

Proximate composition and elemental analysis in soft tissues of freshwater mussels (*Anodonta anatina*) from the Chashma Lake, River Indus Pakistan

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BACKGROUND: Aquatic invertebrates are playing an important role in assessment of the water contaminants and also serve as a major component of food chain. Freshwater mussels are considered to be the good bioindicator species of aquatic environment and widely used to determine the metals load.

METHODS: Proximate composition and elemental analysis were carried out in edible (foot, mantle) and non-edible portion (gills) of freshwater mussels (*Anodonta anatina*) harvested from various site of Chashma Lake, River Indus Pakistan.

RESULTS: The nutritional components were varied among the studied portion and muscular foot found to be the best part for consumption. Protein and fat contents were significantly higher in foot ($15.90 \pm 0.88\%$, $1.19 \pm 0.26\%$) as compared to mantle ($10.78 \pm 2.24\%$, $0.27 \pm 0.09\%$) and gills ($6.44 \pm 1.22\%$, $0.53 \pm 0.15\%$) respectively. For the macro minerals mantle had high concentration of Ca (46838 ± 984 mg/kg), Na (2706 ± 343 mg/kg), P (6921 ± 1063 mg/kg) and Mn (7207 ± 1046 mg/kg) as compared to foot.

CONCLUSIONS: Heavy metals (Cd, Cu, Cr) concentration in edible portions were lower than the permissible limit by WHO whereas the concentration of Pb was slightly higher than the recommended value that might be the risk for the consumers. Being filter feeder gills accumulated the high concentration of all the metals and found to be the key portion for biomonitoring studies. Freshwater mussels of Chashma Lake Indus River are the rich source of protein and all the other micro and macro minerals therefore could be used as an excellent source of food.

Keywords proximate composition, minerals, metals, River Indus, freshwater mussel

Introduction

Chashma Lake is located at River Indus, in Province Punjab. This lake has been reported for its richness in fauna and flora but only few studies have been conducted on invertebrates including freshwater mussels. Aquatic invertebrates are the major component of food chain and also play a significant role to assess the water contamination. Freshwater mussels are considered as a bioindicator species of aquatic environment and widely used to determine the metals load in water. Biomonitoring is a scientific tool to assess the environmental health that is based on the analysis of tissues and body fluids of different organisms. The health of aquatic ecosystem has

been monitored through the chemical analysis of pollutant level in soft tissues of bivalves (Goldberg, 1986; Cantillo, 1998). Being sessile, more endurance rate, possibility to kept in laboratory and capable to accrue wide range of pollutants makes mussels ecologically imperative for environmental studies (Boening, 1999; Kimbrough et al., 2008; Sarkar et al., 2008; Zhou et al., 2008). They can be used to assess the toxicity, even when the concentration of metals in water is shortly available or near to reckonable levels (Viarengo and Canesi, 1991; Blackmore and Wang, 2003).

Heavy metals are supposed as major contaminants in water because of toxicity and non-degradability. Agricultural runoff, mining and industries contributes in elevating the toxicant load in aquatic environs which may directly or indirectly affect the aquatic organism and most of the metals can accumulate in animal tissues (Usero et al., 2005). Some of these metals such as mercury, chromium, cadmium, nickel, copper, lead and zinc could be convert into tenacious metallic

substances and are highly toxic (Hyun et al., 2006; Maanan, 2007). Mineral elements like Zn, Fe, Mg, P and Cu are very important as they play considerable role in biological system while other metals like Hg, Cd and Pb are non-essential and are toxic even in minute quantity. Some biologically required metals (e.g., Cu, Zn, Na, K, P, Mn) may prove lethal when the concentration increased than the optimal level. Turkmen et al. (2008) reported that some metals like mercury, cadmium and lead could prove dangerous at trace quantities and said to be harmful toxicant for the aquatic ecosystem. The same site at Indus River was investigated in one of the previous study and reported the high level of bioaccumulation of various metals in different organs of fish (Jabeen and Chaudhry, 2010).

Bivalves also a rich source of nutritional components and could be used as human diet in Pakistan to reduce the pressure on fish. Bivalves are valuable aquatic organism for environmental health as well as the rich source of food. Freshwater mussels are abundantly present in Chashma Lake River Indus Pakistan and there is need to make use of this imperiled fauna to cope with challenges of food scarcity. Bivalve especially mussels are used for food in different part of world and due to high nutritive values their demands increasing globally (Orban et al., 2002; Fuentes et al., 2009; Karnjanapratum et al., 2013; Pogoda et al., 2013). Mussels are the affluent source of dietetic components such as proteins, mineral, good 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) described that the mussels are the economical sources of proteins and contains many essential vitamins and minerals. For inquiry of food value of mussels, the chemical composition is good indicator to assess the commercial and nutritional quality as per narration of Orban et al. (2006).

Chashma Lake continuously receiving many industrial effluent, pesticides and sewage from the neighboring communities that may deteriorate the water quality and cause decline in diversity of aquatic species including freshwater mussels. This study was carried out to estimate the metal load, mineral elements and food components in foot, mantle and gills of freshwater mussels and to evaluate the capacity of these tissues to store various chemicals. It is important to investigate the aquatic health by using this animal as bioindicator and this study was also spot the top indicator part of freshwater mussels for biomonitoring in aquatic environment.

Materials and methods

Sampling site

The study was conducted at Chashma Lake River Indus, 25 Km south-west from Mianwali City of Pakistan. This site of

River Indus is heavily use for the inland fisheries and is economically important reservoir for irrigation and generation of electricity. The samples of freshwater mussels were collected from the various sites of Chashma Lake (30°16'45" N, 66°57'23" E). Samples were collected trained fisherman or by hand picking where the water was shallow and then transported to laboratory in cool plastic bags. Shell was cleaned from epiphytes and inside was rinsed with deionized water to remove sand and other particle from the shell body. The mussels was left to dry on blotting paper about two hours before dissection.

Sample preparation

Isolation of soft tissues

By using blade and small steel rod, mussel's shells were opened to eradicate the unwanted particles from sample species animals by washing well with water in laboratory. Foot, gill and mantle were removed cautiously with the help of sharp scissor and each part after weighing was put into small plastic bottles and refrigerated at -4°C . The frozen tissues samples were carried to the Newcastle University, UK and stored at -20 . All the samples were weighed and then put into freeze dryer for 7 days and after that dried samples were ground, weighed and placed in desiccators until digestion.

Sample analysis

Metals and minerals analysis

The sample preparation for heavy metals and proximate composition was done in the laboratory of school of agriculture, food and rural development whereas the heavy metals analysis was carried out in school of civil engineering and geosciences Newcastle University, UK. A weight of 0.2 g of each dried out sample was carried in a digestion flask and performed acid digestion in combination of 9 mL concentrated nitric acid (HNO_3) + 1mL perchloric acid (HClO_4). The digested sample flasks were placed in fuming hood for overnight and then heated on hot plate at 100°C till evaporated to near dryness. The left over were allowed to cool at room temperature and then diluted with 10 mL distilled water. The supernatants were separated in volumetric plastic vials after centrifugation at 2000 rpm for 10 min. All prepared samples were then kept in refrigerator at -4°C until analysis. The concentrations of Cadmium (Cd), Calcium (Ca), Chromium (Cr), Copper (Cu), Lead (Pb), Manganese (Mn), Phosphorus (P), Potassium (K), Sodium (Na), and Zinc (Zn) were quantified by inductively coupled plasma spectrometer (ICP-OES, Vista-MPX simultaneous) according to the method of AOAC (2000). The calibration of apparatus was achieved over related concentration with certified standard from the Sigma-Aldrich, UK. The concentrations of metals in soft tissues of freshwater mussels were presented as mg/kg.

Proximate analysis

Freeze-dried samples of gills, mantle, and foot were also examined for the content levels of moisture, fat, proteins, and ash according to AOAC (1997). For estimation of the percentage of carbon and nitrogen Elementar Vario Macro Cube was used. Protein contents were calculated by multiplying the nitrogen value with 6.25. The ash contents were determined by placing the samples in the muffle furnace at 500°C. For fat extraction the Soxhlet apparatus was used and carbohydrates were determined with by difference method.

Statistical analysis

The data were statistically analyzed by general linear model and the means comparison of nutrients and metals in soft tissues of freshwater mussels were examined by Tukey pairwise test. The relationship among minerals and bivalve tissues were investigated by Pearson correlation coefficient (r). Statistical significance was defined at $p < 0.05$ and all the test were carried out by using Minitab 17 software. Tables were drawn with the help of Microsoft excel 2007.

Results and discussion

Proximate composition

Proximate composition of foot, mantle and gills of freshwater mussels (*Anodonta anatina*) are shown in Table 1 and 2. The % of carbon, nitrogen and their ratios were investigated and result revealed in Table 1. Foot had the greater value of Carbon (21.83–43.01%) and nitrogen (4.43–11.72%) as compared to mantle and gills whereas the gills had the lowest value of carbon and nitrogen as it was the poor source of protein. Among all the studied parts, mantle had the lowest moisture content with the highest carbohydrate ($p < 0.05$) (Table 2). On the other hand, the foot contained the highest protein and lipid content with the lowest carbohydrate content ($p < 0.05$). All the bivalve portions contained protein as the chief constituent like in foot (14.69–17.63%) followed by mantle (6.39–12.56%) and gills (4.76–7.82%). Percentage of ash was significantly higher in gills (6.13–11.18%) as compared to mantle (4.67–5.79%) and foot (0.37–0.58%). The values of carbohydrate content were highest in mantle (4.36–12.48%) than gills (4.51–9.33%) and foot (1.85–6.18%). In view of fat content, it was found that the foot had the highest content (0.89–1.69%) when compared with gills (0.28–0.74%) and mantle (0.15–0.44%). Moisture content of gills, foot and mantle was 74.38–82.39%, 74.34–80.19% and 70.85–81.37% respectively (Table 2). Table 3 showed the correlation coefficient of various nutrients in soft tissues of freshwater mussels. There were strong positive correlation was found between fat, moisture and protein and between ash and carbohydrates whereas the negative correlation was found between carbohydrates and other studied nutrients (proteins, fats and moisture). Ersoy and

Sereflisan (2010) reported that whole body of 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. For striped venus clam, *C. gallina*, harvested from Adriatic sea contained 8.55–10.75 g/100 g protein, 0.73–1.59 g/100 g lipids and 2.25–4.96 g/100 g carbohydrates (Orban et al., 2006). Karnjanapratum et al. (2013) also reported that Asian hard clam, *M. lusoria*, collected from the coast of Andaman sea contained 9.09–12.75% protein, 0.32–7.89% carbohydrate, 1.58–6.58% fat and 1.23–2.58% ash. Protein was found to be highest in foot as compared to mantle and viscera. In one of the previous study siphon and mantle of Pacific clam, *Panopea abrupta*, were evaluated for proximate composition and observed value of protein and moisture was 14.32–15.29% and 74.82–80.37% respectively (Oliveira et al., 2011). *Mytilus galloprovincialis* from Bosphorus of the Sea of Marmara, Turkey contained 79.76–87.46 g/100 g moisture, 7.28–12.65 g/100 g protein, 0.33–3.49 g/100 g fat and 1.06–2.06 g/100 g ash (Ozden et al., 2010). Furthermore it was reported that the proteins and fat concentration were highest in the summer whereas the moisture and ash value were found higher in the winter. In one of the previous study the seasonal affects on proximate composition of offshore cultivated oysters was described and results concluded that the value of protein did not show any significant seasonal variations while the lipids contents increased during summer and then gradually decreased in autumn (Pogoda et al., 2013). Nutritional quality was enquired in *Mytilus galloprovincialis* harvested from the gulf of Trieste and it was found that the dietary components were varied seasonally; moreover mussels contained 7.5–11.6 g/100 g protein, 1.0–2.2 g/100 g lipids and 2.2–3.3 g/100 g ash (Bongiorno et al., 2015). The results of present study indicated that protein in muscular foot is a major constituent in Freshwater mussels (*Anodonta anatina*) harvested from the Chashma Lake River Indus Pakistan.

Table 1 Variation in Carbon and Nitrogen contents (%) among soft tissues of freshwater Mussels (*Anodonta anatina*) from Chashma Lake River Indus Pakistan

Soft tissues	Composition %		
	Carbon	Nitrogen	C/N ratio
Foot	37.24±8.74 ^a	9.31±3.01 ^a	4.146±0.60 ^a
Gills	32.12±5.12 ^a	6.806±1.51 ^a	4.787±0.51 ^a
Mantle	38.86±4.08 ^a	8.79±2.49 ^a	4.63±0.98 ^a

Data are mean±standard deviation. Values in the same column with different superscripts indicates significant difference ($p < 0.05$).

Mineral elements

The concentration of Pb, Cu, Cr, Cd, Mn, Zn, Ca, K, Na, P in the soft tissues of freshwater mussels *Anodonta anatina* are summarized in Table 4. The means concentration of all the

Table 2 Variation in Proximate composition (%) among soft tissues of freshwater mussels (*Anodonta anatina*) from Chashma Lake River Indus Pakistan

Soft tissues	Compositions (%)				
	Moisture	Protein	Carbohydrate	Lipids	Ash
Foot	78.22±1.70 ^{ab}	15.90±0.88 ^a	4.23±1.45 ^b	1.19±0.26 ^a	0.46±0.05 ^c
Gills	78.96±2.86 ^a	6.44±1.22 ^c	6.55±1.69 ^{ab}	0.53±0.15 ^b	7.51±1.88 ^a
Mantle	75.12±3.97 ^b	10.78±2.24 ^b	8.54±3.11 ^a	0.27±0.09 ^c	5.29±0.39 ^b

Data are mean±standard deviation

Values in the same column with different superscripts indicate significant difference ($p < 0.05$).

Table 3 Pearson correlation coefficient (r) among nutrients in soft tissues of freshwater mussels (*Anodonta anatina*) from Chashma Lake River Indus Pakistan

Nutrients	Moisture	Protein	Fat	Carbohydrates
Protein	-0.231			
P-value	0.22			
Fats	0.154	0.661		
P-value	0.417	0.000		
Carbohydrates	-0.669	-0.396	-0.551	
P-value	0.000	0.030	0.002	
Ash	-0.183	-0.823	-0.690	0.429
P-value	0.334	0.000	0.000	0.018

Table 4 Variation in elements concentration (mg/kg) among soft tissues of freshwater mussels (*Anodonta anatina*) from Chashma Lake River Indus Pakistan

Metals	Concentration (mg/kg)		
	Foot	Gills	Mantle
Lead (Pb)	3.781±1.555 ^a	4.037±2.348 ^a	4.831±2.661 ^a
Copper (Cu)	15.60±4.61 ^a	29.06±16.01 ^a	30.07±13.70 ^a
Chromium (Cr)	0.8278±0.1447 ^b	1.977±0.951 ^{ab}	2.659±2.459 ^a
Cadmium (Cd)	0.6821±0.2216 ^b	1.503±0.396 ^a	2.011±0.768 ^a
Manganese (Mn)	47.2±30.3 ^c	23519±1050 ^a	7207±1046 ^b
Zinc (Zn)	84.85±11.59 ^b	222.4±51.6 ^a	212.4±56.2 ^a
Calcium (Ca)	2157.6±235.4 ^c	177698±1799 ^a	46838±984 ^b
Potassium (K)	610.2±66.9 ^b	849.1±71.5 ^a	420.6±38.2 ^c
Sodium (Na)	1147.5±225.7 ^b	2261±575 ^a	2706±343 ^a
Phosphorus (P)	2711±487 ^c	47097±1278 ^a	6921±1063 ^b

Data are mean±standard deviation.

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

minerals was varied among the tissues. Among three studied portions, gills had the highest concentration for all the minerals followed by mantle and foot. The concentration of

Ca was significantly higher in gills (175346–179864 mg/kg) as compared to mantle (45117–47850 mg/kg) and foot (1823.5–2407.4 mg/kg) ($p < 0.05$). No significant difference was found among the soft tissues for Pb and Cu but gills had slightly higher value than mantle and foot ($p > 0.05$). Gills and mantle had higher value of Cr (0.967–3.728 mg/kg, 0.518–6.873 mg/kg), Cd (1.089–2.270 mg/kg, 1.055–3.175 mg/kg), Zn (155.2–300.1 mg/kg, 152.1–326.4 mg/kg) and Na (1541–3077 mg/kg, 2277–3136 mg/kg) respectively as compared with foot (Cr 0.7007–1.0511 mg/kg, Cd 0.3646–0.8903 mg/kg, Zn 70.83–106.69 mg/kg, Na 953.4–1454.5 mg/kg). The value of K had again maximum in gills (740.3–963.5 mg/kg) followed by foot (530.0–722.3 mg/kg) and mantle (366.3–489.1 mg/kg). The concentration of Mn and P was significantly higher in gills (22179–25430 mg/kg, 45404–49080 mg/kg) with respect to mantle (5559–9146 mg/kg, 5399–8402 mg/kg) and foot (18.7–86.8 mg/kg, 2198–3381 mg/kg) respectively. In present study the comparative analysis of trace metals and minerals were carried out in various portion of freshwater mussels including the edible parts (Foot and Mantle). Among the studied minerals (Ca, Na, P, K and Zn) mantle had the higher values than foot. In bioaccumulation of different trace metals (Pb, Cu, Cr, Cd and Mn) gills showed the higher concentrations of these metals as compared to foot and mantle. The strongly positive correlation was observed among most of the studied elements (Table 5) whereas some negative correlation were also found like Lead (Pb) showed the negative correlation with the Cu, Cd, Zn, Ca, K and P. K also showed the negative correlation with the Cu, Cr, Cd and Na as well. Jabeen and Chaudhry (2010) investigated the heavy metals in different tissues of fish species harvested from the Chashma Lake River Indus Pakistan and compared the value of Mn and Cr in fish organs with the WHO and Federal environmental agencies standard and found that the concentration were higher than recommended. Our results resemble to another previous study that showed that the mussels are excellent source of Ca, Mg and P (King et al., 1990; Karakoltsidis et al., 1995). There were several studies was conducted on fish fauna for metals, minerals and nutrients analysis on the same site but the bivalves fauna would not be exploited so far and this was the first study on freshwater mussels of Chashma Lake Indus River Pakistan. Biologically important minerals (Ca, K, P, Mn, Na, and Cu) were abundantly observed in edible parts (Foot, Mantle) of freshwater mussels from the Chashma Lake

Table 5 Pearson correlation coefficient (r) among elements in soft tissues of freshwater mussels (*Anodonta anatina*) from the Chashma Lake, River Indus Pakistan

Metals	Pb	Cu	Cr	Cd	Mn	Zn	Ca	K	Na
Cu	-0.032								
<i>p</i> -value	0.876								
Cr	0.084	0.305							
<i>p</i> -value	0.676	0.123							
Cd	-0.012	0.068	0.005						
<i>p</i> -value	0.952	0.735	0.979						
Mn	0.027	0.388	0.227	0.28					
<i>p</i> -value	0.893	0.046	0.254	0.157					
Zn	-0.093	0.412	0.004	0.489	0.634				
<i>p</i> -value	0.644	0.033	0.531	0.01	0.000				
Ca	-0.004	0.316	0.174	0.282	0.995	0.611			
<i>p</i> -value	0.644	0.108	0.385	0.154	0.000	0.001			
K	-0.054	-0.068	-0.152	-0.264	0.685	0.000	0.726		
<i>p</i> -value	0.789	0.735	0.449	0.183	0.000	1.000	0.000		
Na	0.131	0.263	0.157	0.87	0.42	0.548	0.409	-0.127	
<i>p</i> -value	0.515	0.185	0.434	0.000	0.029	0.003	0.034	0.528	
P	-0.051	0.255	0.111	0.164	0.972	0.520	0.986	0.815	0.28
<i>p</i> -value	0.799	0.198	0.582	0.413	0.000	0.005	0.000	0.000	0.157

Indus River Pakistan that might be the nutritive source for the consumers. The concentration of Pb in edible parts of freshwater mussels from Indus river was higher than the permissible value of WHO so it might be cause health hazards to consumers whereas the accumulation of other metals were below the permissible level. Another aim of our study was to use the freshwater mussels as a pollution indicator species and results indicates the gills are best part for biomonitoring study as it had higher accumulation of heavy metals when compared with foot and mantle. Vincent-Hubert et al. (2011) reported that the gills due to direct exposure to water are the sensitive organs for chemicals. *Mytilus galloprovincialis* harvested from Marmara sea turkey contained Na (2146.764–4530.101 mg/kg) and K (1809.336–2930.825 mg/kg) in their soft tissues (Ozden et al., 2010). In another comparative study on bivalve mussels it was concluded that the gills are the best accumulator parts for zinc, Lead, and Cadmium than the other visceral parts and are the good bioindicators of aquatic pollution (Yap et al., 2006). Wadige et al. (2014) illustrated that the concentration of lead (Pb) varied among tissues of bivalves and maximum concentration were observed in gills as compared to other tissues. Jebali et al. (2014) reported the comparative bioaccumulation of trace metals in various tissues of bivalve *pinna nobilis* and stated that the digestive gland stored maximum metals followed by gills and muscles and freshwater mussels are sensitive animal for biomonitoring studies. Espana et al. (2007) compared different minerals and trace elements in molluscs harvested from the Strait of Megellan (Chile) and reported the high concentration of Na, Ca, Zn, Mn and Cd in *Perunytitus purpuratus*. Elements composition of *Mytilus galloprovin-*

cialis collected from the different coast of Spain and the authors reported the significant difference in metals concentrations among the three sites. All the samples showed the high concentrations of Na, K, Ca and P whereas the concentration of Cu and Mn was low (Fuentes et al., 2009).

Usero et al. 2005 reported the high concentration of Cr, Cu, Pb, Zn in *D. trunculus* as compared to *C. gallina* collected from the Atlantic coast of southern Spain. Chemical composition of *Chamelea gallina* harvested from the various sites of Southern Coast of the Marmara Sea has been studied and reported the 67% moisture, 10.12% protein, 2.57% lipids and 1.66% ash. They also reported the perfect concentration Cu, Cr, Mn and Cd tissues in bivalves at most of the sites whereas the concentration of Pb and Zn was higher than the critical concentration at some sites (Colakoglu et al., 2011). Our present findings propose that freshwater mussels from the Chashma Lake Indus River Pakistan were the rich source of nutrients like proteins carbohydrates and fats. The concentration of all the accumulated metals was below the dangerous level except the Lead (Pb) which was slightly higher than the recommended value by WHO. Gills (Non-edible part) had stored maximum concentrated of all the metals that prove that it was the best parts to monitor the aquatic pollution by using freshwater mussels as a key species.

Conclusions

The freshwater mussels especially the *Anodonta anatina* from the study spot was rich source of nutrients. Foot of freshwater mussels was the best nutritive portion as it had the maximum

value of protein among the studied parts. Most of the macro and micro minerals were found adequately in foot and mantle so recommended for the human diet. Ca was one of the most prevalent element in soft tissues of fresh water mussels as compared to the other metals. The concentration of Pb was slightly higher than the recommended value that could be the risk to human health. There is need to sort out the point and non point sources of pollution in Chashma Lake Pakistan. In biomonitoring study gills found to be the best accumulator part as compared to foot and mantle so it made the freshwater mussels *Anodonta anatina* a sentinel animal to assess the aquatic health.

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Compliance with ethics guidelines

Muhammad Sohail, Muhammad Naeem Khan, Abdul Shakoor Chaudhry and Khurram Shahzad declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

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