

Bioaccumulation of heavy metals and analysis of mineral element alongside proximate composition in foot, gills and mantle of freshwater mussels (*Anodonta anatina*)

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Abstract Bioaccumulation of heavy metals and analysis of mineral element alongside proximate composition were studied in tissues of freshwater mussels (*Anodonta anatina*) exposed to various doses of Pb, Cu and Cr in water. The concentrations of all the studied heavy metals in soft tissues of the mussels increased as the metal doses were increased from 0 to 360 µg/L of water. The highest concentration of Cu was observed in the gills of mussels at the highest dose (360 µg/L), whereas the lowest concentration was observed in the Cr-exposed mussels at lower dose (120 µg/L). Amongst mineral elements, Ca was found to be the most abundant element in all tissues. The maximum Ca (156,906 ± 736 mg/kg) was observed in the gills. The abundance order of the other mineral elements was P > Mn > Na > K > Zn. Proximate analysis showed that the protein (15.45 ± 1.13 %), fat (0.97 ± 0.10 %) and moisture (77.78 ± 1.20 %) contents were significantly higher in the foot, whereas the carbohydrate (15.15 ± 1.30 %) and ash (10.55 ± 1.11 %) contents were higher in the mantle and gills, respectively. It was found that the low-dose exposure of Pb and Cu and the high-dose exposure of Cr caused higher protein content in the foot. It

appears that freshwater mussels (*Anodonta anatina*) are an essential tool for biomonitoring studies. However, specific evaluation of mussel tissues was more effective than using the whole animal in these studies.

Keywords *Anodonta anatina* · Bioaccumulation · Heavy metals · Proximate composition · Mineral element analysis

1 Introduction

Bivalve molluscs are usually known to be very useful animals to monitor pollution by heavy metals. Freshwater mussels are a good choice for assessing aquatic pollutants, due to their filter feeding activity and continuous exposure of tissues to ambient water. Analysis of soft tissues and body fluids of aquatic animals were investigated in various biomonitoring studies for assessing environmental health. The accumulation of various metals and their effects on biochemical components of soft tissues were also analysed to identify their interactions. Bivalve mussels are biologically very important for biomonitoring studies due to their high survival rate, feasibility to be maintained in the laboratory, and their ability to accumulate high concentrations of a wide range of pollutants (Boening 1999; Kimbrough et al. 2008; Sarkar et al. 2008; Zhou et al. 2008). In previous biomonitoring studies, it was noted that toxicity could be assessed using molluscs (Goldberg 1986; Cantillo 1998), even when the pollutant concentrations in water are very low or close to their detection limits (Viarengo and Canesi 1991; Blackmore and Wang 2003).

Heavy metals occur in water naturally, but anthropogenic activities related to various industries, mining, agricultural practices and household waste also contribute to an increase of metals in the aquatic environment. This

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may directly affect aquatic life because heavy metals accumulate in different tissues (Usero et al. 2005). Some metals are biologically required (e.g., Cu, Zn, Na, K, P, Mn), although they may prove to be toxic at high levels and could be harmful for the aquatic ecosystem due to their persistence (Turkmen et al. 2008). Toxic metals such as lead nitrate can cause adverse effects in freshwater mussels at various levels, including metabolic defects and decrease in enzymatic activity (Mosher et al. 2012). A significant increase in lipid peroxidation and lysosomal membrane destruction was observed in Pb-exposed mussels, while the antioxidant capacity was greatly reduced (Wadige et al. 2014).

Bivalves, especially mussels, are used as human food in various parts of the world, and due to high nutritive values their demand is increasing internationally (Orban et al. 2002; Fuentes et al. 2009; Karnjanapratum et al. 2013; Pogoda et al. 2013). Mussels are a rich source of dietary components such as protein, minerals, fatty acids and carbohydrates (Fernandez-Reiriz et al. 1996; Orban et al. 2002; Grienke et al. 2014). Espana et al. (2007) and Fuentes et al. (2009) reported that mussels were a cheap source of protein and many essential vitamins and minerals. To investigate the nutritional and commercial value of mussels, analysis of their chemical composition could be a good indicator as reported by Orban et al. (2006). However, the chemical composition of mussels may be affected if they are exposed to metal-polluted waters causing metal-induced changes in their body functions. Nevertheless, potential interactions between metal concentrations and chemical composition of mussels have not been reported in the past.

Therefore, the objective of this study was to estimate the bioaccumulation of trace metals in foot, gills and mantle of freshwater mussels to evaluate the capacity of these tissues to store various metals and their impact on body compositions. Furthermore, the effect of different doses of various metals on mineral elements and proximate composition of freshwater mussels was also investigated in this study.

2 Materials and methods

2.1 Animal collection and placement

Freshwater mussels (*Anodonta anatina*) of average size (64.5 ± 2 g) were collected from an unpolluted university research pond having an average temperature of 20.5 ± 1.5 °C. The mussel samples were immediately carried to the fish hatchery at Manawa fisheries research and training institute in cool plastic bags and then placed in large rectangular cemented tanks with filtered pond water. These mussels were fed green algae, which were harvested

from the same fish pond. An acclimatization period of 10 days was adopted to minimize stress before the start of experimentation. The mussels were kept at 16.8 ± 1.2 °C, and water was changed daily during the acclimatization period when no mortality was observed.

2.2 Experimental design

Freshwater mussels were distributed into glass tanks ($24'' \times 18'' \times 24''$) in such a way that each treatment was allocated to three tanks where each tank had five mussels. The mussels in these tanks were then exposed separately to 0 µg/L, 120 µg/L, 240 µg/L and 360 µg/L of lead (Pb), copper (Cu) and chromium (Cr) for 15 days. Primary stock solutions of Pb, Cu and Cr were prepared in distilled water using Pb (NO₃), Cr (NO₃). 9H₂O and CuSO₄. 5H₂O, respectively. The stock solutions were diluted in distilled water to achieve the required doses of these metals being used for exposure. The water was changed every 5 days with the renewal of each chemical. The mussels were killed at the end of the experiment and the samples of their gills, foot and mantle were collected for the estimation of heavy metals, mineral elements and proximate composition.

2.3 Isolation of soft tissues

The mussel shells were opened with a scalpel and a small steel rod. The animal samples were then washed in water to remove any unwanted particles. The gills, foot and mantle were excised carefully with the help of sharp scissors and washed well with double distilled water. Tissue samples were then put into small plastic bottles which were kept in a refrigerator at -4 °C. All the samples were weighed and then dried in a freeze dryer for 7 days. Thereafter, the dried samples were weighed, ground and placed in a desiccator until analysis.

2.4 Metal and mineral analysis

About 0.2 g of freeze-dried sample was weighed into a digestion flask to which 9 mL concentrated nitric acid (HNO₃) and 1 mL perchloric acid (HClO₄) were added. The digestion flasks were left in a fume hood overnight. Each sample was then heated on a hot plate at 100 °C until it was nearly dry. The samples were cooled and diluted to 10 mL with distilled water and centrifuged at 2000 rpm for 10 min. The supernatants were transferred to separate volumetric plastic vials and stored in a refrigerator until analysis. The concentrations of potassium (K), phosphorus (P), sodium (Na), calcium (Ca), manganese (Mn) and zinc (Zn), lead (Pb), copper (Cu) and chromium (Cr) were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES Vista-Mpx) as recommended by

AOAC (2000). The calibration of the machine was achieved over an appropriate range of concentration with a certified standard containing the target mineral elements from Sigma-Aldrich, UK. The metal concentrations in foot, gills and mantle of freshwater mussels were reported as mg/kg dry weight. These analyses were carried out at the School of Agriculture, Food and Rural Development, Newcastle University, UK.

2.5 Proximate analysis

The freeze-dried samples of foot, gills and mantle were analysed for moisture, protein, fat and ash contents according to AOAC (1997). Elementar Vario Macro Cube was used to estimate the percentage of carbon and nitrogen. Protein contents were obtained by multiplying the nitrogen value with 6.25. The ash contents were analysed by igniting the samples in a muffle furnace at 500 °C. The fats were extracted by Soxhlet apparatus and carbohydrate contents were determined by subtracting the sum of protein, fat and ash contents from the total dry weight of each sample.

2.6 Statistical analysis

The data were examined using two-way analysis of variance (ANOVA) and the means were compared using Tukey's pairwise test to assess the mean difference between the control and different doses of the studied metals. Statistical significance was declared if $p < 0.05$. All the statistical analyses were carried out by using the Minitab 17 software.

3 Results

The mussels were exposed to four separate concentrations of Pb, Cu and Cr which were identified as T₀ (0 µg/L), T₁ (120 µg/L, low), T₂ (240 µg/L, medium) and T₃ (360 µg/L, high). Since T₀ (0 µg/L) contained no metal, it was considered as a control. The comparison was carried out among the various doses of different metals and with the control conditions. The mean values of temperature, pH and dissolved oxygen (DO) of aquarium water were 17.6 ± 1.3 °C, 7.15 ± 0.15 and 6.32 ± 0.42 mg/L, respectively.

3.1 Bioaccumulation of metals

The accumulation of heavy metals in foot, gills and mantle of the studied mussels was observed to be increased for all metals in all tissues as the dose was increased. Among the studied portions, gills stored the highest concentration of

metals in all cases as compared to the foot and mantle (Table 1). The overall accumulation of Cu was found to be significantly higher as compared to Pb and Cr. The gills had the maximum mean value of Cu (149.57 ± 5.34 mg/kg), Pb (30.00 ± 3.45 mg/kg) and Cr (22.192 ± 1.228 mg/kg) at the highest dose (360 µg/L) ($p < 0.05$). The maximum concentration of Cu, Pb and Cr in the foot was 35.271 ± 0.0720 , 17.149 ± 1.521 and 4.539 ± 0.520 mg/kg, respectively. Although no significant difference was found in the foot portion for different doses of Cu, the values in T₁ (29.210 ± 0.514 mg/kg), T₂ (32.888 ± 0.173 mg/kg) and T₃ (35.271 ± 0.0720 mg/kg) were significantly higher than in the control T₀ (10.584 ± 0.963 mg/kg). Cr showed the least accumulation as compared to Pb and Cu. The lowest concentration was found in the mantle at various doses [T₀ (0.837 ± 0.296 mg/kg), T₁ (1.307 ± 0.500 mg/kg), T₂ (1.681 ± 0.516 mg/kg) and T₃ (3.324 ± 0.302 mg/kg)] (Table 1). The order of tissues in the case of Pb and Cr accumulation was gills > foot > mantle, whereas in Cu-exposed mussels the mantle had a higher concentration than the foot in most cases (Table 1).

3.2 Mineral element analysis

Mean mineral element compositions of various tissues of freshwater mussels from the control tank are shown in Table 2. Some variations were observed in the samples of gills, foot and mantle for different mineral elements. The concentration of Na was higher in the mantle (1879.9 ± 50.6 mg/kg) than in the foot (1680.6 ± 2.39 mg/kg) and gills (1577 ± 57.3 mg/kg), whereas the value of Ca was found to be greater in the gills ($156,906 \pm 736$ mg/kg) than in the mantle ($45,207 \pm 241$ mg/kg) and foot (3052 ± 29.7 mg/kg) ($p < 0.05$). The same trend was also observed for P, Mn and Zn, for which gills showed higher values than foot and mantle; the exception was K, which was higher in the foot than other tissues (Table 2). Amongst all the studied mineral elements, Zn showed the lowest concentrations (gills: 282.2 ± 49.5 mg/kg; mantle: 128.7 ± 17.3 mg/kg, and foot: 66.16 ± 5.94 mg/kg).

Mineral elements were also analysed in tissues of metal-exposed mussels. The results are shown in Figs. 1, 2 and 3. The concentration of Ca in gills of mussels was significantly higher ($p < 0.05$) in Pb-exposed mussels at all doses, namely: at 120 µg/L: $185,616 \pm 590$ mg/kg; at 240 µg/L: $183,854 \pm 96$ mg/kg; and at 360 µg/L: $185,098 \pm 795$ mg/kg (Supplementary Fig. 1) as compared to the control (Table 2). The concentrations of Na and K in the foot, gills and mantle were significantly lower ($p < 0.05$) than the control at all the doses of Cu-exposed mussels (Table 2, Fig. 2). No significant difference ($p > 0.05$) was found for Zn, Mn and P between the control and the Cu-

Table 1 Bioaccumulation of metals in foot, gills and mantle of freshwater mussels (*Anodonta anatina*) exposed to different doses of lead (Pb), copper (Cu) and chromium (Cr)

Metals	Tissue	Concentration (mg/kg)			
		T0 (0 µg/L)	T1 (120 µg/L)	T2 (240 µg/L)	T3 (360 µg/L)
Lead (Pb)	Foot	3.959 ± 0.757 ^{ef}	6.955 ± 1.394 ^{def}	11.208 ± 1.450 ^{bcd}	17.149 ± 1.521 ^a
	Gills	4.523 ± 1.057 ^{ef}	10.95 ± 2.99 ^{bcd}	16.06 ± 3.25 ^{bc}	30.00 ± 3.45 ^a
	Mantle	3.23 ± 1.99 ^f	4.386 ± 1.718 ^{ef}	5.71 ± 2.01 ^{def}	10.30 ± 2.50 ^{cde}
Copper (Cu)	Foot	10.584 ± 0.963 ^h	29.210 ± 0.514 ^g	32.888 ± 0.173 ^g	35.271 ± 0.0720 ^g
	Gills	15.50 ± 5.64 ^h	123.75 ± 6.87 ^c	136.53 ± 6.16 ^b	149.57 ± 5.34 ^a
	Mantle	6.983 ± 1.722 ^h	76.33 ± 5.00 ^f	93.90 ± 4.15 ^e	107.87 ± 5.35 ^d
Chromium (Cr)	Foot	0.852 ± 0.068 ^d	2.111 ± 0.583 ^{cd}	2.889 ± 0.235 ^{cd}	4.539 ± 0.520 ^c
	Gills	1.357 ± 1.013 ^d	4.377 ± 0.933 ^c	13.72 ± 2.72 ^b	22.192 ± 1.228 ^a
	Mantle	0.837 ± 0.296 ^d	1.307 ± 0.500 ^d	1.681 ± 0.516 ^{cd}	3.324 ± 0.302 ^{cd}

Data are mean ± standard deviation

Values in the same rows with different superscripts indicate significant differences ($p < 0.05$)

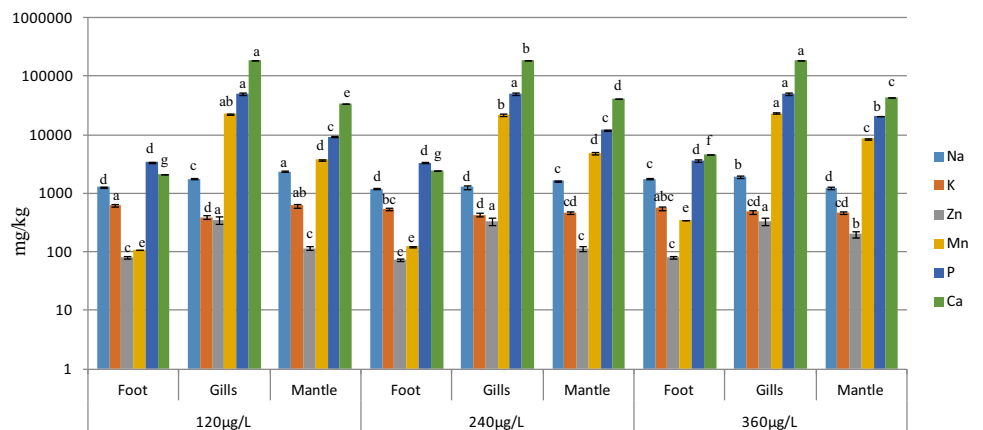
Table 2 Mineral element composition of different portions of freshwater mussels (*Anodonta anatina*) collected from the control treatment

Minerals	Concentration (mg/kg)		
	Foot	Gills	Mantle
Sodium (Na)	1680.6 ± 2.39 ^b	1577 ± 57.3 ^c	1879.9 ± 50.6 ^a
Potassium (K)	675 ± 30.6 ^a	655 ± 36.3 ^a	527.3 ± 32.3 ^b
Calcium (Ca)	3052 ± 29.7 ^c	156,906 ± 736 ^a	45,207 ± 241 ^b
Zinc (Zn)	66.16 ± 5.94 ^c	282.2 ± 49.5 ^a	128.7 ± 17.3 ^b
Manganese (Mn)	192.45 ± 1.81 ^c	20,106 ± 752 ^a	6270 ± 227 ^b
Phosphorus (P)	3468.7 ± 56.1 ^c	44,076 ± 1607 ^a	14,457 ± 517 ^b

Data are mean ± standard deviation

Values in the same rows with different superscripts indicate significant differences ($p < 0.05$)

Fig. 1 Mineral element composition of different portions of freshwater mussels (*Anodonta anatina*) exposed to different doses of lead (Pb). Graph drawn by log base 10



exposed mussels to all the doses. The same trend was also observed in the Cr-exposed mussels (Table 2, Fig. 3).

3.3 Proximate composition

The mean values of proximate composition of foot, gills and mantle of freshwater mussels from the control

treatment are shown in Table 3. The moisture, protein and fat contents were highest in the foot as compared to the mantle and gills ($p < 0.05$), but on the contrary carbohydrates were higher in the mantle and ash in gills ($p < 0.05$) than the other parts. The range of water content was 76.32–79.45, 71.49–74.28 and 72.85–76.25 % for the foot, gills and mantle, respectively. Based on the wet weight, the

Fig. 2 Mineral element composition of different portions of freshwater mussels (*Anodonta anatina*) exposed to different doses of copper (Cu). Graph drawn by log base 10

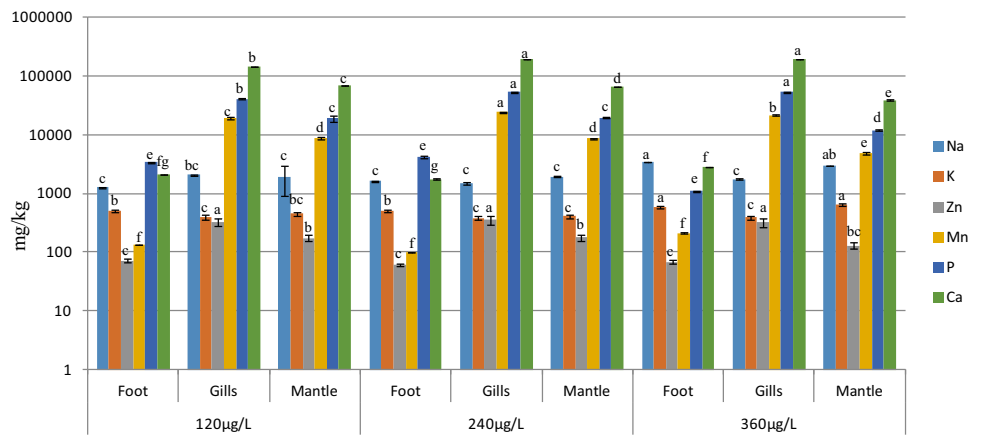


Fig. 3 Mineral element composition of different portions of freshwater mussels (*Anodonta anatina*) exposed to different doses of chromium (Cr). Graph drawn by log base 10

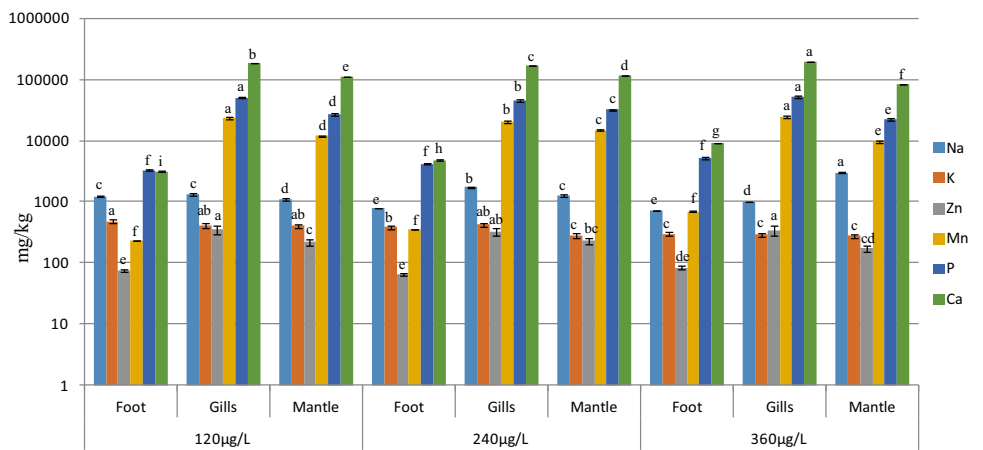


Table 3 Proximate composition of different portions of freshwater mussels (*Anodonta anatina*) collected from the control treatment

Tissues	Compositions (%)				
	Moisture	Ash	Proteins	Fats	Carbohydrates
Foot	77.78 ± 1.208 ^A	0.57 ± 0.1078 ^c (2.62 ± 0.390) ^C	15.45 ± 1.132 ^a (71.39 ± 1904) ^A	0.97 ± 0.1037 ^a (4.47 ± 0.421) ^A	5.23 ± 1.114 ^c (24.32 ± 5.75) ^B
Gills	72.73 ± 1.285 ^B	10.55 ± 1.113 ^a (45.33 ± 0.651) ^A	6.53 ± 0.575 ^b (28.10 ± 1.498) ^B	0.72 ± 0.1427 ^b (3.07 ± 0.539) ^B	9.47 ± 2.66 ^b (42.03 ± 15.99) ^A
Mantle	74.74 ± 1.237 ^B	3.40 ± 0.224 ^b (13.12 ± 0.543) ^B	6.24 ± 0.229 ^b (24.11 ± 1.360) ^C	0.47 ± 0.1725 ^c (1.83 ± 0.706) ^C	15.15 ± 1.304 ^a (58.72 ± 7.32) ^A

Data are mean ± standard deviation

Values in the same columns with different superscripts indicate significant differences ($p < 0.05$)

In parenthesis the values are shown in dry matter (DM)

protein contents were 13.88–16.93 % in the foot, 6.05–7.41 % in the gills and 5.90–6.475 % in the mantle. The range of fat contents in the foot, gills and mantle were 0.91–1.15, 0.56–0.89 and 0.28–0.62 %, respectively. The values of carbohydrates in the foot, gills and mantle were 3.85–6.46, 6.04–12.30 and 13.80–17.20 %, respectively. The ash contents in the foot, gills and mantle were 0.44–0.73, 9.34–11.82 and 3.05–3.63 %, respectively (Table 3).

The proximate composition was also analysed in mussels exposed to different doses of metals. The protein content was significantly increased ($p < 0.05$) in the foot portion of the Pb- (22.39 ± 1.44 %) and Cr (21.46 ± 0.97 %)-exposed mussels at the low dose (120 µg/L) and then decreased at a medium dose (240 µg/L) of Pb (20.23 ± 1.75 %) and Cr (17.67 ± 1.23 %). The protein content was lowest (10.54 ± 1.14 %) at a high dose (360 µg/L) of Pb ($p < 0.05$). There were no

Fig. 4 Proximate composition of different portions of freshwater mussels (*Anodonta anatina*) exposed to different doses of lead (Pb). Graph drawn by log base 10

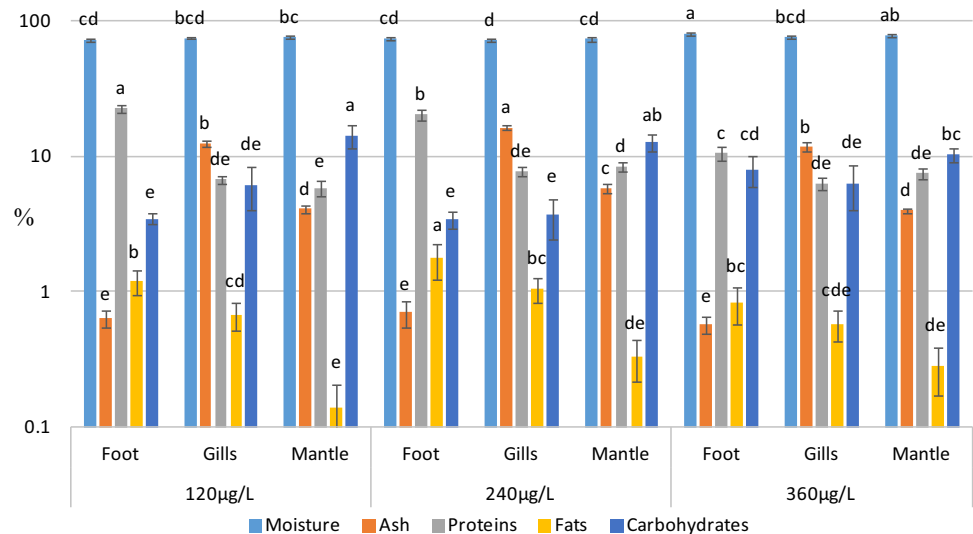
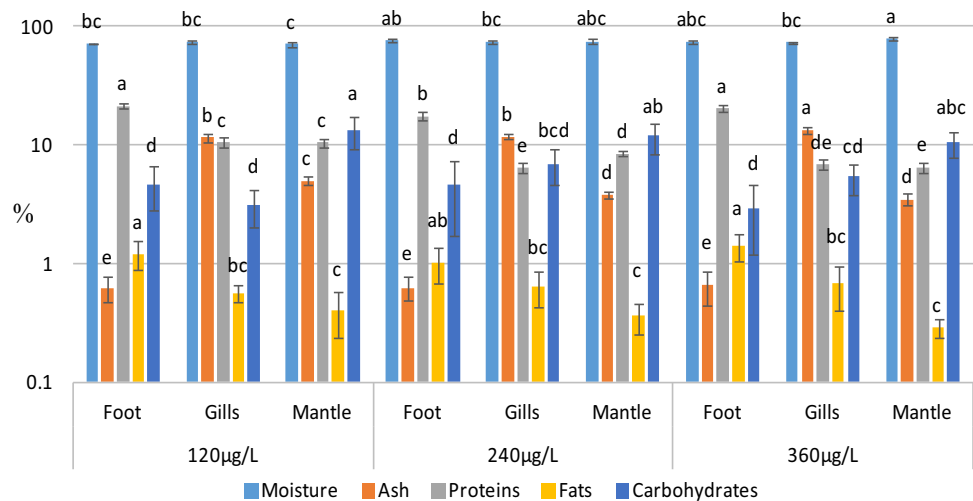


Fig. 5 Proximate composition of different portions of freshwater mussels (*Anodonta anatina*) exposed to different doses of chromium (Cr). Graph drawn by log base 10



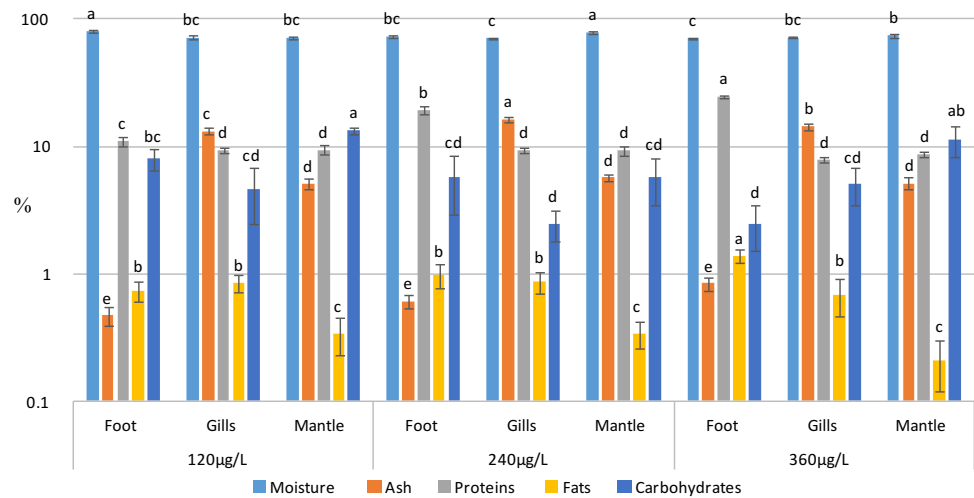
significant differences ($p > 0.05$) in any other nutrients amongst tissues of Pb- and Cr-exposed mussels (Figs. 4, 5). In Cu-exposed mussels again, the protein content was significantly higher in the foot (24.70 ± 0.61 %) at the high dose ($p < 0.05$) than the values of 19.28 ± 1.32 and 10.95 ± 0.98 % at the medium and low doses, respectively. Nutrients other than protein in Cu-exposed mussels did not vary significantly with respect to the control level (Fig. 6; Table 3).

4 Discussion

It is recognized that the bivalve mussels can accumulate high levels of metals in their soft tissue. Thus, bivalves are good candidates to investigate water pollutants because of their sedentary lifestyle and the greater degree to

accumulate the toxicants in their soft tissues. In the present study, Cu was prevalent among the studied metals and the gills were found to be the highest accumulating tissue. Usero et al. (2005) noted that bivalves were the desirable species for the biomonitoring of environmental pollutants in aquatic systems. In various other studies, different tissues of freshwater were used to indicate the metal loads in the surrounding environment. According to Wadige et al. (2014), the bioaccumulation of Pb varied amongst tissues of bivalves and the highest concentrations were observed in labial palps and gills as compared to other tissues. In our results, gills contained higher concentration of all metals than foot and mantle. This is in agreement with the findings by Vincent-Hubert et al. (2011) who demonstrated that the gills were the most sensitive organs for accumulating chemicals, because they are directly exposed to water pollutants in their immediate environment. Jones and

Fig. 6 Proximate composition of different portions of freshwater mussels (*Anodonta anatina*) exposed to different doses of copper (Cu). Graph drawn by log base 10



Walker (1979) also reported that the highest levels of bioaccumulation in freshwater mussels were observed in gills and the lowest values were observed in their foot. In another study (Yap et al. 2006), it was concluded that the gills were the greater accumulator for zinc, lead and cadmium than the other visceral parts, and they were good bioindicators of aquatic pollution. Sasikumar et al. (2006) investigated the trace metal contaminants in green mussels collected from various sites of southwest coast of India. Their results showed a significant input of cadmium, chromium and lead from industrial sites. Yap et al. (2004) studied the heavy metal accumulation in green-lipped mussel (*Perna viridis*) collected from the coast of Peninsular Malaysia and reported the concentrations of different heavy metals. The values ranged from 0.68 to 1.25 mg/g for cadmium, 7.76–20.1 mg/g for copper, 2.51–8.76 mg/g for lead and 75.1–129 mg/g for zinc. In this study, higher level of Cu accumulation was observed than the other studied metals. Our results resemble a previous study where a high level of copper was reported in *D. trunculus* as compared to other metals (Baraj et al. 2003). In our findings, the accumulation of metals increased with increasing doses; similar results were reported by Guidi et al. (2010) who used freshwater mussels in biomonitoring studies and stated that metal bioaccumulation in tissues increased with elevated levels of metals in sediments. Maanan (2007) investigated the heavy metal concentration in *Mytilus galloprovincialis* harvested from the Safi coastal water in Morocco. High levels of metals were observed in mussel tissues collected from the area near industrial coastal settlements, which was due to heavy concentration of metals in water. Variations were observed among bivalve tissues for metal accumulation; the same concept was reported by another study on freshwater mussels where it was stated that the bioaccumulation of metals was tissue specific (Labrot et al. 1999). Patel and Anthony (1991) also

reported a very high concentration of Cd in the gills of *Anadara granosa* and concluded that the gills could uptake most metals. Yap et al. (2003) reported that the Pb and Zn were dominantly found in the gills of *M. edulis*. Another study concluded that the gills and mantle had the highest levels of various metals such as Pb, Cu, Zn, Fe and Mn, whereas the lowest value was observed in the foot samples of adductor muscles (Tessier et al. 1984). Our findings also agreed with a previous study where significant bioaccumulation of Pb in mussel tissues was reported at the highest dose and least accumulation was observed at the lowest dose (Rahnama et al. 2010). Furthermore, it was stated that the initial concentration of Pb had a direct influence on its bioaccumulation in the soft tissue of the mussels.

In mineral elements composition Ca was found to be higher in gills of bivalves, but our results are different from a previous study where the order of mineral elements was Na > K > Ca > Mg (Karnjanapratum et al. 2013). Conversely in our results, the pattern of mineral elements composition was similar to that found in other studies on bivalves (España et al. 2007). Fuentes et al. (2009) reported a higher concentration of Ca in mussels as compared to other mineral elements. Our results agree with studies which showed that the mussels were good sources of Ca, Mg and P (King et al. 1990; Karakoltsidis et al. 1995). Amongst different tissues, gills accumulated the most minerals when compared with the mantle and foot as reported by Karnjanapratum et al. (2013). Mineral elements play a very important role in homeostasis, so these are essential for maintaining various biological processes such as osmotic balancing, membrane potentials and muscular contractions in animals.

In this study, the values of mineral elements were also investigated in bivalves exposed to different doses of Pb, Cu and Cr. There were no significant differences in mineral elements except for Ca that appeared higher in metal-

exposed mussels than the control. The burden of heavy metals in the tissue of mussels can correlate to biological dysfunctioning (Chandurvelan et al. 2012). Heavy metals including Cu showed adverse effects on Ca homeostasis in gills of mussels and also resulted in the deterioration of enzyme action (Viarengo et al. 1996). Exposure to copper produces a variety of serious defects in cell functioning by altering the Ca signalling which can inhibit biochemical reactions (Burlando et al. 2004). Lead accumulates in tissues and interferes with other bioelements such as Ca, Zn and Cu, causing a variety of pathophysiological effects (Berrahal et al. 2011).

Lower values of Na and K were observed in tissues of controlled as compared to the exposed mussels, but very few published data were found on this subject. Fluctuation in mineral elements were shown when the mussels were exposed to different doses of heavy metals, but almost no effort was reported to find such associations between the exposure of organisms to heavy metals and mineral composition of their tissues. Rahnama et al. (2010) reported the effect of Pb accumulation on the filtration rate of zebra mussels harvested from the Anzali wetland in the Caspian Sea and showed that the filtration rate was reduced by 40 % at 455 µg/L concentration of Pb. Amongst proximate composition, protein contents were highest in the foot region of *Anodonta anatina*. Similar results were reported in a previous study where different portions of bivalves were studied for proximate composition (Karnjanapratum et al. 2013). In our study, the highest values of carbohydrates were found in the mantle as compared to gills and foot, which is contrary to a previous study where higher value of carbohydrates was found in the foot than the mantle (Karnjanapratum et al. 2013). The ash contents were higher in gills as compared to the mantle and foot, because of the higher accumulation of metals in the gills of bivalves. Ersoy and Serefislan (2010) reported that the minced whole freshwater mussels, *Potamida littoralis* and *Unio terminalis*, contained 11.9–12.0 % protein, 1.6–1.7 % ash, 1.1–2.6 % fat and 80.4–81.7 % moisture. Striped Venus clam, *C. gallina*, harvested from the Adriatic Sea contained 8.55–10.75 % protein, 0.73–1.59 % lipids and 2.25–4.96 % carbohydrates (Orban et al. 2006). Karnjanapratum et al. (2013) also reported that the Asian hard clam, *M. lusoria*, collected from the coast of the Andaman Sea contained 9.09–12.75 % protein, 0.32–7.89 % carbohydrate, 1.58–6.58 % fat and 1.23–2.58 % ash.

In our study, the values of nutrients were also investigated in tissue of freshwater mussels exposed to different doses of metals. The levels of protein were significantly increased in the Pb-, Cu- and Cr-exposed mussels at the low dose (120 µg/L) and then decreased at the high doses (360 µg/L) of heavy metals; thus the lower dose may be more effective for protein formation in mussels. This is

perhaps the first study where the interaction between metal and dietary components is reported. Therefore, there is a need to further explore a synchronized effect of metal exposure of mussels on body composition, reflecting their nutritional value for safe human consumption. The foot of freshwater mussels (*Anodonta anatina*) is a good source of protein; thus, this animal could play a vital role in coping with the challenge of nutritional deficiency in developing countries. Furthermore, it is believed that the mussels are potentially a desirable choice to evaluate aquatic health by assessing metal loads in their soft tissues.

5 Conclusion

Freshwater mussels (*Anodonta anatina*) were analysed for bioaccumulation of metals, proximate composition and mineral elements analysis. Amongst the studied metals, Cu showed more accumulation than Pb and Cr; the highest metal bioaccumulation was observed when mussels were exposed to higher doses of metals. Although the bioaccumulation of Cr was lower than other metals, it was still higher than in the control mussels receiving no added metals in water. Some fluctuations were observed in mineral elements and chemical composition of mussels following their exposure to different doses of heavy metals. Amongst the studied mineral elements, Ca was significantly higher in all the tissues; its concentration was greatly increased after exposure to various doses of Pb, while the values of Na and K were surprisingly lowered by the exposure to Cu. Protein was found to be greater in the foot portion than the other tissues. Thus, the mussel foot is suggested to be a more nutritious part for the consumers. Gills, rather than foot and mantle, were the main sites for the accumulation of metals perhaps due to their direct contact with their immediate environment containing metals. Overall, *Anodonta anatina* seems to be a good candidate for the assessment of aquatic health. It appears that the tissue-specific investigation of metals, mineral elements and chemical composition is more effective than using the whole animal for similar investigations in the future.

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