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Differences in metal profiles revealed by native mussels and artificial mussels in Sarıçay Stream, Turkey: implications for pollution monitoring

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Abstract. Using the native mussel *Unio crassus* and artificial mussels (AMs), profiles of 11 metals (Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U, Zn) were determined and compared in winter and summer along a pollution gradient in Sarıçay Stream, Turkey. Principal components analysis and correlation analysis showed that metal profiles in the native mussels and AMs were different. Concentrations of most metals were significantly higher in the native mussels compared with AMs, suggesting that metals in Sarıçay Stream predominantly existed in suspended particulates and food compartments, rather than in dissolved form. Although U was not readily accumulated by the native mussels, it could be taken up by AMs. Overall, the results suggest that the use of native mussels and AMs in water quality monitoring can provide complementary information and a better estimate and coverage of different metal species and forms in aquatic environments.

Additional keywords: environmental monitoring, heavy metals.

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Introduction

In Turkey, water pollution has led to major public health and environmental problems (Oglu *et al.* 2015; Yorulmaz *et al.* 2015). In particular, bioaccumulation and trophic transfer of water pollutants in seafood harvested from contaminated waters have caused considerable public health concern (Islam *et al.* 2014). Biomonitors have a remarkable ability to accumulate metals from water and food. Hence, their metal body burden has often been used to provide a time-integrated estimate of metal concentrations in the aquatic environment (Gonzalez-Rey *et al.* 2011; Genç *et al.* 2015). Mussels have been extensively used as biomonitors for metal pollution in marine habitats since the 1980s, most notably by the Global Mussel Watch Program (Goldberg and Bertine 2000; Nakata *et al.* 2012; Marigómez *et al.* 2013; Melwani *et al.* 2014; Regoli *et al.* 2014; Lopes-Lima *et al.* 2017). Nevertheless, metal accumulation in biomonitors can be affected by both abiotic (e.g. temperature, pH, salinity, level of pollution) and biotic (e.g. growth, reproduction, metabolism, excretion) factors, which are difficult or impossible to control (Casas and Bacherb 2006; Wu *et al.* 2007; Degger *et al.* 2011; Melwani *et al.* 2014; Richir and Gobert 2016). The most intractable problem is that the limit of natural distribution of biomonitors often makes it impossible to compare metal concentrations over large geographical areas (Wu and Lau 1996; Wu *et al.* 2007). Furthermore, there is a lack of cosmopolitan biomonitors in freshwater habitats (equivalent to *Mytilus* spp. and *Perna* spp. in marine habitats) to allow for the biomonitoring of metals.

Wu *et al.* (2007) developed a passive chemical device called the ‘artificial mussel’ (AM), which can take up and release dissolved metals in proportion to their respective concentrations in ambient water. Results of anodic stripping voltammetry (ASV) further showed that AMs were able to accumulate the ASV labile fractions, including free metal ions, metal ions associated with inorganic and organic species and the bioavailable fraction of metals. Therefore, AMs can enable assessment and direct comparison of metal concentrations in aquatic environments worldwide. AMs have now been used to determine the spatial and temporal variations of metals in both marine and freshwater systems and identify metal pollution ‘hot spots’ in many countries and regions, including Hong Kong (Wu *et al.* 2007), Iceland, the UK (Leung *et al.* 2008), Portugal (Gonzalez-Rey *et al.* 2011), South Africa (Degger *et al.* 2011; Claassens *et al.* 2016), Australia (Kibria *et al.* 2012, 2016), South Korea (Ra *et al.* 2014), China (Degger *et al.* 2016) and Bangladesh (Kibria *et al.* 2016). The results of all these field studies showed that metals accumulated by AMs can provide a reliable indication on metal levels in marine, estuarine and freshwater environments with contrasting hydrographic conditions. Significant correlations were also found between metals accumulated in AMs and different native mussel species.

Sarıçay Stream is of great ecological and socioeconomic importance in Turkey. It serves as a major source of irrigation (Yılmaz *et al.* 2007), but it is heavily polluted by domestic and industrial waste generated from agricultural and urban activities in the catchment area (Dalman *et al.* 2006; Tuna *et al.* 2007). Using the native mussel *Unio crassus* and AMs, the aims of the present study were to: (1) determine the spatial and temporal variations of 11 metals along the pollution gradient in Sarıçay Stream; and (2) compare metal accumulation and profiles in native mussels and AMs, to shed light on future water quality monitoring programs.

Materials and methods

Study area

Three sites along Sarıçay Stream in Muğla were chosen for the present study: Site 1 (37°19′16.3″N, 27°48′44.5″E) was situated 20 km upstream, remote from any agricultural and anthropogenic activities; Site 2 (37°20′44.40″N, 27°44′17.75″E) was near the city, yet supported a high diversity of aquatic organisms (Oglu *et al.* 2015); and Site 3 (37°18′97.00″N, 27°42′45.43″E) was in close proximity to marble and olive oil industrial plants, and known to be polluted by industrial and agricultural run-off (Fig. 1).

Native mussels and AMs

The native mussel *U. crassus* has a wide geographical distribution, spanning from central, south-eastern to northern Europe (Lopes-Lima *et al.* 2017), and is abundant in Sarıçay Stream. This species is highly tolerant of contamination and fluctuations in salinity and temperature, and therefore was chosen for the present study. Native mussels were harvested from a clean site in Sarıçay Stream (37°19′16.3″N, 27°48′44.5″E) and depurated in ultrapure water (Direct-Q 8 UV; Merck, Darmstadt, Germany) in the laboratory (mean ± s.d., 12.0 ± 0.5°C) for 30 days before field deployment. During the depuration period, mussels were fed with diatoms (*Skeletonema costatum*) *ad libitum* once daily.

AMs were prepared according to the methods of Wu *et al.* (2007). Briefly, each AM consisted of a non-permeable Perspex tube (length 6 cm; diameter 2.5 cm), in which 200 mg of Chelex 100 (50–100 mesh; Bio-Rad, Hercules, CA, USA) was suspended in 8 mL of artificial seawater (salinity = 35‰) inside the tubing. Both ends of the plastic tube were capped with a 1-cm layer of polyacrylamide gel and a perforated plastic cap.

Field deployment

Field deployment and subsequent retrieval were performed once in winter (December 2013–February 2014) and once in summer (June–August 2014). For each season, 90 native mussels (winter deployment: mean (±s.d.) shell length 66.5 ± 0.3 mm and wet weight 50.9 ± 0.8 g; summer deployment: shell length 66.0 ± 0.4 mm and wet weight 54.4 ± 0.8 g) were put in a plastic cage (18 × 17 × 16 cm) and deployed side by side with 60 AMs in another cage 2 m below the water surface at each of the three sites. At the time of deployment, physicochemical parameters (pH, water temperature and conductivity) at each site were measured using a portable multimeter (Model HQ40D; Hach Lange, Duesseldorf, Germany; Table 1). Native mussels and AMs were retrieved for metal determination after 28 days.

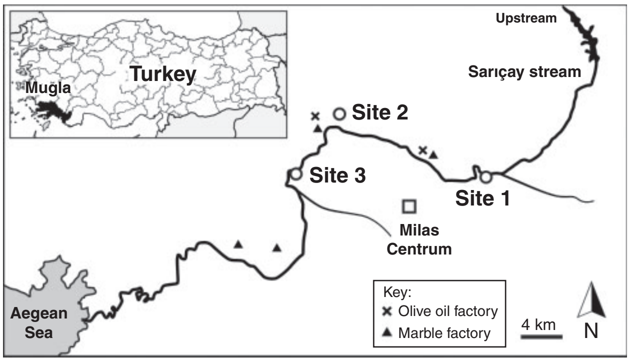

Fig. 1. Location of the three sites along the Sarıçay Stream in Muğla, Turkey.

Table 1. Mean pH, temperature and conductivity at the three sites in winter (December 2013–February 2014) and summer (June–August 2014)

	Site	pH	Temperature (°C)	Conductivity (μS cm^{-1})
Winter	1	8.19	15.8	968.3
	2	8.88	17.4	1436.0
	3	8.27	17.5	1866.7
Summer	1	7.35	16.5	749.3
	2	7.37	21.4	1266.0
	3	7.26	27.1	1567.3

Sample preparation and metal analysis

All metal analyses were conducted following the protocol described by Wu *et al.* (2007), and all laboratory ware was rinsed with 1 M HNO_3 followed by double-distilled water before use. Briefly, the contents of each AM were emptied into a sintered glass filter followed by elution twice with 12.5 mL of 6 M nitric acid (analytical grade). The eluent was made up to a known volume with deionized double-distilled water and the metal concentration was determined using inductively coupled plasma–atomic emission spectrometry (ICP-AES; Optima 2100 DV; Perkin–Elmer, Foster City, CA, USA; plasma flow 15 L min^{-1}, auxiliary flow 0.3 L min^{-1}, nebuliser flow 0.8 L min^{-1}, radiofrequency flow 1300 W, pump rate 1.0 mL min^{-1}).

In the laboratory, the soft tissue of native mussels was dissected with a plastic knife, rinsed with deionized double-distilled water, dried and weighed before acid digestion in a block digester (Techne DG-1; Camlab, Cambridge, UK), using 30% hydrogen peroxide and 70% nitric acid (1 : 1 v/v). Metal concentrations were determined using ICP-AES as described above. Dried oyster tissues (US Standard Reference Material 1566a; National Institute of Standards and Technology, Gaithersburg, MD, USA) were used as reference material, and the recovery rate was >99%.

The concentration of each of the metals in AMs and native mussels is expressed in terms of micrograms per gram of Chelex and micrograms per gram of dry tissue weight respectively.

Data analyses

Kruskal–Wallis tests followed by the Mann–Whitney *U*-test were used to test the significance of differences between sites and seasons (significance for all tests was set at two-tailed $P < 0.05$). Relationship between metals in native mussels and AMs were determined using Spearman's ρ correlation tests. After square root transformation and normalization of data, principal components analysis (PCA) was used to identify differences in metal profiles and concentrations in native mussels and AMs between sites and seasons (R, ver.1.3; R Foundation for Statistical Computing, Vienna, Austria). Analysis of similarities (ANOSIM) was used to test the hypothesis that metal profiles in the native mussels and AMs are similar. All statistical tests were performed using PRIMER 6 (ver. 6.1.5; PRIMER-E, Auckland, New Zealand; Clarke and Gorley 2006) and SPSS (ver. 20.0, IBM, Chicago, IL, USA).

Results

Metal profiles in native mussels and AMs

The concentration of each metal (mean ± s.d.) in native mussels and AMs at the three sites in summer and winter is shown in Fig. 2. Hg was below the limit of detection in native mussels throughout the study, as well as in AMs in winter. Cd and Cr were measured in native mussels but were below the limit of detection in AMs (except for a very low level of Cr recorded at Site 3 in summer). In contrast, U was only found in AMs and was below the limit of detection in all native mussel samples. Notably, the concentrations of all other metals found in native mussels were significantly higher than in AMs.

The correlation of individual metals in native mussels and AMs are given in Table 2. Positive correlations were only found for Zn ($r = 0.608$) and Fe ($r = 0.591$). In addition, the level of Zn in AMs was positively correlated with Fe ($r = 0.474$) and Mn ($r = 0.560$) in native mussels. However, clear discrepancies were found in the accumulation profiles of most other metals, demonstrating that factors governing metal uptake in the native mussels and AMs may be different in the study area.

Results of the PCA on the metal profiles of native mussels and AMs are shown in Fig. 3 and 4 respectively. Notably, the metal profiles of the native mussels and AMs are different. In the native mussels, 78.6% of the total variance could be explained by the first two components (PC1: 58.9%; PC2: 19.7%). In the AMs, 57.3% of the total variance could be explained by the first two components: (PC1: 37.4%; PC2: 19.9%). Results of the PCA further showed that the metal profiles of native mussels in the reference site (Site 1) could be separated from the intermediate site (Site 2) and the polluted site (Site 3) in winter, but not in summer. The metal profiles in the AMs also showed a clear separation between sites in winter.

The eigenvector values of metals are given in Table 3, and correlation values above 0.4 and below −0.4 are taken as the discriminating value in the present study. In native mussels, PC1 is strongly correlated with Fe and Cr, whereas PC2 is strongly correlated with both Cu and Pb. In AMs, PC1 is strongly correlated with Mn, Fe and Ni, whereas PC2 is strongly correlated with Cu and U.

Results of ANOSIM showed that global *R* values for AMs (temporal: 0.959; spatial: 0.7) are much higher than those for native mussels (temporal: 0.48; spatial: 0.276). A comparison of *R* statistics also indicated that the resemblance of metal profiles was lower in native mussels than AMs (Table 4).

Temporal and spatial variations in metals in native mussels and AMs

The patterns of temporal variations between the native mussels and AMs appeared to be different (Fig. 2). Considerable temporal variations in metal concentrations were found in native mussels, and the levels of Fe, Mn, Zn and Cd at all sites were significantly higher in summer. Temporal variations were less obvious in AMs, and higher levels of Mn, Ni and Co were only found in Sites 2 and 3 during winter.

In winter, levels of Fe, Mn and Zn in both native mussels and AMs showed a significant increase from the reference site (Site 1) to the polluted site (Site 3). This spatial trend was much less obvious in the summer, when levels of Fe, Ni and Co showed a significant increase from the reference site to the polluted site in the native mussels, but not in AMs.

Discussion

Metal concentrations found in the native mussels in Sarıçay Stream are much higher compared with other species of freshwater and marine bivalves, such as in *Unio* sp. in Muğla City and Van Lake, Turkey (Yarsan *et al.* 2000; Genç *et al.* 2015), *Perna viridis* along the west coast of Malaysia (Yap *et al.* 2004) and *Anodonta anatina*, *A. cygnea* and *Unio tumidus* in Poland (Rzymski *et al.* 2014), suggesting that Sarıçay Stream may be more polluted with metals than the other study areas. Oglu *et al.* (2015) reported high concentrations of Fe, Mn and Zn in the water, sediment and fish (*Squalis cephalus)* in Sarıçay Stream. This is consistent with the high concentrations of Mn, Fe and Zn found in both native mussels and AMs in the present study. The high levels of metal pollution revealed by these studies together may pose a significant threat to both ecosystem and public health in Sarıçay Stream and the surrounding area.

Both PCA and correlation analysis show that the metal profiles of the native mussels and AMs are different, and this may be attributed to several factors. There are different chemical forms of metals in the aquatic environment. Notably, AMs only take up the dissolved fraction of metals, whereas the native mussels can take up metals in dissolved form, as well as in particulates and food through filtering and feeding. Thus, metal accumulation in native mussels and AMs may depend not only on the total metal concentration, but also on the proportion of specific metal forms that prevail in the natural environment. Furthermore, metal uptake by mussels could be confounded by food selection and metal

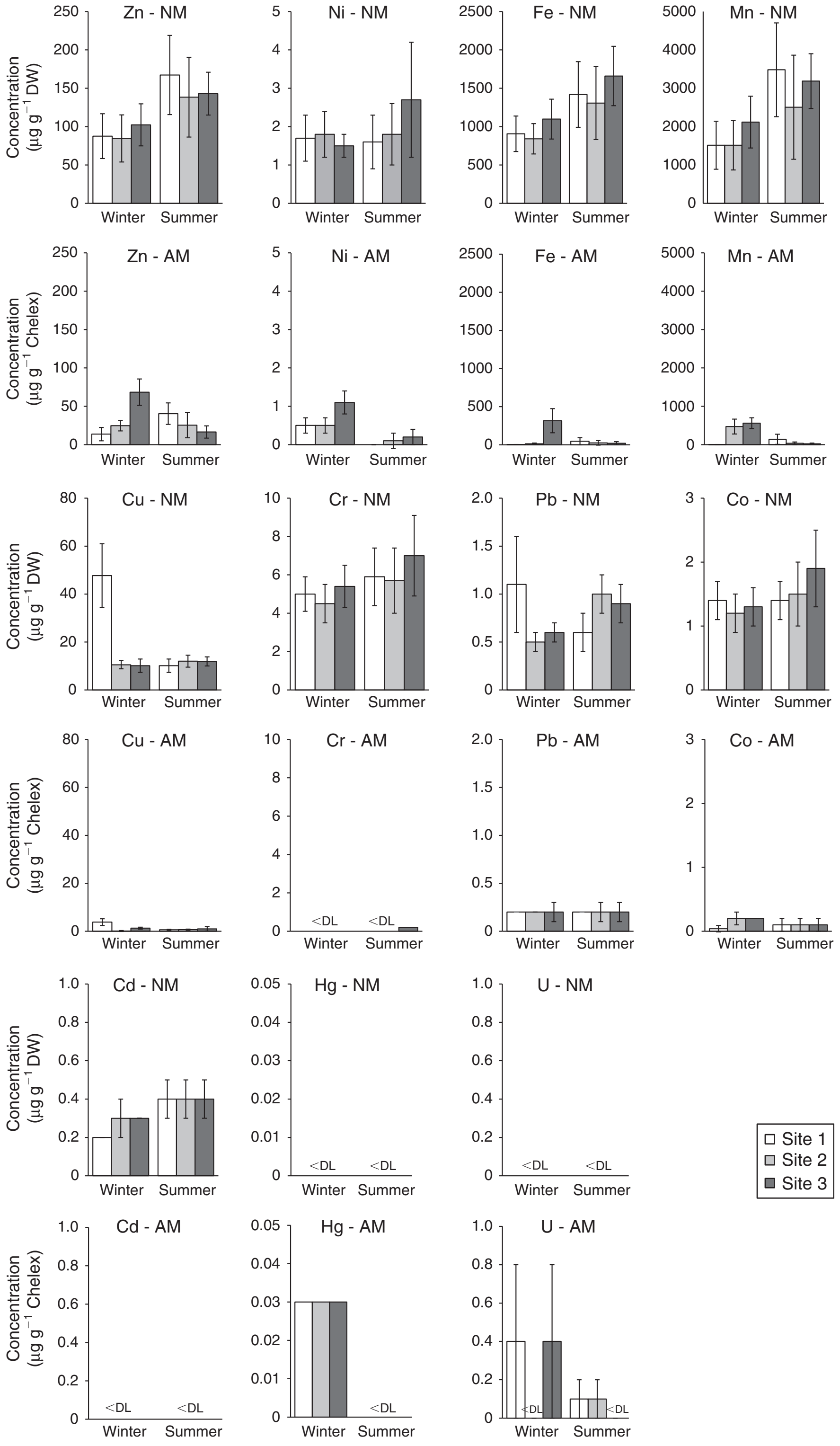

Fig. 2. Metal concentrations in native mussels (NM; *Unio crassus*) and artificial mussels (AM) at the three sites in winter (December 2013–February 2014) and summer (June–August 2014). Site 1 was the reference site, Site 2 was an intermediate site and Site 3 was a polluted site. Data are the mean ± s.d. <DL, below the limit of detection; DW, dry weight.

Table 2. Correlation (*r*) matrices of metal concentration in native mussels (*Unio crassus*) and artificial mussels

**, $P < 0.001$; *, $P < 0.05$. AM, artificial mussel

AM	Native mussel						
	Zn	Ni	Fe	Mn	Cu	Pb	Co
Zn	0.608**	0.187	0.474*	0.560*	−0.602**	−0.555*	−0.373
Ni	−0.077	−0.091	−0.142	−0.131	−0.309	−0.470*	−0.492*
Fe	0.645**	0.214	0.591**	0.670**	−0.399	−0.246	−0.179
Mn	0.251	−0.201	0.119	0.292	−0.311	−0.396	−0.420
Cu	−0.340	−0.123	−0.230	−0.364	0.267	0.264	0.116
Pb	−0.415	−0.524*	−0.420	−0.361	0.254	0.121	−0.101
Co	−0.365	−0.742**	−0.429	−0.242	0.068	−0.222	−0.393

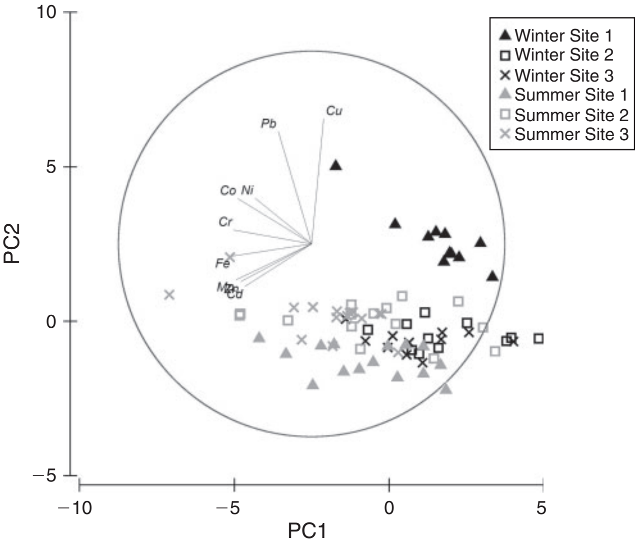

Fig. 3. Principal components analysis for metal profiles in native mussels (*Unio crassus*). Site 1 was the reference site, Site 2 was an intermediate site and Site 3 was a polluted site. PC1, principal component 1; PC2, principal component 2.

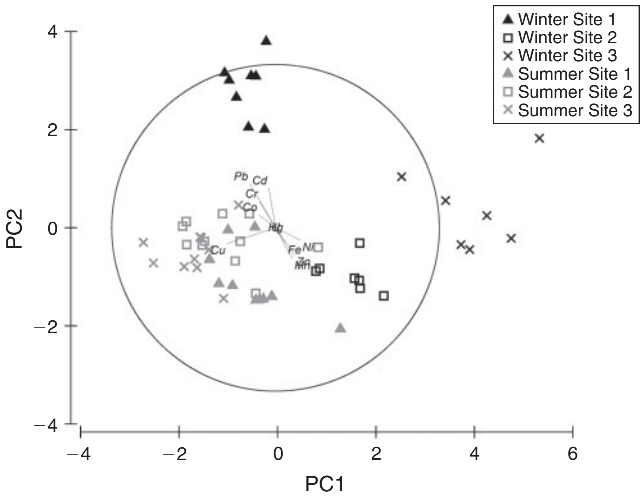

Fig. 4. Principal components analysis for metal profiles in artificial mussels. Site 1 was the reference site, Site 2 was an intermediate site and Site 3 was a polluted site. PC1, principal component 1; PC2, principal component 2.

Table 3. Eigenvector values of principal components (PC) 1 and 2 of native mussels and artificial mussels (*Unio crassus*)

Variable	Native mussel		Artificial mussel	
	PC1	PC2	PC1	PC2
Zn	−0.366	−0.195	0.358	−0.176
Ni	−0.291	0.236	0.377	0.348
Fe	−0.411	−0.063	0.418	−0.097
Mn	−0.390	−0.186	0.458	−0.266
Cu	0.062	0.648	−0.038	0.597
Cr	−0.402	0.072	−0.213	−0.122
Pb	−0.172	0.582	0.002	0.207
Co	−0.381	0.235	0.416	−0.176
Cd	−0.346	−0.221	0.344	0.364
U	–	–	0.087	0.436

regulation, which are often metal and species specific (Goldberg and Bertine 2000; Wu *et al.* 2007; Leung *et al.* 2008). The observed differences in metal accumulation between the native mussels and AMs may be further confounded by physicochemical factors (pH, temperature, alkalinity, humic acids and water hardness) prevailing in the environment, which may affect not only metal speciation (and hence bioavailability), but also the feeding, growth and reproduction (and hence metal retention) of the native mussels (Smith *et al.* 2015).

In Portugal, Gonzalez-Rey *et al.* (2011) found that the accumulation of Cu and Cd was similar between AMs and *Mytilus galloprovincialis*, whereas higher concentrations (~10-fold higher) of Zn were observed in the native mussels and the reverse was shown for Pb. In the present study, concentrations of all metals were higher in native mussels than in AMs. This, coupled with the marked difference in metal profile revealed between native mussels and AMs, indicates that food and suspended particulate matters are the major sinks of metals in the waters of Sarıçay Stream.

The present study showed that the spatial and temporal variations of metal levels are more marked in native mussels than AMs, which may be attributed, in part, to the seasonal

Table 4. *R* statistics indicating resemblance of metal profiles in native mussels (*Unio crassus*) and artificial mussels at the three sites in winter (December 2013-February 2014) and summer (June–August 2014)

Site 1 was situated 20 km upstream, remote from any agricultural and anthropogenic activities, and was considered the reference site. Site 2 was near the city, yet supported a high diversity of aquatic organisms. Site 3 was in close proximity to marble and olive oil industrial plants, and was known to be polluted by industrial and agricultural run-off

Comparisons	Native mussel		Artificial mussel	
	R-statistic	*P*	*R*-statistic	*P*
Site 1 *v.* Site 2	0.342	0.1	0.443	0.1
Site 1 *v.* Site 3	0.422	0.1	0.912	0.1
Site 2 *v.* Site 3	0.070	2.4	0.774	0.1

availability of food and particulate matters in the Sarıçay Stream and the different biological responses of the native mussels in different seasons. In general, higher levels of Fe, Mn and Zn were found in both native mussels and AMs at the polluted site (Site 3) compared with the reference site (Site 1) in winter, which can be attributed to an increase in agricultural activities at this time of year.

Conclusion

To the best of our knowledge, the present study is the first time that AMs have been used for metal monitoring in the Middle East, following the Artificial Mussel Watch Programme conducted in the UK, Iceland, Portugal, South Africa, China, Korea, Bangladesh and Australia. Although previous studies showed similar metal profiles between AMs and native mussels (*Mytilus* spp. and *Perna* spp.) in marine environments, the results of this study showed that metal profiles in AMs and the native mussel *U. crassus* were different in the freshwater environment. For example, Hg and U were measured in AMs but not in *U. crassus*. However, it must be noted that even different species of mussels may take up different fractions of metals in the environment. It is generally accepted that no standard or model biomonitor species can be considered representative (Degger *et al.* 2016). From the viewpoint of pollution monitoring and environmental risk assessment, the bioavailable fraction of metals, which is the most toxic fraction and bioaccumulatable, is of primary concern, and AMs can take up free ions as well as the organic and inorganic liable fractions of metals (Wu *et al.* 2007). Therefore, the use of both native mussels and AMs can provide complementary information and a better coverage and risk estimation of metals in the aquatic environment. Furthermore, the results of the present study showed that AMs can be used in practical field monitoring of metal contamination in freshwater environments in addition to marine environments.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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