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Toxic Metals' Accumulation and Elemental Characterization in Mollusc and Crustacean Species' Shells Found Along the Coastline of Pakistan

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ABSTRACT

Pakistan has the coastline of 990 km, rapid industrialization and economic growth have resulted in increased water pollution in the coastal areas. Present study characterized natural and xenobiotic elements in the shells of molluscs and crustaceans found along the coastline of Pakistan. The objective of this study was to highlight the scope of these shells to be used as bio-indicators or nutrient source, instead of getting waste after seasonal washed up along the coastline.These washed up shell were collected from 09 locations in year the 2023 and were analyzed through scanning electron microscopy energy dispersive x-ray spectroscopy and atomic absorption spectrometry. This study presented a comprehensive elemental and morphological analysis of the selected species' shells, which hasn't been reported earlier. The mass percentages of elements were compared by one-way ANOVA, among species. Results suggested that these shells can also be used as bio-indicator for those elements whose toxicity is usually ignored, such as Al. Silicon and aluminum showed similar trend among species in mass percentages i.e., Mussel>Crab>Scallop>Clam>Shrimp. Variation in levels of elements in natural shell composition may influence the attachment of xenobiotic metals. While, this study also reflected shells that could be used as a nutrient source. Water pH was found to be an influencing factor on the solubility of elements. The morphological analysis of shells helped in understanding the transport of organic and inorganic materials between the body and shell.

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INTRODUCTION

Pakistan has very productive fishery sector that has the potential to produce approximately one million per year sea food. Sindh and Balochistan coastlines are the two main resources for these fishery items to earn foreign exchanges (Nazir et al., 2016; Noman et al., 2018). The total coastline of Pakistan is approximately 990 km. About 30 km of the total coastline has been contaminated by the discharge of industrial and domestic wastewater (Ali & Dinshaw,2016; Saher et al., 2019). Marine pollution is a worldwide environmental issue due to anthropogenic activities. It has been studied and reported that toxic pollutants reach marine environment through dumping of industrial waste, municipal waste, agriculture activities and oil spills. All these sources are the major threat to the living organisms in the marine ecosystem and coastal environment (Ali et al., 2019; Baig & Askari, 2024; Dąbrowska et al., 2021).

Karachi is one of the big cities of Pakistan, around 60% industries of Pakistan are found in

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Karachi due to which discharge of million gallons waste and untreated water into the coastline occurs. The major sites of Karachi coastline where wastewater discharge occurs include Karachi Harbor via Lyari River and Gizri Korangi Creeks via Malir River (Figure 1) (Ramzan et al., 2024). Karachi Harbor which is present on the Lyari River's estuary is now considered as the most polluted area along the Karachi coastline by the regular discharge of wastewater (Jilani, 2014; Nergis et al., 2021).

Heavy metals particularly are significant pollutants that are stable and persistent in the environment because they cannot be destroyed or degraded. As a result, they tend to accumulate in water bodies, marine organisms, soils, and sediments, disrupting the ecological balance (Aqeel et al., 2023; Khalid et al., 2021). Although acute poisoning from heavy metals through ingestion or skin contact is rare, exposure is typically chronic due to their transfer through the food chain (Briffa et al., 2020).

The pollutants get accumulated into marine organisms by the process of bioaccumulation and concentrate up to toxic levels through biomagnifications in tissues and shells (Reckendorfet al., 2023; Yarsan &Yipel, 2013). Shell is a part of the body (the exoskeleton) of bivalves, molluscs and crustaceans that are commonly washed up on beaches and along the edges of lakes, rivers, and streams (Phillips, 1976). Figure 2 showed tons of shells washed up on the Seaview beach of Karachi, Pakistan.

In present study, washed up shells of molluscs (including clams oysters, mussels, scallops etc.) and crustaceans (including crabs, shrimps etc.) species were collected from the coastline of Pakistan and were characterized for their morphology, elemental composition and xenobiotic elements to obtain information regarding their exposure to marine pollutants.

Fig. 1. Satellite image of Karachi coastline showing Lyari and Malir rivers.

Fig. 2. Photographs of shells washed up on Seaview beach of Karachi, Pakistan.

MATERIALS AND METHODS

Sample collection

The sampling points along the coastline of Pakistan from where the shells were collected include Gwadar, Pasni, Kund Malir, Mubarak village, Paradise point, Hawksbay, Sandspit, Kemari harbor and Sea view. Crab shells (*Scylla olivacea*), shrimp shells (*Parapenaeopsis stylifera)* andbivalves' shells (*Perna viridis, Anadara antiquate, Marcia cordata and Indothai lacera*)were collected via hand picking (Figure 3), in winter and summer seasons of the year 2023. Since not all species' shells were found at each location, therefore species were selected according to their abundance along the coastline of Pakistan throughout the year, irrespective of their gender. Shells were placed in small plastic bags and were properly labeled with relevant details. pH and temperature of sea water at the time of sampling were also noted.

Selected species

The description of each selected species is presented in Table 1 (Psomadakis et al., 2015).

Reagents and glassware

Metals' stock standard solutions and other chemicals used were of analytical pure grade, acquired from Sigma, Merck, ChemTechAnalyticals and Fisher Scientific.

Fig. 3. Photographs of shells collection during sampling.

Table 1. Description of selected species.

Species	Local name(s)	Habitat	Color
	Selected species of crab		
Scylla olivacea	Kaikara, Khunkhna (Bal), Khunkhna (Sin), Kukri	Mangroves	Brownish green to brownish (sometimes orangish)
	Selected species of shrimp		
Parapenaeopsis stylifera	Kalri, Saana, Madak (Bal), Kiddi (Sin)	Depth of about 90 m in water and 50 m in mud or sandy-mud	Pinkish white or pale brownish (sometimes grayish)
	Selected bivalves species		
Anadara antiquate (Scallop)	Dandari (Bal), Seep (Sin)	Muddy bottoms, sublittoral and intertidal zone (25 m)	Grayish white
Marcia cordata (Clam)	Kuchki (Bal), Badam (Sin)	Shallow and intertidal subtidal zone	Buff or whitish to brown
Pernaviridis (Mussel)	Kuchki, Seep (Sin), Kharkunt (Bal)	Sublittoral and littoral zone (20 m)	Whitish shell
Indothaislacera (Oyster)	Gur (Bal), Star shell (Sin)	Subtidally and muddy littoral rocks (25 m)	Yellowish brown, pale gray and brown

The apparatus used in the study were cleaned with the help of tap water followed by rinsing with distilled water. After that, the apparatus were soaked in HNO_3 (10%), rinsed with de-ionized water and dried in electric oven at 60 °C.

Samples treatment and analysis

Washing of samples (bivalve, shrimp and crab shells) was done by running tap water to eliminate impurities like sand or clay then sun dried. The length and width of each shell were measured. Each sample was divided into two equal halves to be analyzed by two different techniques.

Morphological and elemental analysis of samples was done by SEM-EDS (Scanning Electron Microscopy Energy Dispersive X-ray Spectroscopy),model number of SEM was JSM-6380A and that of EDS detector was EX-54175jMU.

In order to analyze the bio-accumulated heavy metals concentrations, the other half part of each shell sample was grinded and then mineralized by the wet digestion method via nitric acid and perchloric acid, followed by Mititelu et al. (2008). In which, 5 g of each sample of shell powder was predigested in 65% HNO_3 (2 mL) and kept for 24 hours at room temperature. After that 98% perchloric acid (2 mL) was added in the mixture and heating was done until a transparent solution achieved. The solution was cooled and the volume of the sample was made up to 50 mL with de-ionized water. The prepared samples were analyzed by Atomic Absorption Spectrometer (Thermo scientific ICE 3000 series).

The acquired data was analyzed by Minitab-16 software through different statistical techniques.

RESULTS AND DISCUSSION

The details about the average length and width of each species shellscollected for this study are given in Table 2.

The temperature of seawater ranged between 19.8–30.1 °C, while the pH of seawater ranged from 8.1–8.4, throughout the year (Table 3). The lowest pH values were observed for the seawater of Kemari harbor and Sandspit locations; this could be due to the mixing of industrial and domestic wastewater into seawater, through Lyari River.

Table 2. Measurements of collected species' shells.

Species	Average Length (cm)	Average Width (cm)
Crab (*Scylla olivacea*)	10	6.1
Shrimp (*Parapenaeopsis stylifera*)	20	3.4
Scallop (*Anadara antiquate*)	4.6	4.6
Clam (*Marcia cordata*)	3.85	5
Mussel (*Perna viridis*)	5.45	3
Oyster (*Indothais lacera*)	4.1	2.6

Table 3. Details of seawater's pH and temperature at each sampling point.

Sampling Points	pH	Temperature (°C)
Gwadar	8.2-8.3	20.1-30.1
Pasni	8.2-8.3	20.1-30.1
Kund Malir	8.2-8.3	20-30
Mubarak village	8.3-8.4	20-30
Paradise point	8.2-8.3	19.8-29.5
Hawksbay	8.2-8.3	19.8-29
Sandspit	8.1-8.2	19.8-29
Kemari harbor	8.1-8.2	20-30
Sea view	8.3-8.4	20-30

Assessment of toxic metals in shells by AAS

In the marine environment, heavy metals are absorbed by cellular membrane of protein from the surrounding water because the protein is hydrophilic in nature and favors the transport of metal ions. The other route of metal entrance into the body of marine organisms is by the process of endocytosis that absorbs the metal particles. Bioaccumulation of metals occurs by up taking food, which is absorbed in the intestine. Excess of heavy metals are stored in the lysosomes situated along with hepatopancreas in bivalves and gastropods, as detoxified metals (Abdullah, 2014).

Industrial effluent's discharge contributes about 60 percent of the total sources of heavy metals (like nickel, cadmium, copper, chromium, lead and manganese) which degrades the water quality and causes marine organisms' fatality (Sunday et al., 2013). Toxic heavy metals were analyzed by AAS and the obtained concentrations of each metal were compared among species by one-way ANOVA.

Lead (Pb)

Lead compounds exist in water, like $PbCO_3$ or $Pb(CO_3)_2$. A familiar example of a water dissolved lead complex is lead sugar [lead (II) acetate]. Shrimp exoskeleton showed significantly higher concentration of lead than clam species (Figure 4). Shrimp shells also found to contained highest carbon mass percent (mentioned earlier) which showed their protein enrichment. The attachment of metals depends upon the binding sites i.e. hydroxyl group and ammine group present in the shells of crustaceans.

Lead is known as the highly toxic metal for the marine ecosystem (Madkour, 2005). It causes severe health effects in the living organisms like change in the behavior, disturbed metabolism process and growth survival (Kamal et al., 2015). The legal permissible concentrations limit for lead reported by Stankovic et al. (2011) in seafood is 1.0-6.0 µg/g. The lead concentrations in the shells of species considered in present study were found within the range of legal permissible limits.

Cadmium (Cd)

Figure 5 revealed that mussels exhibit the highest cadmium concentration among the marine species tested, while shrimp and crab show no detectable cadmium in their shells. Cadmium

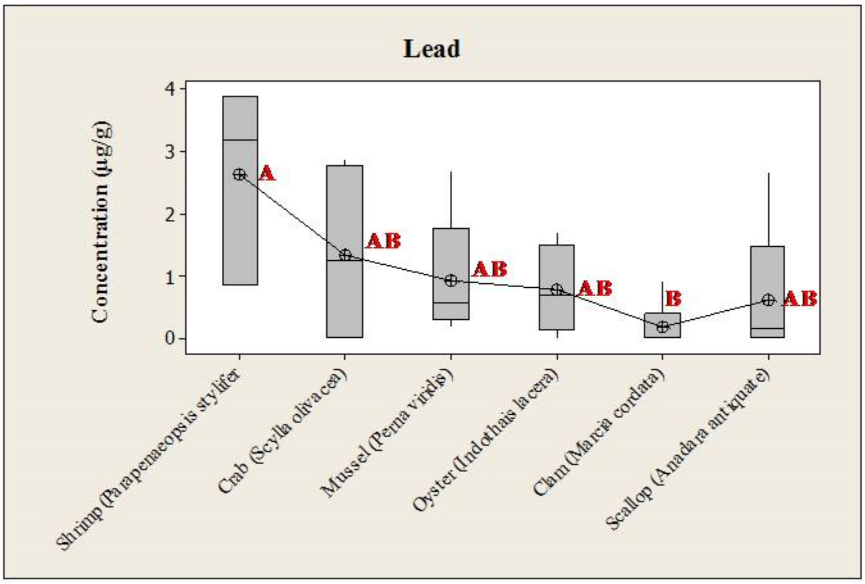

Fig. 4. under the heading of "Lead (Pb)"

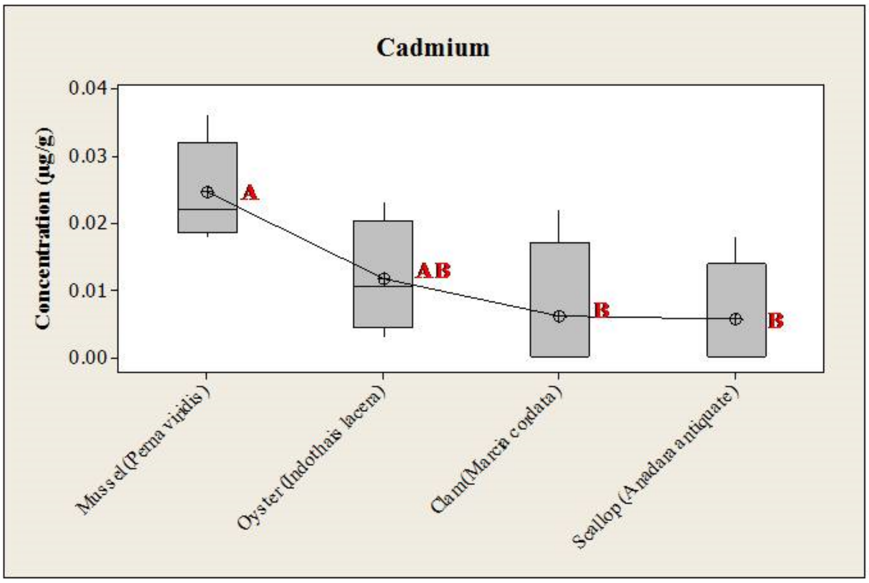

Fig. 5. Box plot of cadmium concentration in selected species' shells.

binds with proteins and nucleic acids, and in mussels, the high oxygen content in their shells may explain the elevated cadmium levels (Andersen, 1984). The binding properties of cadmium allow it to form complexes with proteins rich in oxygen, sulfur, or nitrogen, which could account for its higher accumulation in mussel shells compared to other species, as discussed previously.

Cadmium naturally occurs in water as inorganic salts and competes with calcium in fish gills, reducing calcium uptake, which leads to hypocalcemia (Madkour, 2005). Industrial wastewater, particularly from electroplating activities, is a significant source of cadmium contamination in coastal areas, and untreated domestic wastewater and agricultural fertilizers also contribute to environmental cadmium levels. Cadmium is considered one of the main toxic pollutants in aquatic ecosystems, and its accumulation in seafood poses risks to human health, affecting organs like the kidney, liver, bones, and lungs through ingestion or occupational exposure (Kamal et al., 2015; Mahurpawar, 2015).

Cadmium absorption in humans is facilitated by the Natural Resistance Associated Macrophage Protein (NRAMP 2) known as Divalent Metal Transporter 1 (DMTI), which also affects iron absorption, leading to iron deficiency and blood diseases (Bressler et al., 2004). Cadmium accumulation can cause calcium deficiency and bone demineralization, increasing the risk of fractures (Silva et al., 2005). Despite these risks, cadmium levels in the species analyzed were found to be within legal permissible limits for seafood safety, with guidelines suggesting a concentration range of 0.1-4.0 µg/g (Stankovic et al., 2011).

Copper (Cu)

No significant difference ($p > 0.05$) was observed in copper concentrations among selected species (Figure 6). The copper concentrations were found below the detection limit for species other than crab, shrimp, oyster and mussel. The shells consist of certain proportion of proteins for which copper is an important element. These proteins need trace amount of copper for their proper functioning in building up of tissues or cells in the living body. Copper is very important element for all living organisms in aquatic and other ecosystems. Sources of copper emission in environment are both natural (weathering of rocks and earth crust) and anthropogenic such as fertilizers, power stations, fungicides and industrial emissions (Aselage, 2011).

Accumulation of copper depends upon its binding sites in the tissues of marine organisms; also

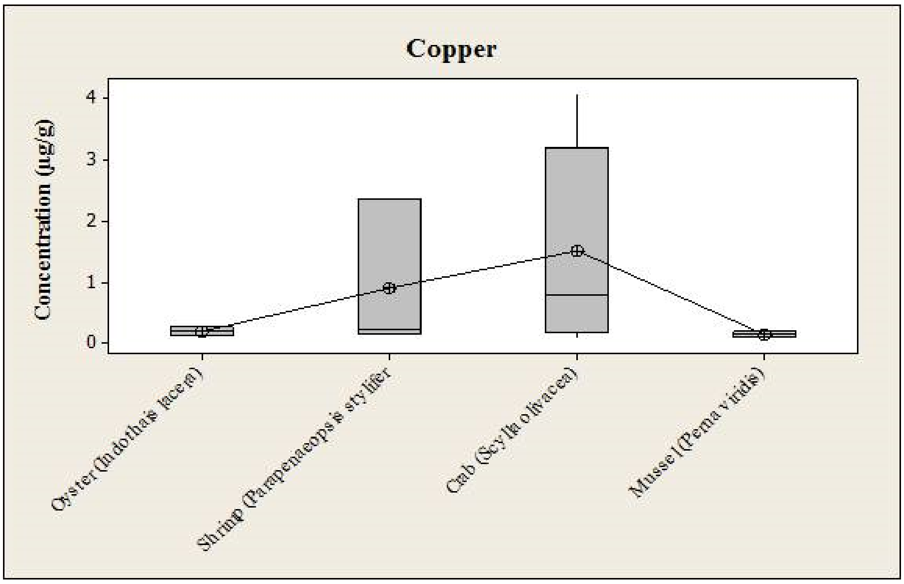

Fig. 6. Box plot of copper concentration in selected species' shells.

the presence of zinc in the living body does not allow large amount of copper accumulation in the tissues (Ahsanullah et al., 1981; Wang & Lu, 2017). Copper is found in the marine organisms in the form of complexes chelated with phytochelatin, glutathione and metallothioneins (MT), by the detoxification mechanism (Bioteau et al., 2016). It is found in the water and soil as organic and inorganic complexes and bind with organic compounds and sediments. Ionic copper exists in water to make complex with carbonate. In water, copper is present in the form of different oxidation states i.e. Cu^{+1} (cuprous ion) and Cu^{+2} (cupric ion) while Cu^{+3} is found in solid state. Copper has the potential to make complexes with the sulfhydryl group of proteins, ethylene diamine and clays.

The copper concentration in each species in the present study was found within the legal permissible value (i.e., 30 µg/g) in seafood (Aselage, 2011).

Elemental composition of shells by EDS

Representative graphs of elemental composition by EDS for each species are given in Figures 7(a-f). The obtained mean mass percentages of each element were compared among species by one-way ANOVA.Tukey's test further elaborated the significant differences in mass percentages of elements among species (means that do not share a letter are significantly different).

Aluminium (Al)

Figure 8 demonstrated that significant differences ($p< 0.05$) in the mean mass percentages of aluminum among marine species, with mussels showing the highest aluminum content in their shells, while oysters contained none. A study conducted by Wang et al. (2018) disclosed that mussel and amphipod species were very responsive to aluminium in chronic exposure. Mussels in this study were primarily collected from the Sea View area, a highly polluted coastline due to untreated wastewater discharge and recreational activities.

Aluminum, commonly found in domestic wastewater due to its use as a water coagulant, is also abundant in the Earth's crust (8.8%), naturally occurring in various forms such as inorganic, monomeric, and polynuclear species (Gensemer & Playle, 1999; Gidde et al., 2012; ATSDR, 2008). In surface water, aluminium is also attached with colloidal high molecular weight organic compounds (Fawell, 1993). The solubility of aluminum increases in either highly acidic or basic water conditions. The solubility product (K_{sp}) values of different aluminium species at 25°C

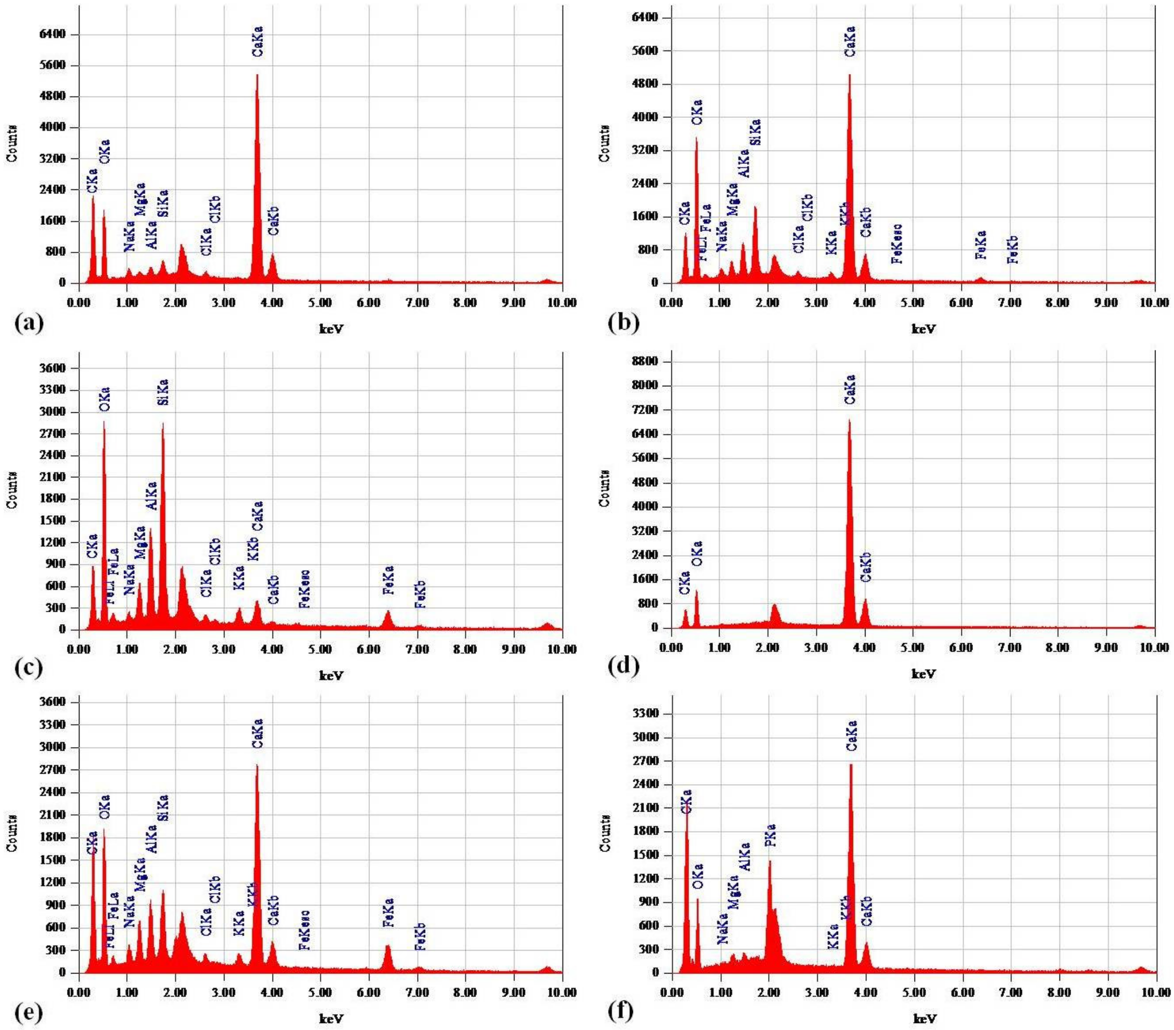

Fig. 7. Belongs to the heading "Elemental composition of shells by EDS"

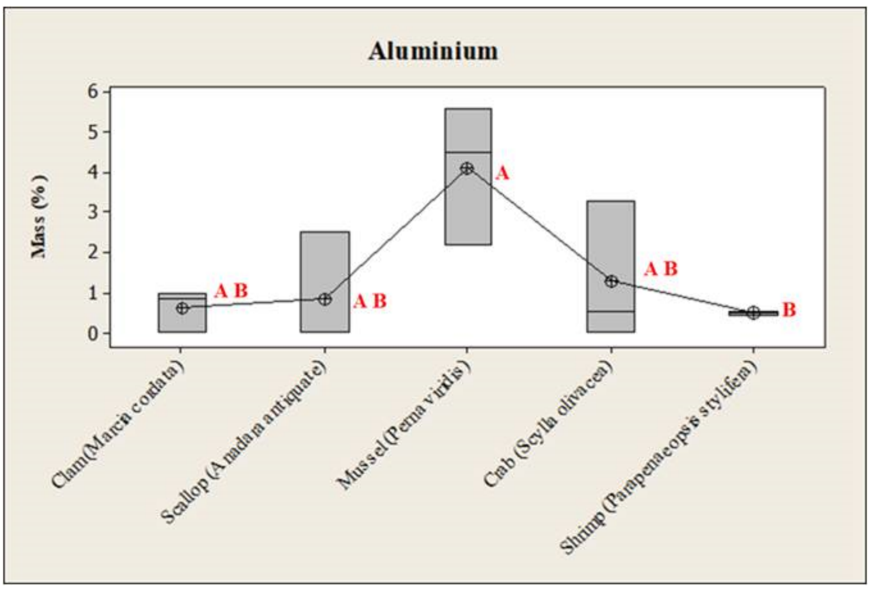

Fig. 8. Box plot of aluminium mass percent in shells.

Table 4. K_{sp} values of aluminium salts at 25°C (Lide, 2000).

Aluminum species	K_{sp} value at 25°C
Aluminum chloride	2.04×10^{4}
Aluminum nitrate	2.16×10^{3}
Aluminum sulfate	6.92×10^{1}
Aluminum hydroxide (gibbsite)	1.06×10^{-33}

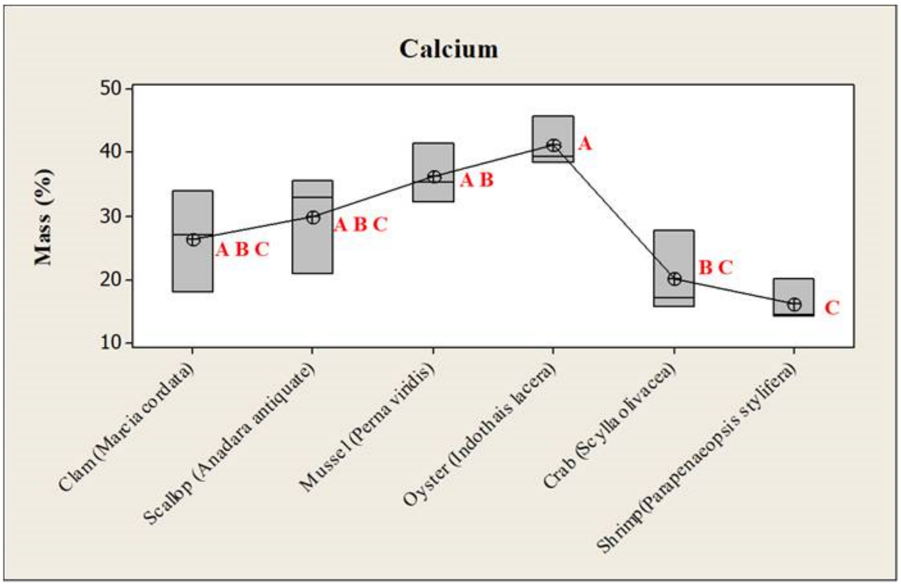

Fig. 9. Under the heading "Calcium (Ca)"

are given in Table 4 (Lide, 2000). The seawater pH (8.1–8.4) and temperature (19.8–30.1°C) conditions of present study (mentioned in table 3) served as contributing factors in solubility of aluminium.

Bioaccumulation of aluminum in invertebrates is primarily through adsorption rather than diet, and aluminum toxicity increases at higher pH levels (Handy, 1993; Herrmann & Frick, 1995; Poston, 1991). At basic pH (>8), aluminate anions dominate, leading to salt regulation issues and sodium loss in invertebrates, especially sensitive species (Hornstrom et al., 1995; Sparling & Lowe, 1996). In fish, aluminum toxicity causes gill dysfunction, epithelial necrosis, and impaired gas exchange, ultimately resulting in death (Dietrich, 1988; Dietrich & Schlatter, 1989; Mallatt, 1985). Thus, aluminum acts as a toxic agent for marine organisms, particularly affecting invertebrates and fish.

Calcium (Ca)

The comparison of mass percentages of calcium in the shells of different species showed significantly highest ($p < 0.05$) mean mass percentage of calcium in oyster's shell than in all other species (Figure 9). The shell of an oyster is high in thickness and mainly composed of calcium carbonate, due to which it is highly calcium enriched. Calcium is found in the shell as calcite or aragonite. Study conducted by John and Mary (2016) also revealed that oyster shell contains largest constituents of calcium (i.e., 47.49%). Shrimp shell showed lowest mean Camass percentage than the shells of the rest of species. This is because crustacean species contain lower calcium in their shells than in meat. The hardness in their exoskeleton is majorly by the composite of chitin with calcium carbonate (Abidin et al., 2020).

Carbon (C)

Mass percentage of carbon was found in all considered species because carbon is the main component of organic compounds in the living organisms. It is present in the form of carbohydrates, lipids, proteins and calcium carbonate in the exoskeleton of the marine species. It exists in the living body as both organic and inorganic forms.

Figure 10 represented mean mass percentages of carbon in the shells of selected species; shrimp shell containedsignificantly highest ($p< 0.05$) carbon than other species because shrimp shells contain high amount of protein (Gopan et al., 2020). Ravichandran et al. (2009) conducted the study on chemical composition of shrimp and revealed that shrimps were enriched with proteins and the shrimp shell was made up to 32.5% of protein.

Chlorine (Cl)

No significant difference ($p> 0.05$) was observed among the mean mass percentages of chlorine in shells, by one-way ANOVA (Figure 11). Chlorine is an essential element; chloride

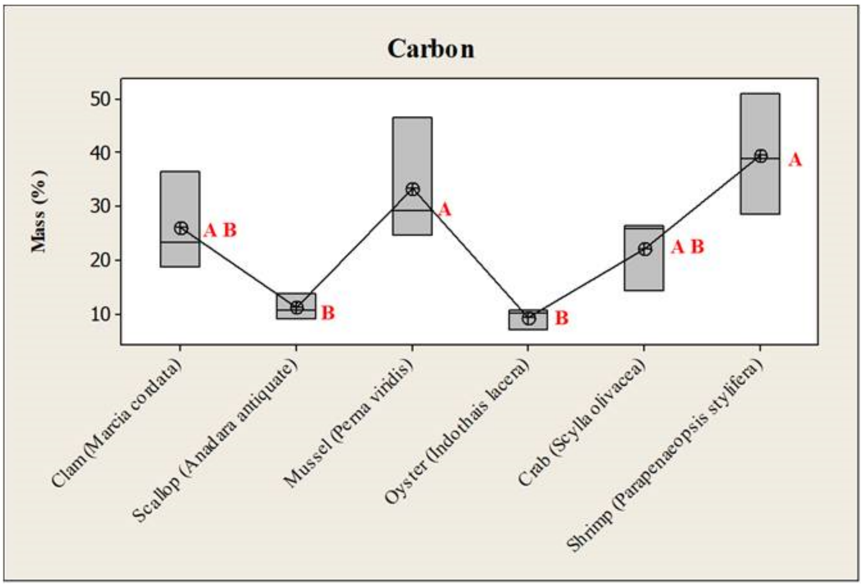

Fig. 10. Under the heading "Carbon (C)"

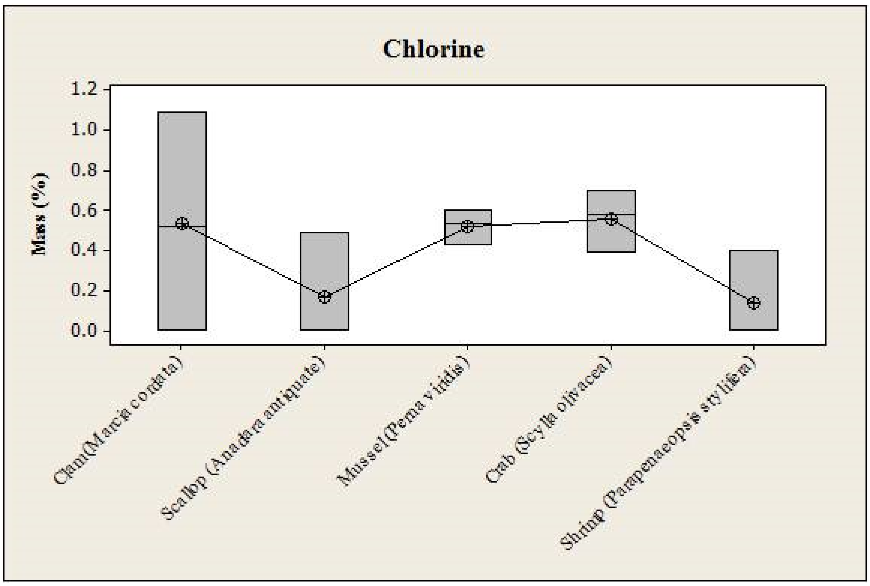

Fig. 11 Box plot of chlorine mass percent in shells.

ions are dissolved in water majorly in the form of sodium chloride. These ions are necessary as electrolytes in body fluid and are responsible for the acid-base balance. Chloride ion is also found in the proteins and some enzyme (amylase) (Shrimanker & Bhattarai, 2023).

Iron (Fe)

According to Tukey's method, the mean mass percentage of iron in exoskeleton of crab was found to be significantly highest ($p< 0.05$) than the other species (Figure 12). While, shrimp species have the lowest mean mass percentage of iron. Literature has suggested crab shell as a good source of iron and magnesium than meat (Ahmed, 2022). While others have reported maximum concentrations of iron and magnesium in the female crab than in males (Soundarapandian et al.,2013).

Iron is one of the most important elements and occurs in surface water around the world. Iron exists as Fe^{+2} and Fe^{+3} in the water; thus it is bioavailable for species. Sea water may also get exposed to iron from domestic discharge, geological sources and industrial wastes (Tiwari et al., 2013). Oxygen is transported in blood via iron as it is an important part of haemoglobin, the red color in blood is due to the presence of oxygen in blood. Iron also plays important role in cellular respiration because it is the main part of cytochromes (Soetan et al., 2010).

Magnesium (Mg)

The mean mass percentage of magnesium was recorded to be significantly highest ($p< 0.05$) in crab species, as evaluated by one-way ANOVA (Figure 13). While, clams contained lowest content of magnesium in shells.Whereas, magnesium content in their shells of oyster species were below detectable limit. In previous study, other species of oyster *Crassostrea madrasensis* showed very low percentage of Mg in shell (i.e., 0.619 %) in the form of MgO (John & Mary, 2016).

As discussed earlier, crabshells are rich in iron and magnesium, therefore the considered species of crab (*Scylla olivacea)* can be a good source of magnesium and iron. Magnesium is the second mainly abundant intercellular cation. A normal human body holds 20-28 grams of magnesium, 1% of it is present in the extracellular fluid and 60-65% is present in the skeleton. It plays multiple functions in the body and behaves as a cofactor in enzymatic reaction. It is involves in the functions of enzymes associated with lipids, carbohydrates, nucleic acid

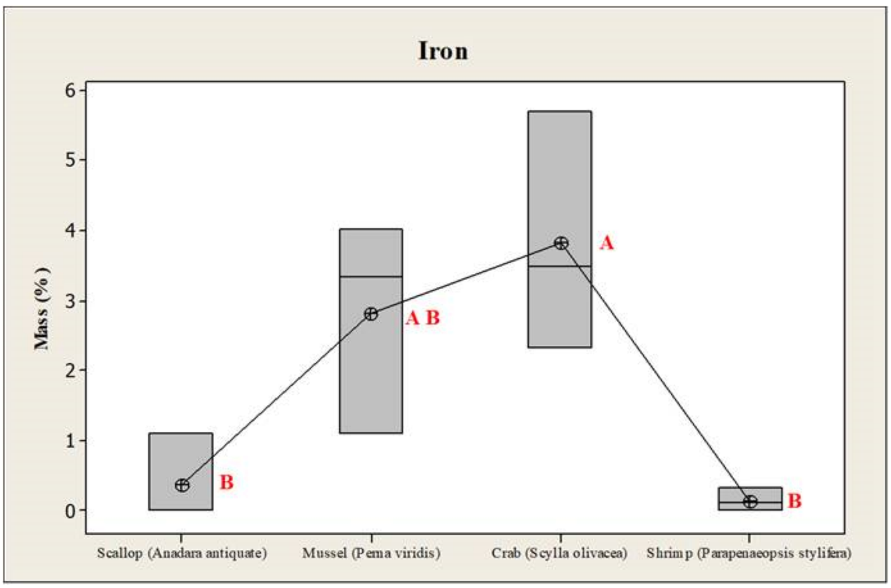

Fig. 12. Under the heading "Iron (Fe)"

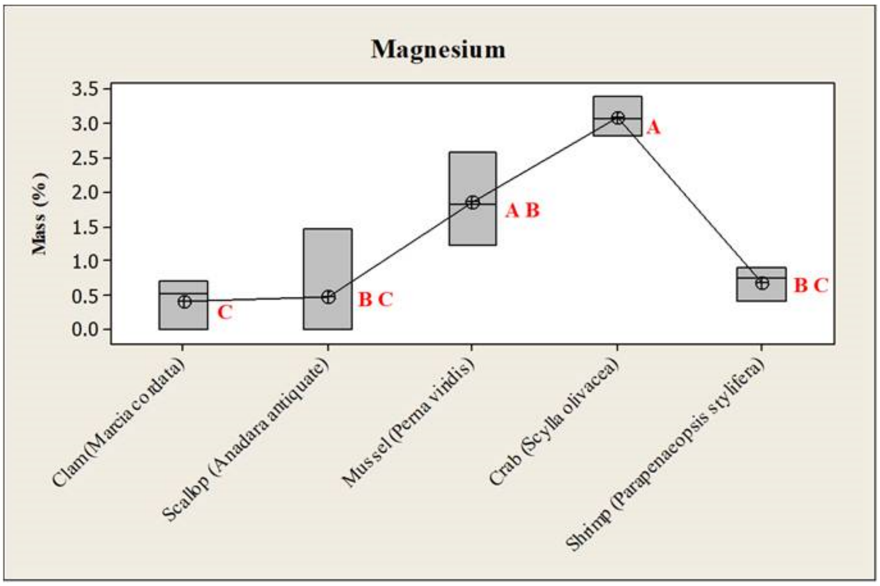

Fig. 13. Box plot of magnesium mass percent in shells.

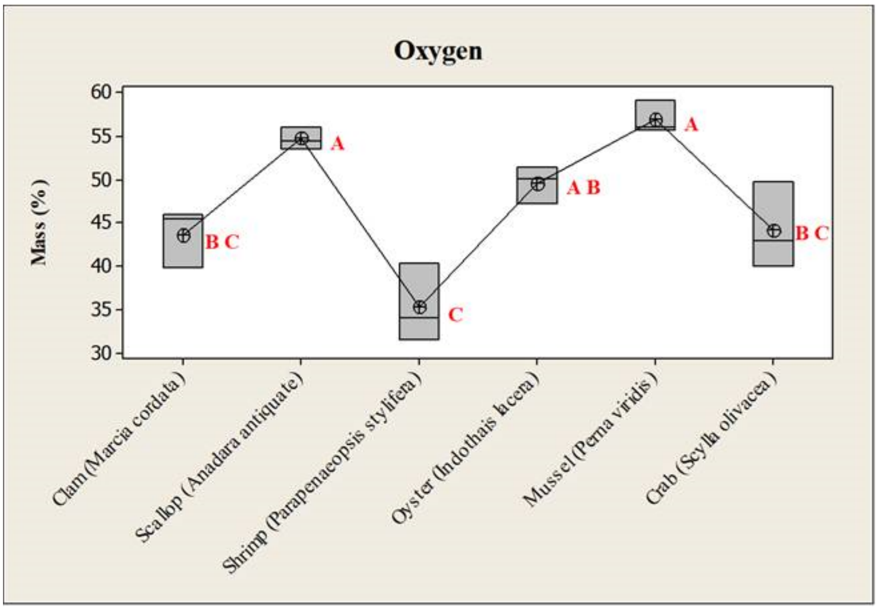

Fig. 14. Under the heading "Oxygen (O)"

metabolisms and protein. It also plays vital role in the development and mineralization of skeleton, neuromuscular excitability and cellular permeability (Olivares & Uauy, 2005).

Oxygen (O)

Mussel showed the highest ($p< 0.05$)mean mass percentage of oxygen than other species' shell (Figure 14).

Shells of musseland oyster contain a large proportion of oxides of calcium, which can be attributed to the highest oxygen content in their shells. Table 5 showed the chemical composition of mussel and oyster shells reported by Hamester et al. (2012), the results supported the highest oxygen mean mass percent in mussel shells than the other species. This can also be attributed for the highest mean mass percentage of calcium in oyster shells (mentioned previously). The

Table 5. Chemical composition of mussel and oyster (Hamester et al., 2012).

Oxides	Mussel (%)	Oyster (%)
CaO	95.7	98.2
K_2O	0.5	-
SiO_2	0.9	-
SrO	0.4	-
Fe_2O_3	0.7	-
SO_3	0.7	0.7
MgO	0.6	-
Al_2O_3	0.4	-

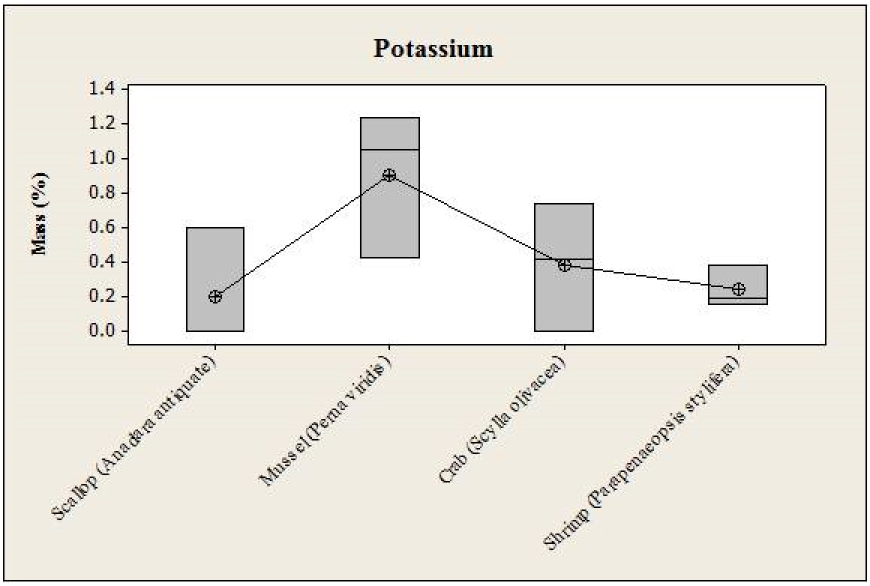

Fig. 15. Under the heading "Potassium (K)"

shrimp showed the least mean mass percentage of oxygen as compared to the other species' shell because of lesser calcium oxide content.

Phosphorus (P)

Ravichandran et al. (2009) conducted the study on chemical composition of shrimp and revealed high value of phosphorous in shrimp shells. Present study supported these results as phosphorus was found only in the shrimp species shell (i.e., 5.02%), while the mass percentage of phosphorus was below the detectable limit in other species' shells.

Phosphorus exists in the exoskeleton of the marine life and takes part in a significant function of bone formation. It is an essential component of nucleic acid, enzymes, phospholipids and phosphoprotein. Inorganic phosphorus helps to regulate the acid-base balance in the fluid of animal body.

Potassium (K)

The mean mass percentages of potassium showed no significant difference ($p > 0.05$) by one-way ANOVA (Figure 15). Potassium is found in the living system alliance with the sodium and chloride ions and helps in balancing electrolyte in the body. Mass percentages of potassium in clam and oyster shells were below the detectable limit (Shrimanker & Bhattarai, 2023).

Silicon (Si)

According to Tukey's test, the mean mass percentage of silicon in mussel was found to be significantly higher than clam and shrimp ($p < 0.05$). While, oysters did not show silicon content in their shells (Figure16).

Silicon is one of the most important elements in animal and plant body, it was accepted as an essential element in 1972. If the uptake of silicon decreases, the body weight also starts decreasing. It has biological functions such as a cross linked agent of structure and maintaining the resiliency of connective tissue (Soetan et al., 2010).

Vazquez et al. (2010) examined that aquatic species with high aluminium contamination have significantly higher silicon levels as well. Bioavailability of silicon in the living organism and the toxicity of aluminium have been widely noticed in the organisms (Cocker et al., 1998). Aluminium bonded with the silicon can amend the toxicity of metal by forming a bio-unavailable compound like HAS (hydroxyaluminosilicates) (Exley et al., 2002).

In present study the trend of mass percentage was found similar for Si and Al i.e., Mussel>Crab>Scallop>Clam>Shrimp, which showed the similar interaction of Al and Si with the proteins present in the shells of these species.

Sodium (Na)

Like chlorine, one-way ANOVA showed no significant difference ($p > 0.05$) in the sodium mean mass percentages among all species (Figure 17).

Sodium is present in water mostly in the form of sodium chloride and is bioavailable for to each species, because of being soluble in nature. This can be attributed for no significant difference in the sodium mean mass percentages among all considered species (Shrimanker & Bhattarai, 2023).

Seasonal variation in metal concentrations

Table 6 showed the concentrations of metals in each species, in winter and summer seasons. Paired T-test was applied to determine the significant difference between metal concentrations in both seasons; to evaluate seasonal effects.

No significant difference was revealed between metal concentrations in winter and summer seasons ($p > 0.05$), except for copper concentrations in oyster species. A number of factors influence on the seasonal differences in concentrations of heavy metals in oyster species, such as biochemical activities in organisms, chemical form of element, size and age of oyster,

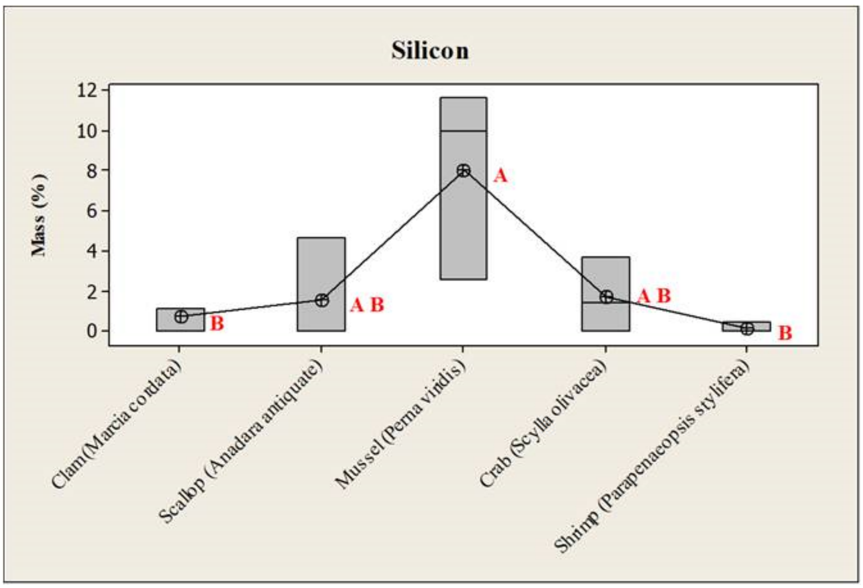

Fig. 16. Under the heading "Silicon (Si)"

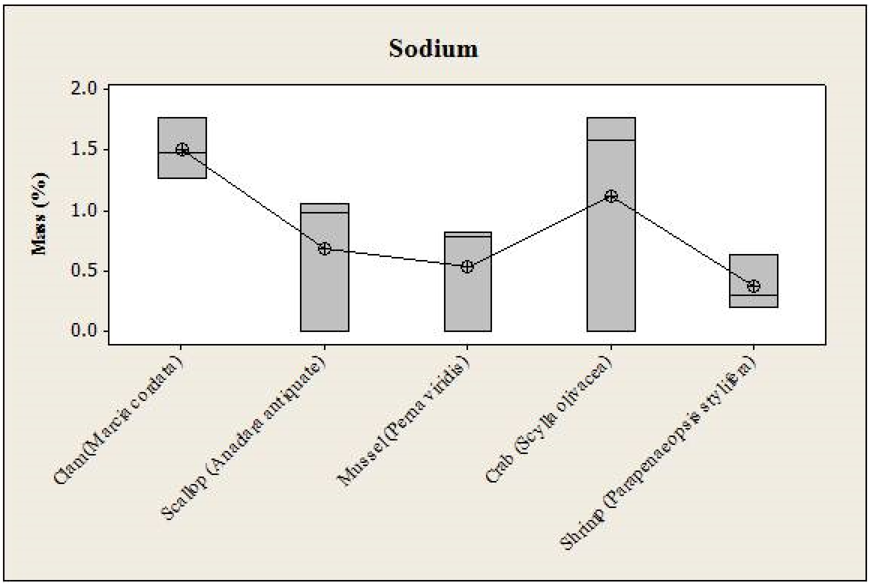

Fig. 17. Under the heading "Sodium (Na)"

Table 6. Seasonal variation in metal concentrations in shells.

Metal and Species	Winter Mean (µg/g)	Summer Mean (µg/g)	T-value	P-value
Pb in Shrimp	2.02	1.95	0.09	0.945
Cu in Shrimp	1.28	0.07	1.06	0.482
Pb in Crab	0.961	0.822	0.09	0.936
Cu in Crab	1.61	0.88	1.27	0.332
Cd in Mussel	0.0227	0.0183	0.58	0.623
Pb in Mussel	1.361	0.190	2.01	0.182
Cu in Mussel	0.0533	0.1133	-1.87	0.203
Cd in Oyster	0.0105	0.0130	-0.20	0.874
Pb in Oyster	1.298	0.240	6.45	0.098
Cu in Oyster	0.11	0.235	-25.00	0.025
Cd in Clam	0.012	0.00	2.15	0.121
Pb in Clam	0.126	0.249	-1.31	0.281
Cd in Scallop	0.00933	0.00	1.79	0.215
Pb in Scallop	0.889	0.148	0.91	0.460

environmental concentrations and filtration rates etc (Ali et al., 2014). Other than these, seasonal variation also depends on phytoplankton productivity. The nutritional status of oyster increases with greater phytoplankton production, which leads to increased metal concentrations in oyster (Ferreira et al., 2005).

Morphology of shells by SEM

Scanning Electron Microscopy (SEM) technique is very useful in examining the microstructure (size of particles and morphology) of the shells of the marine organisms. Surface morphology and structures were studied with the help of SEM technique. It was observed that the molluscs shell are composed of aragonite platelets (elastic biopolymers such as chitin and lustrin; and silk-like proteins) that are scattered all over the shell structure, aragonite has spire like shape of calcium carbonate. The plate structure of calcite and aragonite can be seen in Figure 18 (a).

The shells of mollusc have porous scattered structure (Figures 18 b and c). Pores and little

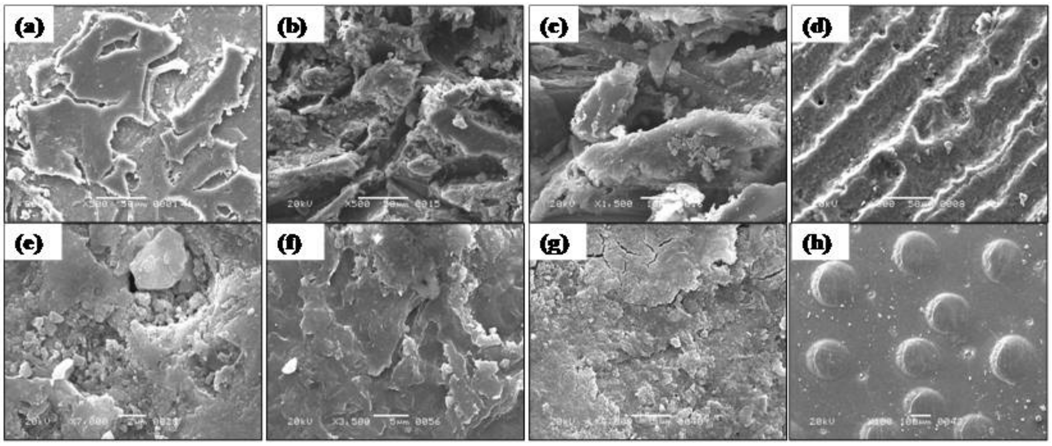

Fig. 18. SEM micrographs of shells. (a) Clam, (b & c) Mussels, (d) Scallop, (e) Oyster, (f) Shrimp and (g & h) Crabs.

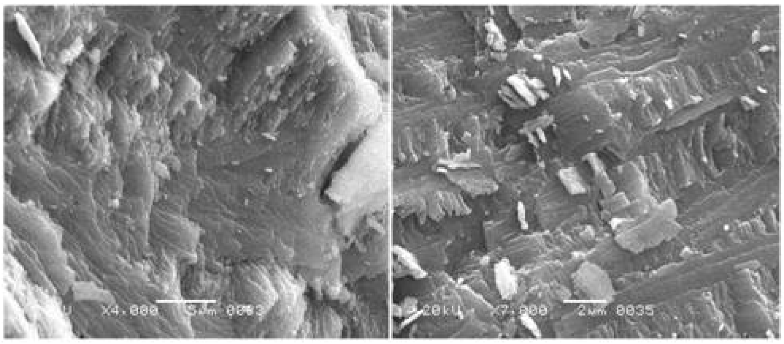

Fig. 19. Structures of the two layers of molluscs shells.

veins in the shells of molluscs help in transportation of materials into body and shell; and provide interaction between water and gases, like carbon dioxide. The calcite crystal has longer shape and is slightly spaced and interconnected with globular material (Figure 18 d). Calcium carbonate is one of the main chemical compounds that exist in marine shells in different forms such as calcite, aragonite and vaterite. The SEM images confirmed that the molluscs shells contain two types of calcium carbonate crystals i.e. calcite and aragonite. This showed that exoskeleton of molluscs is composed of the mixture of calcite and aragonite. Molluscs showed irregular shape and sizes of particles (Figure 18 e).

Crustacean shells didn't have regular shape and structure, but particles were joined together (Figures 18 f and g). Small pores were present among the particles that serve as the path for the transportation of organic and inorganic materials between the body and shell. Crab shell also showed tiny bulges protrude from it (Figure 18 h), these are the epithelium cells which are woven by the chitin fibrils.

Molluscs shells have two types of layers i.e. an external (prismatic) layer and an internal (nacreous, pearly and lamellar) layer. Outer layer is mostly composed of aragonite or calcite while inner layer are parallel long sheet like structure (Figure 19).

CONCLUSION

This study presented a comprehensive elemental and morphological analysis of the selected species' shells, which hasn't been reported earlier.

The presence of toxic metals in the elemental analysis of sea shells suggested that these species could be used as good bio-indicators of environmental pollution as well. Specifically, for those elements whose toxicity is usually ignored, such as Aluminium. Mussels showed higher sensitivity to aluminum, than rest of species. This determined that certain species are more sensitive to certain elements than others, although sharing same environment.

Shells of mussel and oyster contained a large proportion of oxides of calcium. Variation in levels of elements in natural shell composition may influence the attachment of xenobiotic metals; for instance, highest oxygen mass percent in mussel shell indicated highest cadmium attachment. Other than bioindication, these shells are also a rich source of nutrients; like the shells of crab species considered in this study was found to be as a good source of iron and magnesium. Aluminum and silicon showed similar trend i.e., Mussel>Crab>Scallop>Clam>Shrimp, which revealed their similar interaction with proteins in shells.

The pH was found to be an influencing factor affecting the availability of elements in water. While, no significant seasonal variation was observed in mean mass percent of metals, except for copper in oyster shells which increased double in summer than in winter. SEM analysis showed that exoskeleton of molluscs and crustaceans contained irregular shape and sizes of particles. The morphological analysis of shells helped in understanding the transport of organic and inorganic materials between the body and shell.

Pakistan fishery is the earner of foreign exchange and offer high profit margins, therefore the results of this study are inevitable from both environmental and economic perspectives. The results of in-situ toxic metals accumulation analysis in shells showed the exposure ofmolluscs and crustacean to toxic metals, this will help the relevant authorities to reduce the marine pollution along the coastline of Pakistan.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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