day as the highest level of daily Ni intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population (tolerable upper intake level, UL). The UL represents total intake from food, water, and supplements. The amount of Noah's Ark shellfish that should be consumed to exceed the UL threshold was calculated taking into account the average concentration of Ni obtained in this study (0.15 mg/kg w.w.; Table 5). The resulting high value of 6.6 kg/day of Noah's Ark shell meat indicates that at least for the Ni intake, the consummation of Noah's Ark shellfish from this part of the Adriatic should not pose any risk of adverse health effects.

Chromium is not covered by EC regulations for fish and other aquatic products. However, together with As and Ni, Cr is classified as a hazardous element by the USFDA (2001). The concentrations of Cr in Noah's Ark shell tissue from this study (Table 5) were far below the concentration of 13.0 mg Cr/kg w.w. that is considered as health protective for human consumers of molluscan shellfish (USFDA 2001; Sankar et al. 2006). However, there were some studies in which much lower concentrations, such as 0.3–0.4 mg Cr/kg d.w. of molluscan tissues, were proposed as safe for human consumption (Kucuksezgin et al. 2008). Due to the lack of appropriate data, the UL value for Cr was not determined (Trumbo et al. 2001).

Cobalt is essential nutrient to all animals and is a key constituent of vitamin B_{12}, which is the primary biological reservoir of cobalt as an "ultratrace" element (Kazuhiro 2013). Although some health problems, such as polycythemia, heart disease, and anaemia, have been reported in the excessive intake of cobalt (Alexander 1972), cobalt is not usually classified as a hazardous element, and no quantitative recommendation was given for Co intake (Trumbo et al. 2001).

All above calculated values were obtained based on the intake of metals through consummation of Noah's Ark shellfish only. Normal daily food consumption comprises different food and beverage categories, and the shellfish would comprise only smaller proportion. Nevertheless, daily intakes of shellfish could vary according to different dietary habits and cultural origin of tourists visiting the coastal area of the eastern Adriatic Sea. Some of them could be high consumers of shellfish, but even in such instance it is unlikely that the health risk would be very high. Certainly more detailed investigations on the statistics of consumption of Noah's Ark shellfish would be necessary to assess the health impact on humans more exactly.

Conclusions

This study represents the first detailed investigation on heavy metal concentrations in *A. noae* with the accompanying information about the metal concentrations in sediments and seawater, and as such, it provides baseline data for future assessment of the quality of these bivalves as a food and also highlights the importance of possible anthropogenic impacts caused by environmental metal pollution. Concentrations of all analysed trace elements were below the levels recommended for human consumption. However, in the case of cadmium, due to high metal accumulation potential of *A. noae*, some precautions should be taken into account if older shellfish are consumed. Two different periods concerning the intensity of tourist activities, the off-season and the peak tourist season, were evaluated. An increase in metal concentrations in *A. noae* during tourist season was observed for Cd, Cr, and Cu, but before the final conclusion on the potential impact of tourist activities, the influence of other factors, such as the annual reproductive cycle, on metal content in *A. noae* should be further investigated.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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