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SỰ TÍCH LŨY KIM LOẠI NẶNG Ở MỘT SỐ LOÀI NHUYỄN THỂ TẠI VÙNG BIỂN KHÁNH HÒA, VIỆT NAM

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Tóm tắt: Nghiên cứu bao gồm việc xác định thành phần một số kim loại không ảnh hưởng đến sinh vật như sắt (Fe), nhôm (Al) cũng như hàm lượng các kim loại nặng độc hại khác như arsen (As), đồng (Cu), cadmium (Cd), crôm (Cr), chì (Pb) và kẽm (Zn) trong các mẫu trầm tích cũng như thịt của một số loài nhuyễn thể. Mẫu các loài nhuyễn thể cũng như mẫu trầm tích được lấy tại 4 điểm thuộc vùng ven biển từ đầm Nha Phu (thuộc huyện Ninh Hòa) tới đầm Thủy Triều (thuộc huyện Cam Lâm) vào tháng 9/2012. Mẫu 4 loài nhuyễn thể phi cái (*Glauconome virens* (Linnaeus, 1767), phi đực *Laternula anatina* (Linaeus, 1758), ngao đen *Katelysia hiantina* (Lamarck, 1818) và sò lông *Anadara antiquata* (Linaeus, 1758) được mang về phòng thí nghiệm, sấy khô trong 24 giờ ở 60^oC, nhiệt phân hủy với hỗn hợp axit HNO_3 (69%) và HCL (36,46%) và phân tích các kim loại nặng bằng máy khối phổ plasma cảm ứng ICP-OES. Hàm lượng nhôm (Al) và sắt (Fe) không có sự khác biệt nhiều trong thịt các loài nhuyễn thể khác nhau nhưng rất thấp so với hàm lượng kim loại trong trầm tích. Hàm lượng kim loại nặng As, Cu, Cr, Cd trong các mẫu sinh vật nằm dưới ngưỡng cho phép (MPL) theo tiêu chuẩn của Cục an toàn thực phẩm Mỹ FDA nhưng hàm lượng chì (Pb) và kẽm (Zn) cao hơn 4 lần ngưỡng cho phép của FDA.

Từ khóa: *Tích tụ sinh học Kim loại nặng, Nhuyễn thể, Khánh Hòa*.

BIOACCUMULATION OF HEAVY METAL IN SOME MOLLUSK SPECIES AT THE COAST OF KHANH HOA PROVINCE, VIET NAM

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Abstract: Levels of non-critical metals, Iron (Fe) and/or Aluminum (Al), as well as toxic and relatively accessible elements including Arsenic (As), Copper (Cu), Cadmium (Cd), Chrome (Cr), Lead (Pb) and Zinc (Zn) in soft tissues of seven gastropod and bivalve species (*Bufonaria rana, Isonomon perna, Modiolus vaginus, Crassostrea rivularis, Katelysia hiantin, Anadara antiquata* and *Geloina coaxans)* collected from 8 different stations in the Nha Trang Bay, Khanh Hoa province, Viet Nam were analysed. Tissues samples were dried 24 hours at 60^oC, digested in a mixed HNO_3 (69%) and HCL (36%) solution, and then analysed for heavy metals using ICP-OES. The concentrations of heavy metal in the tissues ranged between 33.8-262.9, 0.80-6.24, 0.12-87.6, 8.28-23.7, and 4.31-3525 (µg/g dry wt) for As, Cd, Cr, Cu and Zn, respectively. Mean concentrations of As, Cu, Cr, and Cd in the selected mollusk species were below the maximum permissible level (MPL) when compared with the FDA guidance document.

Key words: *Bioaccumulation, Heavy metal, Mollusk species, Khanh Hoa*.

I. INTRODUCTION

The pollution of aquatic ecosystems by trace metals is a significant problem, as trace metals constitute some of the most hazardous substances that can bioaccumulation Nguyen Van Khanh & Pham Van Hiep (2009). The bioaccumulation of heavy metals by marine mollusc and other marine organisms may reach many orders of magnitude above background concentrations of certain locality. This phenomenon may demonstrate the potential of these species as a biomonitor of heavy metal pollution Chan (1989), Hamed & Emara (2006). Many studies have shown that intertidal molluscs can be good biomonitoring organisms Ismail (2006). Due to heavy metals contamination are very localised and closed to discharge point sources (Ismail et al. 1993) and molluscs inhabit in different microhabitat of intertidal areas, a detail studies on different species and tissues are important (Berandah et al. 2010).

A lot of studies on mollusks associated with heavy metal pollution have been done by many researchers (Abdullah et al. 2007), but studies of this kind have been rare in Vietnam due to local difficulties in organizing major studies and legal restrictions regarding the export to fin digenous biologic material (Wagner & Johan 2004) For example, the concentrations of some heavy metals in the tissues of bivalve molluscs have been observed in the samples collected along the coastal of Viet Nam (Le Thi Mui, 2008; Nguyen Van Khanh & Pham Van Hiep, 2009; Tu et al. 2010). However, none of those studies specifically investigated the bioacumulative factor of such a species.

The objective of this study is to provide important information of the metal distribution in the sediments and different mollusk tissues. Thus, helping in proposing the potential bioaccumulation by determining its Biota-Sediment Accumulation Factor (BSAF) (Dallinger et al. 1993) and suggesting bio-monitor for heavy metals in Khanh Hoa coastal.

The work is based on study of samples of sediments and 4 mollusks species (*Glauconome virens* (Linnaeus, 1767), *Laternula anatina* (Linaeus, 1758), *Katelysia hiantina* (Lamarck, 1818) and *Anadara antiquata* (Linaeus, 1758) taken in September 2012 in 4 locations within Nha Phu lagoon (Ninh Hoa) - Nha Trang bay and Thuy Trieu lagoon (Cam Lam). There locations provided wide intertidal zone across the Khanh Hoa coastal in terms of the sandy-muddy areas, represented high community of the bivalve mollusks.

II. MATERIAL AND METHOD

1. Study sites

Khanh Hoa province – central-southern Viet Nam, which is an important ecological zone and providing a wide range of coastal and marine ecosystems in relation to prevailing oceanographic conditions and gradients in mainland – oceanic influence. Compared with some other areas, species composition of molluscs in Nha Trang Bay (490 species) was more abundant than Cat Ba – Ha Long Bay (372 species) and Tonkin Gulf (470 species) Vo Si Tuat et al. (2002).

Sediments and mollusks samples were collected from four sites: Tan Dao (Ninh Loc, Ninh Hoa) – TD (12°02'17"N; 109°08'14"E), Ngoc Diem (Ninh Ich, Ninh Hoa) – ND (12°23'42"N; 109°11'57"E); Binh Tan (Nha Trang) – BT ($12^0$12'22"; $109^0$11'17"E) and Cam Hai Tay (Cam Lam) – CL ($12^0$04'45"N; $109^0$10'42"E). The locations of sampling sites are showing in Figure 1.

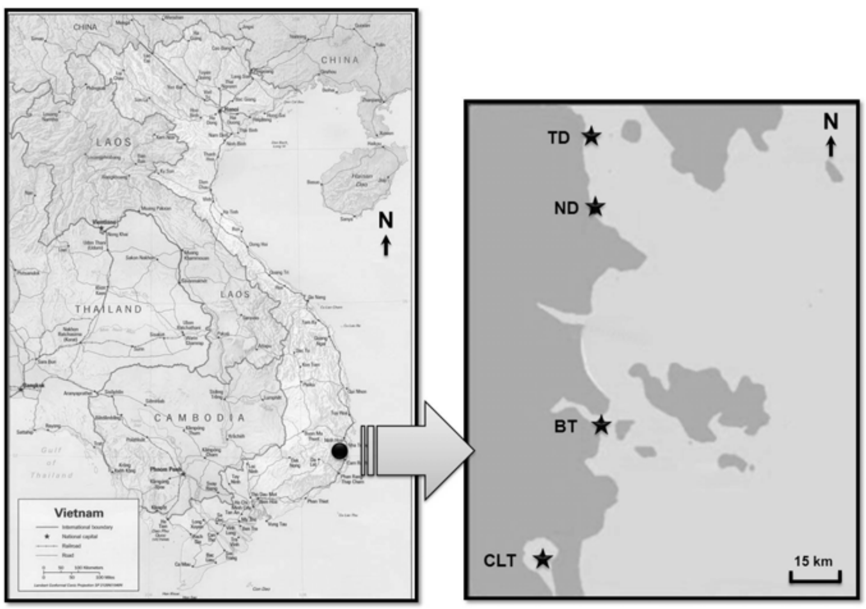


Figure 1: Map and locations of sampling site

2. Sample collection and preparation

2.1 Organisms collection

About 10-20 numbers of the same sizes of each mollusk species (*Glauconome virens* (Linnaeus, 1767), *Laternula anatina* (Linaeus, 1758), *Katelysia hiantina* (Lamarck, 1818) and *Anadara antiquata* (Linaeus, 1758) were picked by hand from four locations and then put in ice box at 4^0C to be transported to the laboratory. The tissues were separated by a plastic knife and dried at 60^oC during two days to constant dry weight. These dried tissues were then stored in a polyethylene bag to be sent in ECOMERS laboratory (UNS-France) for the determination of heavy metals concentrations.

2.2 Sediment collection

The sediment samples of the top 20cm surface centimeter were collected with a polyethylene spool or Ekman grab, then placed in a plastic bag, which were stored in an ice box at 4^0C, to be transported to the laboratory. There sediment samples were dried at room temperature, and then were sieved through a nylon mesh to obtain particles smaller than 0.2mm in diameter for determination of heavy metal concentration. For each sediment sample, 0.1gram was taken for heavy metal analysis.

2.3 Sample digestion and analysis

Sediment digestion

The samples were digested following the guideline from EPA 3050b US EPA (1996) for digestion of sediments using a Microwave 3000. A 0.1 gram of dried and sieved sediment samples were weighed out in the reaction vessel then added with mixed of 9ml concentrated acid HNO_3 (69%) and 3ml of HCl (36,46%). Vessels then were placed in the rotor in the microwave. The vessels were heated to at least 240^{o}C over 35 minutes and then held at 210^{o}C for at least 15 minute. The samples were cooled and mixed to volume to 50 mL with ultra-pure water. The obtained solution was then centrifuged and the concentration of heavy metals measured using ICP-OES (Perkin Elmer). The accuracy of the analytical procedures employed for the analysis of metals in sediment samples was checked using the marine sediment IAEA SP-M1 as certified reference material.

Tissues digestion

The digestion method of organically based matrices used is the one described in the guideline EPA 3052 US EPA (2008). The tissue sample was dried, pulverized and sieved for digestion and determination of metals concentrations. A weight of about 0.5 gram of dry tissue was then transferred in a 100 ml Pyrex beaker and added with 9 ml of acid HNO_3 (69%) and 1ml of HCl (36,46%). Then the beakers were placed on a hot place for reflux extraction at 95^{o}C, for 30min. The digested samples were diluted and filled up with de-ionized water to a total volume of 50ml before metal analyzing. Heavy metals were analyzed by ICP-OES (Perkin Elmer). The accuracy of the analytical procedures employed for the analysis of metals in organic samples was checked using the Fish tissue reference IAEA MA-B-3/TM as certified reference material, and showed good agreement with the certified values.

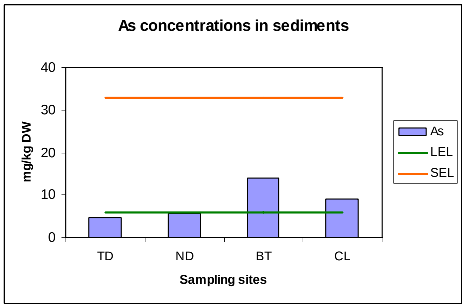
2.4 Data analysis

A mollusk's capacity to accumulate metals from living medium into its tissue can be estimated using Biota-Sediment Accumulation Factor (BSAF). BSAF were calculated for the selected metal in the studied mollusks according to the formula BSAF = Cx/Cs where Cx and Cs were the metal concentrations in the organisms and in associated sediments, respectively (Phillips 1997). By comparing BSAF values, we can compare the ability of those mollusks in taking up metal from sediment. The categories of BSAF are presented as: High potential of accumulation if BSAF>2, Moderate potential if 1<BSAF<2 and Low potential of accumulation if BSAF<1 (Wich & Colvin 1993).

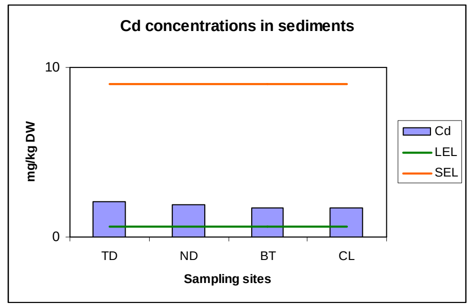
III. RESULTS AND DISCUSSION

1. Sediments quality

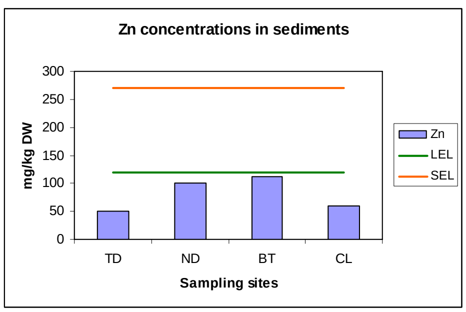
The sediment qualities from the four sites are given in Figure 2.



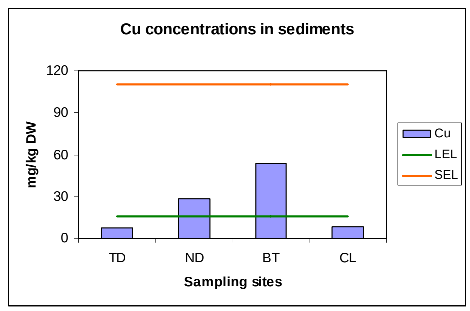
As contents in collected sediments (mg/kg dry wt)



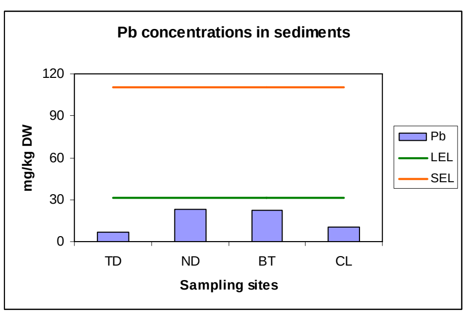
Cd contents in collected sediments (mg/kg dry wt)



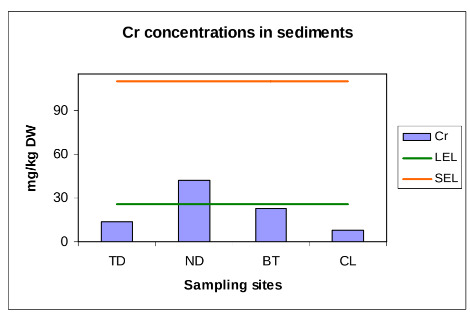
Zn contents in collected sediments (mg/kg dry wt)



Cu contents in collected sediments (mg/kg dry wt)



Pb contents in collected sediments (mg/kg dry wt)



Cr contents in collected sediments (mg/kg dry wt)

Figure 2: Concentrations of heavy metals in collected sediments.

Concentrations of As, Cd, Cr and Cu in collected sediemnts were between LEL (Lowest Effect Level) and SEL (Severe Effect Level) values event the concentrations of Zn and Pb were lower than LEL. It indicated that the coast has been polluted by most of the heavy metal. Indeed, the effect of these sediments is considered to be contaminated, moderate impact to benthic life Fletcher et al. (2008).

2. Heavy metal concentrations in the tissues of 4 studied species

The overall 8 heavy metal concentrations in the 4 analyzed species *Glauconome virens* (Linnaeus, 1767), *Laternula anatina* (Linnaeus, 1758), *Katelysia hiantina* (Lamarck, 1818) and *Anadara antiquata* (Linaeus, 1758), which were collected at 4 locations are included in Table 1 and presented in mg/kg of dry weight.

Table 1. Metal concentrations (mg/kg DW) in tissues of four collected organisms.

Names	As mg.kg^{-1}	Cd mg.kg^{-1}	Cr mg.kg^{-1}	Cu mg.kg^{-1}	Pb mg.kg^{-1}	Zn mg.kg^{-1}	Fe mg.kg^{-1}	Al mg.kg^{-1}
A.antiquata	3,4±1,48	2,72±2,6	3,14±1,9	8,22±1,1	2,53±0,0	48,4±15	879±285	392±134
G.virens	1,7±0,98	1,02±0,9	1,32±0,9	6,96±6,9	6,43±3,9	49,8±13	642±164	398±214
K.hiantina	9,89±4,39	0,24±0,1	4,08±5,1	7,33±2,4	3,52±1,4	34,4±6,5	597±296	388±201
L.anatina	2,72±1,85	0,69±0,2	2,43±0,3	13,5±6,0	8,25±2,0	225±232	1880±112	805±131
FDA	86	4	13	100	1,7	150	-	-

Metal concentrations of Al and Fe in the tissue of the four sites were similar and did not vary significantly and presented very lower compared to concentrations of these metals in the sediments. Concentrations of Al and Fe in the sediments were 20-100 times higher than the concentration in the mollusks. For most of the heavy metals, when mean values were calculated, values were close to FDA maximum permissible level (Rainbow & Phillips, 1993; Rainbow et al., 2000), except for Pb. The Pb concentration in all of samples was more accumulated and was higher than the FDA maximum permissible level (Pb<1.7μg/g dry wt), which eventually may pose hazard to human in term of health risk. The observed concentration of heavy metals in the mollusk samples showed that the magnitude of the heavy metal accumulation in the tissues depend on the type of the heavy metals and the species. There values of BSAF in the different mullusk species are shown in Table 2.

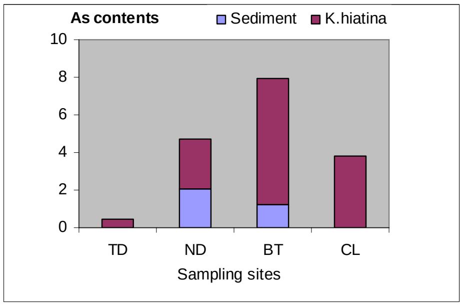
Table 2. The BSAF values in the different mollusk species.

Names mollusks	As	Cd	Cr	Cu	Pb	Zn
Anadara antiquata	0,57±0,55	2,19±2,50	0,39±0,36	1,87±0,92	0,27±0,21	1,09±0,1
Glauconoma virens	9,61±2,63	0,69±0,73	1,19±0,05	4,52±0,32	0,32±0,11	2,08±2,31
Katelysia hiantina	5,01±3,6	4,87±6,76	0,32±0,35	1,38±1,06	0,44±0,29	3,14±3,09
Laternula anatina	12,11±1,67	0,24±0,27	0,44±0,55	0,51±0,44	0,54±0,16	1,95±3,06

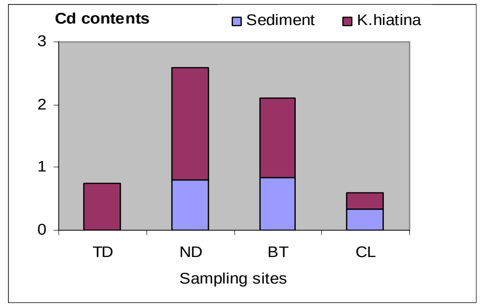
Notes:

High bioaccumulation
Medium bioaccumulation
Low bioaccumulation

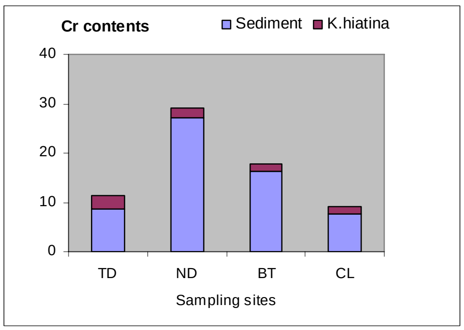
On the other side, when BSAF were calculated, it indicated that different species showed different bioaccumulation of metals levels depending on the geographical location. In general, for all of the studied metals, the BSAF values for As were highest than those for Pb were lowest.



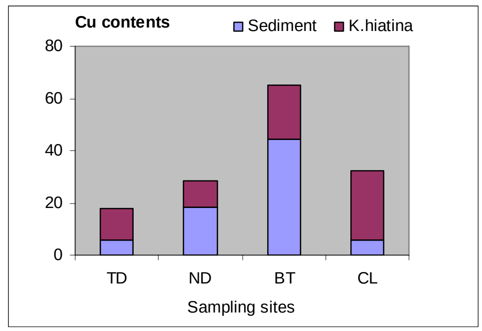
As in tissues and sediments (mg/kg dry wt)



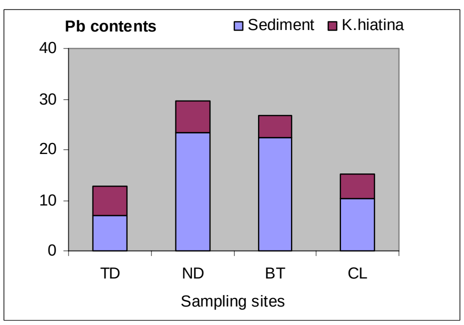
Cd in tissues and sediments (mg/kg dry wt)



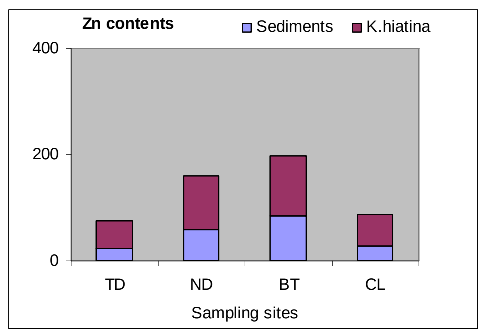
Cr in tissues and sediments (mg/kg dry wt)



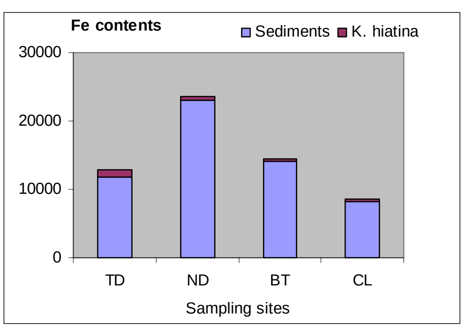
Cu in tissues and sediments (mg/kg dry wt)



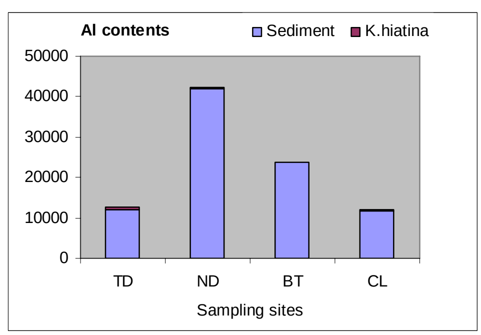
Pb in tissues and sediments (mg/kg dry wt)



Zn in tissues and sediments (mg/kg dry wt)



Fe in tissues and sediments (mg/kg dry wt)



Al in tissues and sediments (mg/kg dry wt)

Figure 3: The contents of metals in soft tissue of the clam and sediments

The BSAF indicated a low potential bioaccumulation for Pb and Cr (BSAF<1) and a very high potential of accumulation for As, Cd, Cu and Zn.

From the results, it was found that clam *Katelysia hiatina* (Lamarck, 1818) highly accumulate As, Cd, Cu and Zn (BSAF>2), and then could be chosen as sensitive indicators for monitoring heavy metal pollutions in Khanh Hoa coastal, Viet Nam.

3. The heavy metal contents in the tissues of *Katelysia hiantina* and sediments

The clam *Katelysia hiantina* (J.B.Lamarck, 1818) also known as other names *Marcia hiantina* and local names "ngao den" or "ngao gia", is an economical important bivalve and can be also represented in Khanh Hoa coast - Nha Trang bay as one of the local abundant mollusca. This clam belongs to the class Bivalve, order Veneroidea in which shell is medium, oval and thick, that live on the loose bottom of water bodies. They are filter feeder and thus very potential to accumulate toxic substances from water and sediment.

The concentrations of metals (mg/kg dry wt) in whole soft tissue of the clam *K.hiantina* and surface sediments from the four sites are given in Figure 3.

It was observed that clam *K.hiatiana* accumulate 8 metals at different concentrations and at different potential of bioaccumulation. The distribution of metals in the tissue of *K.hiatina*, which inhabit in different habitat of intertidal areas, shows that metals contamination are very localized and closed to the anthropogenic influences. The study has concluded that clam *K.hiatina* could be a good bio-indicator of the particular environment through bio-monitoring process.

The higher bioavailability of heavy metals to TD and CL could be explained by the significantly lower organic matter contents, which could potentially affect metal availability, or affect several physiological processes that influence the accumulation of heavy metals by *K.hiatina.*

IV. CONCLUSSION

The coast has been polluted by most of the heavy metal as As, Cd, Cu, Cr. Indeed, the effect of these sediments is considered to be moderate impact to benthic life.
Concentrations of almost heavy metals in 4 species mollusk collected were closed to FDA maximum permissible level except for Pb.
The BSAF indicated a low potential accumulation for Pb and Cr and a high bioaccumulation for As, Cd, Cu and Zn.

Clam *K.hiantina* is represented in study zone as the most local abundant mollusca. This species high accumulate heavy metals and then could be chosen as sensitive indicators for monitoring heavy metal pollutions in Khanh Hoa coastal, Viet Nam.

This preliminary sampling should merit further studies using the BSAF values for assess contamintion in coastal areas by demonstrate the potential of bioaccumulation of mollusk species

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