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Research Article
Distribution of Mineral Contents in the Selected Tissues of *Meretrix lyrata*

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Abstract
Background and Objective: *Meretrix lyrata* is hard clam that is found abundantly at Kuching division in Sarawak and used as delicacy by the local people. This study aimed to determine amount of macro and micro-minerals in the soft tissue of *M. lyrata.* **Materials and Methods:** Macro and micro-minerals extracted from tissues of *Meretrix lyrata*, sediment and seawater were determined using air-acetylene flame Atomic Absorption Spectrophotometer (AAS). The minerals; Na, K, Mg, Ca, Zn, Cu, Mn and Fe were extracted from environment, adductor muscle, foot, gill, mantle and siphon from the clam. Concentration of macro and micro minerals were analysed using one way ANOVA and multivariate analysis. **Results:** The Na (319.552 ± 9.47 µg g^{-1}) and Fe (19.48 ± 4.726 µg g^{-1}) concentration were high in *M. lyrata* tissues compared to other elements. This result suggested that *M. lyrata* tend to accumulate more Na and Fe from the environment and this supported by high concentration of Na and Fe in the seawater. Furthermore, multivariate analysis indicated that tested tissues were grouped according to the mineral elements and not based on tissue variety. Therefore, macro and micro-minerals that accumulated in the *M. lyrata* tissues were non tissue dependent. **Conclusion:** The affinity of hard clam tissue to accumulate other elements was high and it depends on availability of the elements in the seawater. Hence, pristine environment was important to harvest hard clam as the food source to prevent consumption of unwanted elements such as heavy metals.

Key words: Hard clam, macronutrient, *Meretrix lyrata*, micronutrient, component

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Veneridae (hard clam) is listed under mollusca phylum and as one of the important invertebrate that generates animal protein for human consumption in the modern world. Hard clam is among of 60 families of bivalve and approximately 10,000 species, included of oyster, clams, scallop and mussels[1,2]. Hard clam generally found inhabit at marine area particularly intertidal area such as coastal and estuary. Favourable habitat condition for instance nutrient cycle[3], physico-chemical variable[4] and sediment properties[5] are highly associate with number and diversity of bivalve.

In Asian region, hard clam also known as Asiatic clam and grouped under *Meretrix* genus. China, Japan and Korea are countries that abundantly found with *M. petechialis* and *M. lusoria*[6]. Meanwhile, *M. meretrix* and *M. lyrata* distributed in Malaysia and Vietnam[7-9]. *Meretrix lyrata* is discovered copiously in Kuching Sarawak especially at the Santubong region[7]. Santubong area is reach with mollusc and other marine invertebrate because of the pristine condition. Hamli *et al.*[7] reported that 9 types of palatable bivalves found in Kuching. Local people gathering seashell from the wild particularly *M. lyrata* from the intertidal zone for consumption or selling in market and this specified the abundance of clam in nature. *M. lyrata* and other *Meretrix* genus are filter feeder animals those sieving water to trap microscopic food such as phytoplankton and zooplankton. Moreover, the filtered food and other nutrient from the ambient water will be digested and accumulated in clam tissue. Due to this ability, clam is frequently used as bioindicator for environmental monitoring[10,11]. Furthermore, Asiatic clam consist a good tolerance to high concentration of mineral and desiccation resistance which qualified as monitoring tool[12].

Mineral content that deposited in the *M. lyrata* tissue has fundamental capacity to the human. Deficiency of this mineral or malnutrition to human body may cause variety of disease. Mineral content that essential to human can be found in form of macro-minerals and micro-minerals[13]. The macro and micro minerals include sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn)[14]. Due to the importance of *M. lyrata* as the local consumption, knowledge regarding essential mineral content to human in this hard clam become crucial compared to heavy metal evaluation. Furthermore, pristine habitat will be expected to produce natural mineral content deposit in the hard clam tissues. Documentation on macro and micro-mineral in *M. lyrata* tissue is insufficient particularly from Borneo, therefore, present study aimed to evaluate concentration and distribution of macro and micro-minerals content in selected tissues such as adductor muscle, gill, foot, mantel and siphon of *M. lyrata* from Sarawak.

MATERIALS AND METHODS

Total of 20 individuals of *M. lyrata*, sediment and seawater were collected from the natural habitat in the Buntal Village (01°42'18"N, 110°22'03.6"E), Kuching, Sarawak, Malaysia from January to, March 2014 (Fig. 1). Sediment and seawater were collected and stored in the plastic container to prevent any metal contamination. Samples then transported to the laboratory for further analysis.

Sample preparation: Ranged of 10-20 individual of *M. lyrata* with size ranged 55.75-76.58 mm were randomly chosen and immersed in water of room temperature. Shells were washed to remove any dirt and sediment to prevent contamination. Shell valves were slowly opened by cutting the adductor muscle from the posterior area. Tissue was dissected based on 5 different categories namely adductor muscle, gill, foot, mantle and siphon. Afterward, these tissues were dried in oven at 60°C for 72 h until constant weight[15]. Drying procedure was also applied for collected sediment. The dried samples were then pounded using glass mortar and stored in polyethylene boxes until digestion process.

Tissue and sediment digestion: Total of 0.5 g of tissue sample was digested in 10 mL of concentric nitric acid 65% (Merck, CAS number: 7697-37-2)[15]. Total of 5 g dried sediment was digested in 10 mL of nitric acid 65% and hydrochloric acid 37% (Merck, CAS number: 7647-01-0) with 3:1 ratio[16]. Tissues and sediment sample than heated in the hot block for at 40°C for 30 min before increased to 140°C for fully digestion for 3 h. Then samples were cooled at room temperature and diluted with 40 mL of distilled water. Subsequently the cooled samples were filtered with filter paper (Whatman filter paper, Grade 2) before analysed for mineral elements (Na, Mg, K, Mn, Zn, Fe and Cu) using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model Analyst 800 with four standard calibration.

Statistical analysis: Data on mineral concentration on every parts of *M. lyrata* tissues, seawater and sediment were analyzed by one way analysis of variance (ANOVA) using Statistical Analysis Software (SAS), version 9.3[17]. Significant difference ($p<0.05$) in mean was compared using Tukey test.

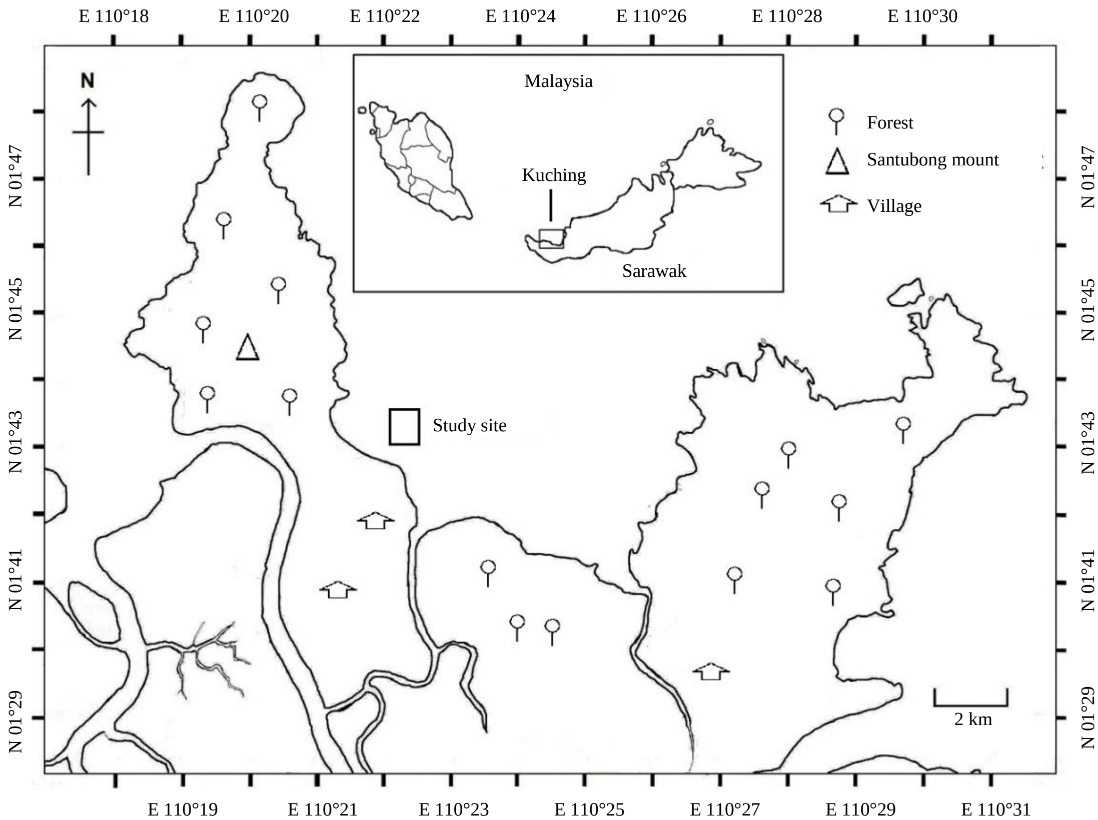

Fig. 1: Natural habitat location of *M. lyrata* for mineral content study

Cluster analysis: Data on mineral composition in selected tissues of *M. lyrata* was analysed using clustering analysis with hierarchical method and similarity distance used to calculate data was based on Bray-Curtis similarity and analysed using computer program PRIMER version 5 (Plymouth Routines In Multivariate Ecological Research)[18].

Principal Component Analysis (PCA): Data on mineral composition in selected tissues of *M. lyrata* was also analysed with Principal Component Analysis (PCA) using the program PRIMER version 5 (Plymouth Routines In Multivariate Ecological Research)[18]. Result on the PCA analysis was then compared with the clustering analysis result.

RESULTS

Minerals content analyzed in the present study was grouped into macro-minerals and micro-mineral based on minerals concentration >100 and <20 $\mu g\ g^{-1}$, respectively that found distributed in the selected tissues of *M. lyrata*. Analysis of variance (ANOVA) on macro-minerals concentration in

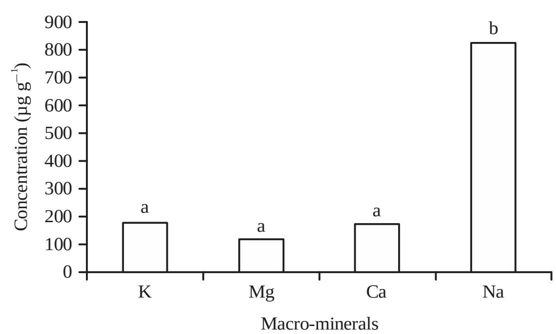

Fig. 2: Macro-minerals concentration in *M. lyrata* tissue
Different alphabets indicate significant difference at $p<0.05$

M. lyrata tissue showed significant difference at $p<0.05$ (Fig. 2). Sodium (Na) level in *M. lyrata* was notably higher compared to potassium (K), magnesium (Mg) and calcium (Ca) within macro minerals group. Furthermore, concentration of K, Mg and Na were higher ($p<0.05$) in seawater compared to sediment (Table 1). However, no variance ($p>0.05$) on macro-mineral concentration and no tissue dependent among the elements (Fig. 3, 4) were found.

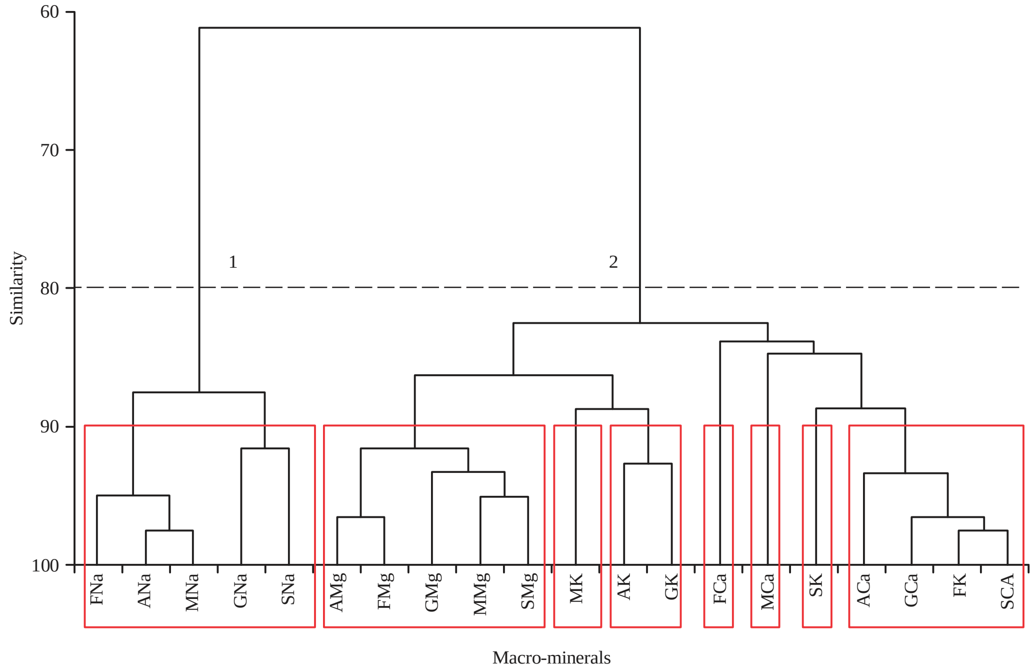

Fig. 3: Hierarchical cluster of macro-minerals composition in *M. lyrata* tissues
A: Adductor, F: Foot, G: Gill, M: Mantel, S: Siphon

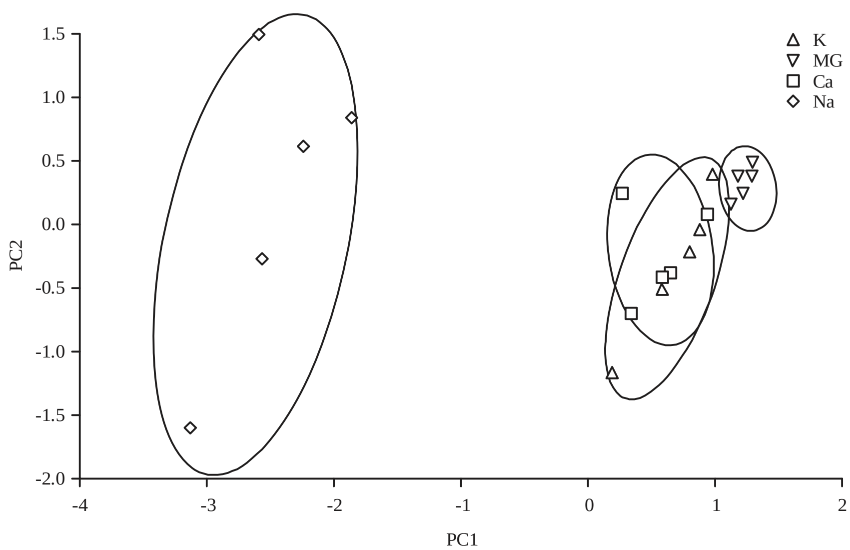

Fig. 4: Principal Component (PC) analysis on macro-minerals in each *M. lyrata* tissue

Table 1: Mean± of macro-minerals concentration in selected tissues of *M. lyrata*, sediment and water

Tissues	K (μg g^{-1})	Mg (μg g^{-1})	Ca (μg g^{-1})	Na (μg g^{-1})
Adductor	245.719±52.0^{a}	161.273±27.9^{ab}	241.771±43.0^{a}	1213.992±288.4^{ab}
Foot	259.886±28.9^{a}	166.8±36.2^{ab}	202.827±54.0^{a}	1390.214±394.6^{ab}
Gill	263.963±89.4^{a}	179.8±55.2^{ab}	304.223±36.0^{a}	1353.809±530.1^{ab}
Mantel	285.386±124.2^{a}	194.078±39.1^{ab}	413.132±86.3^{a}	1109.909±276.5^{ab}
Siphon	310.941±79.4^{a}	190.439±33.0^{ab}	271.882±18.1^{a}	1283.964±264.6^{ab}
Sediment	1.42889±0.2^{a}	14.978±0.1^{a}	971.782±693.0$_{a}$	16.94311±2.4^{a}
Water	1721.161±541.6^{b}	269.878±8.3^{b}	1106.539±543.4^{a}	2378.556±40.1^{b}

^{ab}Different superscript letters between row are significantly different at $p<0.05$

Table 2: Mean± of micro-minerals content in selected tissues of *M. lyrata*, sediment and water

Tissues	Zn (µg g^{-1})	Cu (µg g^{-1})	Mn (µg g^{-1})	Fe (µg g^{-1})
Adductor	1.549±0.6^{ab}	0.153±0.1^{ab}	0.109±0.1^{a}	8.558±2.0^{ab}
Foot	2.314±0.8^{ab}	0.162±0.1^{ab}	0.061±0.1^{a}	5.313±0.7^{ab}
Gill	2.997±0.7^{ab}	0.381±0.1^{a}	0.571±0.4^{a}	14.413±1.6^{ab}
Mantel	3.013±0.5^{a}	0.269±0.1^{ab}	0.484±0.4^{a}	17.299±7.5^{ab}
Siphon	2.055±0.4^{ab}	0.221±0.0^{ab}	0.352±0.3^{a}	32.521±9.7^{a}
Sediment	0.019±0.0^{b}	0.000±0.0^{b}	0.532±0.2^{a}	6.850±0.4^{ab}
Water	0.149±0.0^{ab}	0.101±0.0^{ab}	0.0413±0.0^{a}	0.2652±0.0^{b}

^{ab}Different superscript letters between row are significantly different at p<0.05

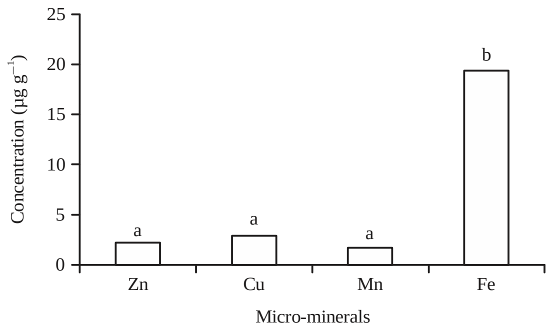

Fig. 5: Micro-minerals concentration in *M. lyrata* tissue
Different alphabets indicate significant difference at p<0.05

Micro-minerals concentration in *M. lyrata* tissue showed significant difference at p<0.05 (Fig. 5). Ferrum (Fe) concentration in *M. lyrata* was considerably high compared to Zn, Cu and Mn within micro minerals group. Subsequently, Zn and Mn amount in sediment was particularly low compared to *M. lyrata* tissues (Table 2). Conversely, Fe level in seawater was notably higher than the other elements and remarkably low analogized (p<0.05) to concentration in *M. lyrata* tissues. Cluster analysis and PCA indicated that micro-mineral that accumulated in the *M. lyrata* tissues were not tissue dependent and result demonstrated each selected tissue was clustered based on element type (Fig. 6, 7).

DISCUSSION

High concentration of Na in the visceral mass of *M. lyrata* is might be due to seawater as the main component in the habitat. Therefore, high Na concentration in *M. lyrata* tissues might be related to the concentration of Na in seawater as shown in Table 1. Meanwhile, accumulation level of macro-minerals similar for each part of *M. lyrata* tissue which suggests maco-minerals that deposited were not tissue dependent. This was supported by multivariate analysis which depicted that each tissue was grouped based on element predominantly Na and not based on type of tissue (Fig. 3, 4). Veiga *et al.*[19] reported that increase of salinity in environment cause alterations to hemolymph osmolality in the mantle tissue of mollusc. Sodium is important electrolyte component in the marine animal visceral mass which always maintains balance to the sodium concentration in seawater. Furthermore, regulating process known as osmoregulation which is based on carrying of ions in specialized organs, allows the level of the internal osmotic concentration to be maintained for an unlimited period of time[20].

Current study on macro and micro-mineral in selected *M. lyrata* tissues indicated Na and Fe as the highest stored element. Accumulations of the elements were affected by the ambient circumstance and food. High Fe amount in the visceral mass is due to the bioaccumulation of the element from the phytoplankton as the producer in the food chain and the availability of Fe in the seawater as well. Phytoplankton requires Fe in the seawater for photosynthetic and respiratory function[21]. Therefore, the higher organisms in the food chain level will accumulate more Fe in the soft tissue. Moreover, high Fe level in every parts of *M. lyrata* tissues are facilitated by high iron binding protein in the soft tissue[22]. High convergence of Fe additionally was accounted for *Meretrix* sp. from Wenzhou, China which was higher than the present review[23].

Veiga *et al.*[19] detailed that *Meretrix* sp. collected from Vietnam coast aggregate high centralization of Zn, Cu and Mn in the tissue contrasted with present review. In addition, other review from China coastal water too demonstrated high grouping of Zn, Cu and Mn in the *Meretrix* sp. tissues[23,24]. High fixation of micro-minerals found in the past review because of *Meretrix* sp. natural surroundings near to industrial areas[11,25]. In this way, industrial activity, for example, reclamation and dredging, sewage discharge, industrial effluents, desalination plants and oil pollution are the significant contributor for the micro-minerals in nature[26]. Consequently, Santubong area is still perfect within the South China Sea locale with low measure of micro-mineral in the environment compared to study conducted on *M. lyrata* from Vietnam[19] and China[23,24]. Undeveloped region and government part to gazette Santubong as National Park is perhaps one of the commitment to this perfect natural surroundings. In any case,

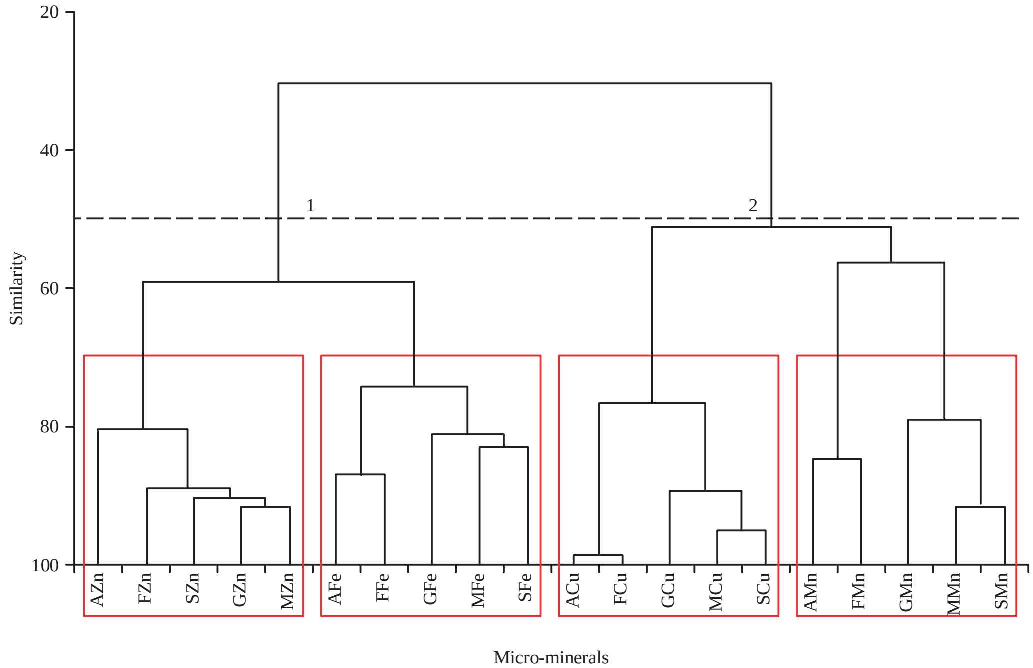

Fig. 6: Hierarchical cluster of micro-minerals composition in *M. lyrata* tissues
A: Adductor, F : Foot, G: Gill, M: Mantel, S: Siphon

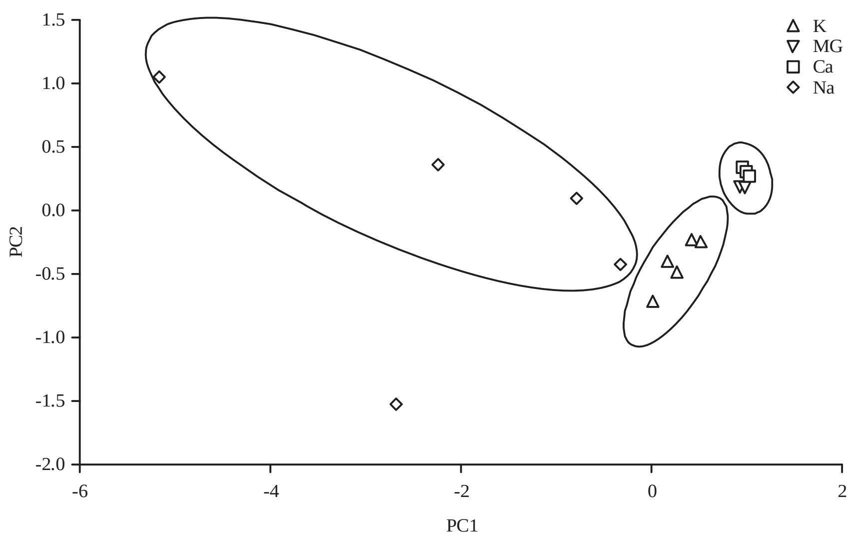

Fig. 7: Principal Component (PC) analysis on micro-minerals in each *M. lyrata* tissue

minerals uptake rate by bivalve relies upon species which is *Anadara granosa*, *Tridacna squamosa* and *Crassostrea gigas* have more contrasted with *Meretrix* sp.[27].

CONCLUSION

Measurable examination uncovered that residue level of macro and micro-minerals not compared to the tissue sort. This proposes that accumulation of component in the *M. lyrata* tissues identified with the measure of component that is present in nature. Along these lines, unblemished environment provides beneficial components that collected in clam tissue which were imperative for human body, for example, Na and Fe, while contaminated zone may contain high substantial metal stored in the shellfish tissue that is harmful for human consumption.

SIGNIFICANCE STATEMENT

This study discovers the prominent minerals found in the *Meretrix lyrata* tissues that can be beneficial as source of nutritious food. This study will help researchers to uncover critical areas related to the bioaccumulation in marine bivalve that many researchers were not able to explore. The accumulation of particular minerals in specific tissue perhaps helps to regulate the physiological activity in bivalve. Thus, a new theory on minerals accretion in shellfish may be emerged.

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