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Bioaccumulation of some trace elements in tropical mangrove plants and snails (Can Gio, Vietnam)

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ABSTRACT

Mangrove sediments can store high amount of pollutants that can be more or less bioavailable depending on environmental conditions. When in available forms, these elements can be subject to an uptake by mangrove biota, and can thus become a problem for human health. The main objective of this study was to assess the distribution of some trace elements (Fe, Mn, Co, Ni, Cr, As, and Cu) in tissues of different plants and snails in a tropical mangrove (Can Gio mangrove Biosphere Reserve) developing downstream a megacity (Ho Chi Minh City, Vietnam). In addition, we were interested in the relationships between mangrove habitats, sediment quality and bioaccumulation in the different tissues studied. Roots and leaves of main mangrove trees (*Avicennia alba* and *Rhizophora apiculate)* were collected, as well as different snail species: *Chicoreus capucinus*, *Littoraria melanostoma*, *Cerithidea obtusa*, *Nerita articulata*. Trace elements concentrations in the different tissues were determined by ICP-MS after digestion with concentrated HNO_3 and H_2O_2. Concentrations differed between stands and tissues, showing the influence of sediment geochemistry, species specific requirements, and eventually adaptation abilities. Regarding plants tissues, the formation of iron plaque on roots may play a key role in preventing Fe and As translocation to the aerial parts of the mangrove trees. Mn presented higher concentrations in the leaves than in the roots, possibly because of physiological requirements. Non-essential elements (Ni, Cr and Co) showed low bioconcentration factors (BCF) in both roots and leaves, probably resulting from their low bioavailability in sediments. Regarding snails, essential elements (Fe, Mn, and Cu) were the dominant ones in their tissues. Most of snails were "macroconcentrators" for Cu, with BCF values reaching up to 42.8 for *Cerithidea*. We suggest that high quantity of As in all snails may result from its high bioavailability and from their ability to metabolize As.

Keywords: Bioavailability; Bioconcentration factor, Translocation; macroconcentrator.

1. Introduction

Mangroves, complex intertidal forests, are considered as sinks for contaminants including trace metals (Tam and Wong 2000). It was previously suggested that mangrove plants can play key roles in metals removal from mangrove sediments (Alongi et al. 2004, Yang et al. 2008). On the one hand, depending on the metals properties and the mangrove species, they can provide "phytostabilization", meaning that metals are immobilized and stored in the sediment or in the below-ground biomass (MacFarlane et al. 2007). On the other hand, they can provide "phytoextraction", and mangrove trees can be considered as accumulators, providing that they can transfer metals from the sediment and concentrate them in above-ground tissues (Kříbek et al. 2011). Because of different rates in metals uptake and the specific influence of mangrove roots on sediment geochemistry, metals dynamic in mangrove sediments may be affected by the composition of plant communities (Verkleij and Schat 1990). In fact, some mangrove trees can oxidize the sediments via the movement of oxygen from their rhizosphere downwards to aerenchyma tissues (Moorhead and Reddy 1988). This oxidation process can remobilize the stable forms of metals, e.g. the ones bound to sulphides (Marchand et al. 2006, Noël et al. 2015), thus increasing metals' bioavailability. The bioaccumulation and/or fixation of trace metals in mangrove plant tissues, especially roots which can then redistribute metals proportion into aboveground tissues (i.e. especially leaves, see review of (Weis and Weis 2004)), may limit metals concentrations in the water column and restrict the transfer of these contaminants into mangrove biota. However, the excess of essential and non-essential trace elements could affect the growth, metabolism activities and cell structure of plants (Cox and Hutchinson 1981, Wang et al. 2003).

Bioaccumulations of trace elements by snail species have been addressed in many research projects in the past two decades (Berandah et al. 2010, Dias and Nayak 2016, Reed-Judkins et al.

1997). The variations of trace metals concentrations in snails depend on their feeding regime, their digging activities, and metals bioavailability within their habitat. Snails can accumulate higher metals concentrations than any other groups of mollusks (Zhou et al. 2008) and can offer the possibility to assess metals contaminations (De Wolf and Rashid 2008, Samsi et al. 2017, Yap and Cheng 2013). It was demonstrated that elevated trace metals concentrations in sediments can influence negatively the number of snail species in an ecosystem as well as the community structure (Amin et al. 2009).

In Vietnam, an emerging country, the fast economic development (i.e. urbanization, industrialization, etc.) and the population growth induce high pressure on rivers and estuaries (Babut et al. 2019, Costa-Boddeker et al. 2017, Strady et al. 2017). Can Gio estuary is located at the edge of the biggest industrial city in Vietnam-Ho Chi Minh City (*i.e*. a megacity of almost 10 million inhabitants), and half of the Can Gio area is covered by mangrove forests. This estuary is also a unique gate for drainages of sewages from the land to the ocean. Recently, Thanh-Nho et al. (2018) highlighted that metals can be transferred over long distance from watersheds to the mangrove forest, and that elevated inputs of metals in the estuary were the result of enhanced runoff and soil leaching during the monsoon season. It was proved that the enrichment of mangrove-derived organic matter played a key role in controlling partitioning and availability of trace elements in the sediment beneath different mangrove species (Thanh-Nho et al. 2019). Consequently, taking into account mangrove specific geochemical characteristics, the lack of wastewater treatment plants in emerging countries as Vietnam and the important local ecosystem services provided by the Can Gio Mangrove (Cormier-Salem et al. 2017, Kuenzer and Tuan 2013), more attention should be paid on metals bioaccumulation and transfer in mangrove ecosystems. Because of their persistence, trace metals may present a major threat to the mangrove diversity

and also to human health via food chain. Notably, the Can Gio mangrove is home to high biodiversity with 20 species of flora, in which two mangrove species are dominant: *Avicennia alba* and *Rhizophora apiculata* (Luong et al. 2015), and with more than 200 species of fauna (e.g. benthic organisms, fish, mollusks, planktonic, etc.). Thus, the knowledge of trace elements' contents accumulated by different mangrove organisms would be useful to get a better understanding on the behaviors of these contaminants in the mangrove, and to contribute to an important information on the protected species of tropical mangroves in Vietnam and their potential element poisoning.

The main objective of this study was to assess the distribution of some trace elements (Fe, Mn, Co, Ni, Cr, As, and Cu) in the tissues of different mangrove plants and snails receiving elevated amounts of contaminants from watersheds, i.e. lateritic soils and Ho Chi Minh City. In addition, we were interested in the relationships between the bioaccumulation in the different tissues studied, mangrove stands, and sediments quality, bioconcentration factors were calculated using data published in a previous paper and concerning trace metals dynamics in the sediment of the studied mangrove stands (Thanh-Nho et al. 2019). To reach our goals, we collected and analyzed different parts (i.e. roots and leaves) of the main mangrove trees (i.e. *Avicennia alba* and *Rhizophora paticulata)* in the Can Gio Biosphere Reserve, and different snail species within mangrove stands: *Chicoreus capucinus (C.capucinus)*, *Littoraria melanostoma (L.melanostoma)*, *Cerithidea obtuse (C.obtusa)* and *Nerita articulata (N.articulata)*. The field sampling was conducted at the end of the monsoon season in 2015, since we showed previously that elements inputs and sediment reactivity were highest at this period, and since our main objective was to compare the two main mangrove stands.

2. Materials and methods

2.1. Study area

The present study was conducted in the Can Gio mangrove Biosphere Reserve, Vietnam (10º22'-10º44'N and 106º46'-107º01'E, Fig. 1). This mangrove covers approximately 35,000 ha (Tuan and Kuenzer 2012), being usually classified as a "Mangrove afforestation and re-forestation area" (Blasco et al. 2001). It is also a district of the densely populated megacity of Ho Chi Minh City (HCMC), with almost 10 million of inhabitants. The Can Gio mangrove is situated 35 km downstream of the city urban center and industrial zones (mainly plastic and rubber production, mechanical engineering, electrical engineering, packaging, textile and dyes industry, oil activities and cement production) (Strady et al. 2017, Vo 2007). The main economic activities of the local people in Can Gio are aquacultures, salt production, fishing, and forest management. The topography of Can Gio mangrove is generally low-lying. This coastal area is subject to an asymmetric semi-diurnal tidal regime and to the typical tropical monsoon climate, with two distinct seasons. The dry season extends from November to April and the wet season lasts from May to October. The climatic conditions were not much different during consecutive years, from 2014 to 2016, prior and after sampling campaign (data from HCMC statistical Years Book, 2016). The highest precipitation can reach up to 500 mm in September or October, while it is usually less than 80 mm per month during the dry season. The annual mean precipitation ranged from 1,800 to 2,000 mm, with almost 90 % of the precipitation falling during the rainy season. The annual mean temperature varied from 28.4 ºC to 30 ºC while the annual humidity ranged from 72 to 74 %. The two dominant mangrove species are *Avicennia alba* and *Rhizophora apiculata.* Because of high commercial values, *Rhizophora apiculata* was widely replanted, being often found on elevated ground. *Avicennia alba* is a pioneering species with high salinity tolerance and ability to grow on weak, unconsolidated sediment. Consequently, there is a zonation of the ecosystem (Fig. 1) with

Rhizophora trees growing at higher elevation that the *Avicennia* ones, and being thus less immerged by tides. Many marine organisms live within and around the mangroves, e.g. shrimp, fish, snail, or crab, etc. Therefore, the mangrove ecosystem provides not only various goods and services to local people such as timber, seedlings, medicines, but also foods (Kuenzer and Tuan 2013).

2.2. Field sampling

The sampling campaign was carried out at the end of the wet season (October 2015) in the center area of the Can Gio mangrove (Fig. 1).

Leaf and root samples were collected from different types of trees (saplings and mature trees of the *Rhizophora apiculata* and *Avicennia alba* species). For both species, each leaf sample consisted of 30 leaves collected from 15 trees, and we analyzed 3 samples per each type of tree. Root samples were collected in the upper 50 cm from sediment cores collected using an Eijkelkamp gouge auger (inner diameter: 80 mm) at low tide (n = 3 per each type of tree). All samples were rinsed with deionized water and were then dried at 50 ^{o}C for > 48 h to constant mass in an oven. Dried samples were then ground and sieved using 100 µm pore size for trace elements analysis.

Four species of snails were collected in the mangrove stands: *C.capucinus*, *L.melanostoma*, *C.obtusa* and *N.articulata*. Notably for *L.melanostoma*, they were collected on mangrove trees. In the *Rhizophora* stand, due to their excessive height (> 15 m), *L.melanostoma* were collected on young trees. In the *Avicennia* stand, due to low density of saplings, *L.melanostoma* were collected on mature trees. For each species, we analyzed 3 samples per mangrove stand, and each sample comprised 15 to 20 snails. Their shell sizes (length and width) were measured prior to taking out their tissues by pegging out (using a clean pestle) the shell carefully. Due to the fragile

characteristic of the shell, a mild force was sufficient to break the shell (strong force might destroy the internal organ of the snail). Snails were chosen in the same range of size throughout the work to reduce possible variations in trace elements concentrations due to size (age). The whole bodies were preserved at -18 ^{o}C until performing processes of freezer-drying. Dried samples were ground and sieved through 100 µm pore size for analysis of trace elements. Snails' soft tissues are food sources for local people, and thus their trace elements contents may be a risk to human health. Therefore, only the soft tissues of snails were analyzed in the present study.

Specific features of selected snail's species

C.capucinus has elaborately textured shell with uniformly dark brown and six convex whorls. These shells are heavy and solid and can reach a size of 40 to 120 mm. They are sculptured with prominent spiral cords, axial ribs and striae. The aperture is rounded or oviform, brown tinged and the inner labial edge show 14 to17 denticles. The siphonal canal is quite long. The operculum is dark brown.

L.melanostoma can reach 2 to 3 cm length. Shell has a narrower tip, smooth, yellowish to moss green or dark brown with fine spiral pattern of brown dots. Sometimes the shell is bleached white. "*Melanostoma*" means "black mouth". There is short fat tentacles on soft head.

C.obtusa has Obtuse Horn Shell (large, solid, broad, periphery rounded; aperture strongly thickened and flared; 14 to 37 rounded axial ribs on penultimate whorl; 5 spiral cords on spire, 5 to 9 above periphery on last whorl). Shell is also known as Mud Creeper. It is a relatively common snail found in muddy coastal areas, growing to around 5 to 6 cm (Suwanjarat and Klepal 2001).

N.articulata is also known as *Nerita lineata* or *N. balteata*, with 2 to 3 cm length. Shell is sturdy oval, spire does not stick out characterized by beige, greyish or pinkish with fine, spiralling and black ribs. The flat underside is smooth, white, sometimes with yellow patches. Small notched

'teeth', usually three, on the straight edge at the shell opening. Operculum thick, evenly covered in tiny bumps, pinkish with black portions. Body pale with fine black bands on the foot and long thin black tentacles (data from wildsingapore).

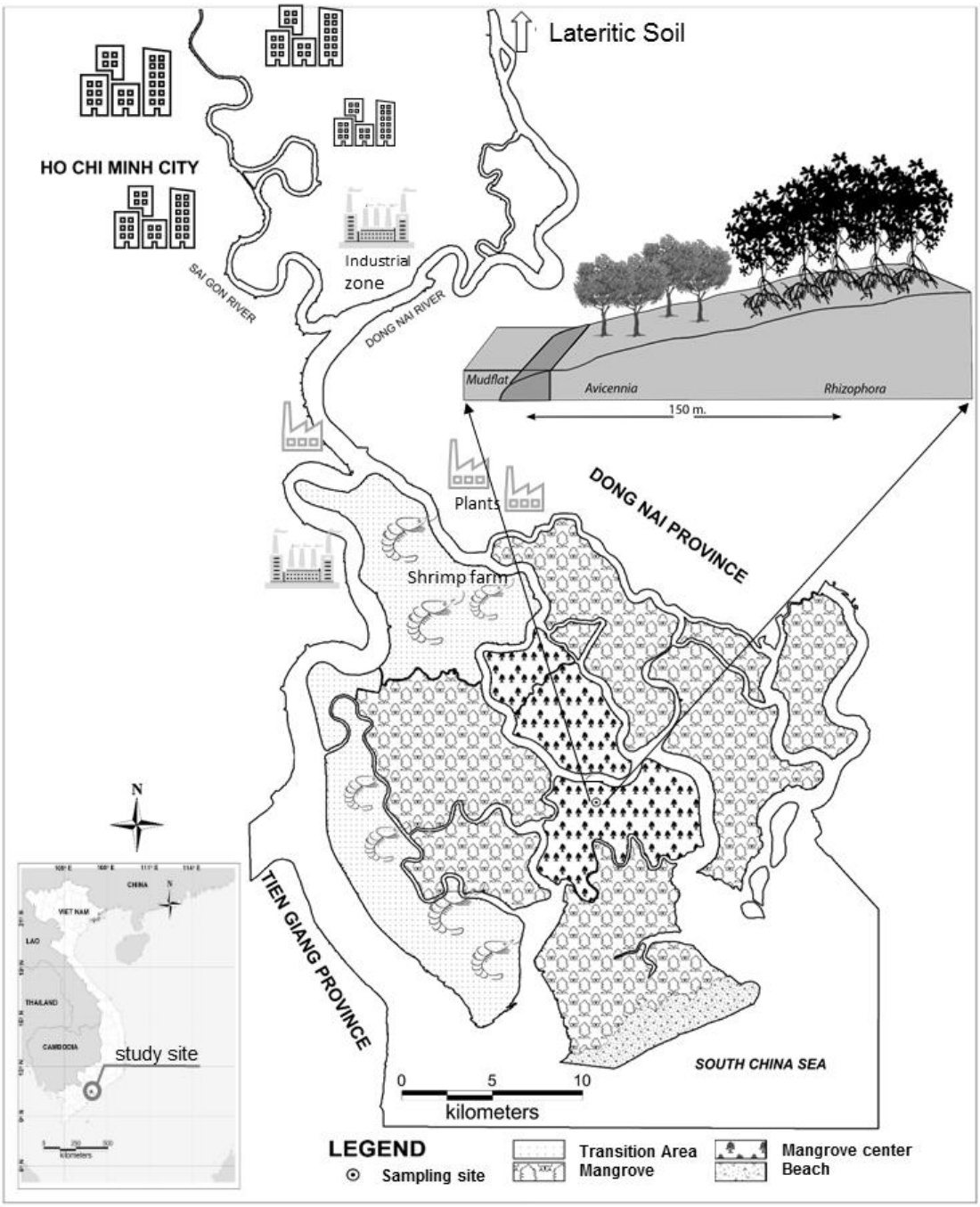

Fig. 1. Map of the study area showing the location of the Can Gio mangrove in Vietnam and the location of the collected biological samples in the mangrove.

2.3. Analytical methods and calculations

2.3.1. Samples digestion and analysis

The plant and snail samples were digested in triplicate with concentrated nitric acid and hydrogen peroxide (MacFarlane et al. 2003). The samples (250 mg to 500 mg of dried weight) were put into PTFE vessels, in which 10 mL of concentrated HNO_3 was added. These samples were homogenized in an ultrasonic bath for 15 min and were then digested at 110 °C for 12h on an electrical oven. After cooling, 2 mL of H_2O_2 was added into these samples, which were again digested at 110 °C for 30 min. The residual HNO_3 was eliminated at 160 °C. The samples were centrifuged to reject any residues and then diluted to 25 mL using deionized water and stored at 4 °C until analysis. The trace elements were determined by ICP – MS (Agilent 7700x at Institute of Public Health, Ho Chi Minh City, Vietnam), using spiked ^{103}Rh and ^{197}Au as internal standards. The precision and accuracy of analytical method were controlled using certified reference material muscle tissues (SRM-2976: certified by National Institute of Standard and Technology/NIST, USA). The recoveries of trace elements were 94 % to 106.5 % with relative standard deviation from 4.2 % to 9.8 % (Table SD1). All chemicals were analytical grade (Merck). HNO_3 was purified using a sub-boiling quartz distillation equipment.

2.3.2. Data calculations

Bioconcentration factor (BCF) was proposed by Babukutty and Chacko (1995) to assess the bioaccumulation of pollutants into an aquatic and terrestrial organisms via any route i.e., by active or/and passive accumulations. It is defined as $BCF = C_{tissues}/C_{sediment}$, where $C_{tissues}$ and $C_{sediment}$ are total concentration of trace element in organism tissues and total concentration of trace element in the sediment, respectively. To do this calculation, we used the trace elements concentrations in the

sediment that we published in a previous paper (Thanh-Nho et al. 2019); sediment samples were collected at the same sites and at the same period of the present study.

BCF of trace elements in roots and leaves

BCF was calculated from a mean concentration of trace element in sediment down to 50 cm depth (Table SD2). This depth was chosen because in mangrove environment, the highest root density is in the upper sediment, between 20 and 50 cm depth (Ha et al. 2018, Komiyama et al. 2000, Tamooh et al. 2012).

Translocation factors (TF) was calculated, which are used to assess the transfer of trace elements from roots to aboveground components and to evaluate the potential phytoextraction of plants (Marchiol et al. 2004). In the present study, it is defined as $TF = C_{leaves}/C_{root}$, where C_{leaves} and C_{root} are total concentration of trace element in leaves and total concentration of trace element in roots, respectively.

BCF of trace elements in snails

Depending on feeding regimes of individual snail species and their habitat, the BCF was calculated from a mean concentration of trace element corresponding to a mean concentration of trace element in each type of feeding source. *L.melanostoma* feeds on mangrove trees, mainly fresh leaves and materials on the plant surfaces as phylloplane and fungi (Lee et al. 2001). BCF of trace element was estimated based on the mean concentration of trace element measured in *Avicennia* and *Rhizophora* leaves. *C.obtusa* is known as sediment eater (Tue et al. 2012), BCF of trace element was calculated based on a mean concentration corresponding to a mean concentration of trace element in available fractions at the upper 5 cm of the sediment (Table SD2). *N.articulata* eats microalgae (Eichhorst 2016), and *C.capucinus* is a versatile predator, feeding on the barnacles growing on mangroves and on mussels, snails and worms in their habitat (Tan and

Oh 2003, Tan 2008). We were not able to sample their feeding sources, consequently, we did not calculate any BCF for two snails' species.

3. Results

3.1. Trace elements in roots and leaves of mangrove plants

Mean concentrations of Fe, Mn, Co, Ni, Cr, As, and Cu (μg g^{-1}) in the roots and leaves of the *Avicennia alba* and the *Rhizophora apiculata* are presented in Table 1. Trace elements in roots of the *Avicennia* saplings and mature trees presented a similar distribution: Fe > Mn > Cu > Cr > As > Ni > Co. For the roots of the *Rhizophora* trees, the concentrations of trace elements were Fe > Mn > As > Cr > Cu ~ Ni > Co in the saplings, and were Fe > Mn > Cr > Ni ~ Cu > As ~ Co in the mature trees. Most trace elements were characterized by low values of BCF in the roots, i.e. less than 1, except for Cu and As for the roots of *Avicennia* saplings (i.e. BCF: 1.02 for As and 2.95 for Cu) (Fig. 2). Leaves of the *Avicennia* saplings were characterized by the following trace elements concentrations: Fe > Mn > Cu > Cr > Ni > Co ~ As, while in the mature trees, the distribution was Mn > Fe > Cu > Cr > Ni > Co ~ As. Mn presented higher concentrations in the leaves of mature trees than in the saplings. For leaves of the *Rhizophora*, the concentration of trace elements were Fe > Mn > Cr ~ Cu > Ni > Co ~ As in the saplings, and were Fe > Mn > Cu > Cr > Ni > As ~ Co in the mature trees. Mn and Cu concentrations were higher in the leaves of the mature trees than in the saplings. The bioconcentration factors were lower than 1 for most trace elements in both mangrove species, with the exception of Mn in saplings and mature trees (Fig. 2). The translocation factors (TF) were higher than 1 for Mn, Cu in the *Rhizophora* saplings and mature trees, for Cr in the *Rhizophora* saplings, while it was lower than 1 for the other trace elements studied (Fig. 3).

Table 1. Mean concentrations of trace elements (n = 3) in the roots and leaves of the Avicennia alba and the Rhizophora apiculata, expressed in µg g^{-1} (mean, SD). The roots of trees were collected in the upper part of 50 cm depth in the sediments.

Roots

	Depth (cm)	Type of trees	Fe	Mn	Co	Ni	Cr	As	Cu
Avicennia	< 50	Saplings	**14,948 ± 239**	**376 ± 4**	2.8 ± 0.06	5.1 ± 0.16	17.8 ± 0.11	**11.9 ± 0.12**	**54.7 ± 1.8**
	< 50	Mature trees	7,433 ± 308	**217 ± 1.7**	5.0 ± 0.13	5.8 ± 0.09	8.9 ± 0.42	7.5 ± 0.19	10.2 ± 0.29
Rhizophora	< 50	Saplings	**19,897 ± 1,265**	60 ± 1.2	2.4 ± 0.05	5.0 ± 0.11	8.8 ± 0.25	**12.7 ± 0.15**	6.3 ± 0.12
	< 50	Mature trees	5,458 ± 145	57 ± 1.1	2.0 ± 0.01	5.4 ± 0.08	11.9 ± 0.33	2.0 ± 0.05	4.7 ± 0.13

Leaves

	Height of tree	Type of trees	Fe	Mn	Co	Ni	Cr	As	Cu
Avicennia	< 80 cm	Saplings	**2,287 ± 39**	583 ± 5	1.43 ± 0.01	3.18 ± 0.28	7.31 ± 0.22	0.61 ± 0.02	**22.14 ± 0.30**
	> 7 m	Mature trees	359 ± 21	**908 ± 20**	0.29 ± 0.10	1.09 ± 0.14	2.13 ± 0.29	0.30 ± 0.03	8.93 ± 0.30
Rhizophora	< 80 cm	Saplings	**1,416 ± 50**	267 ± 3	0.48 ± 0.02	1.60 ± 0.02	13.19 ± 0.79	0.40 ± 0.01	10.63 ± 0.55
	> 15 m	Mature trees	498 ± 21	**405 ± 38**	0.07 ± 0.03	0.70 ± 0.25	3.72 ± 0.30	0.14 ± 0.03	15.92 ± 0.80

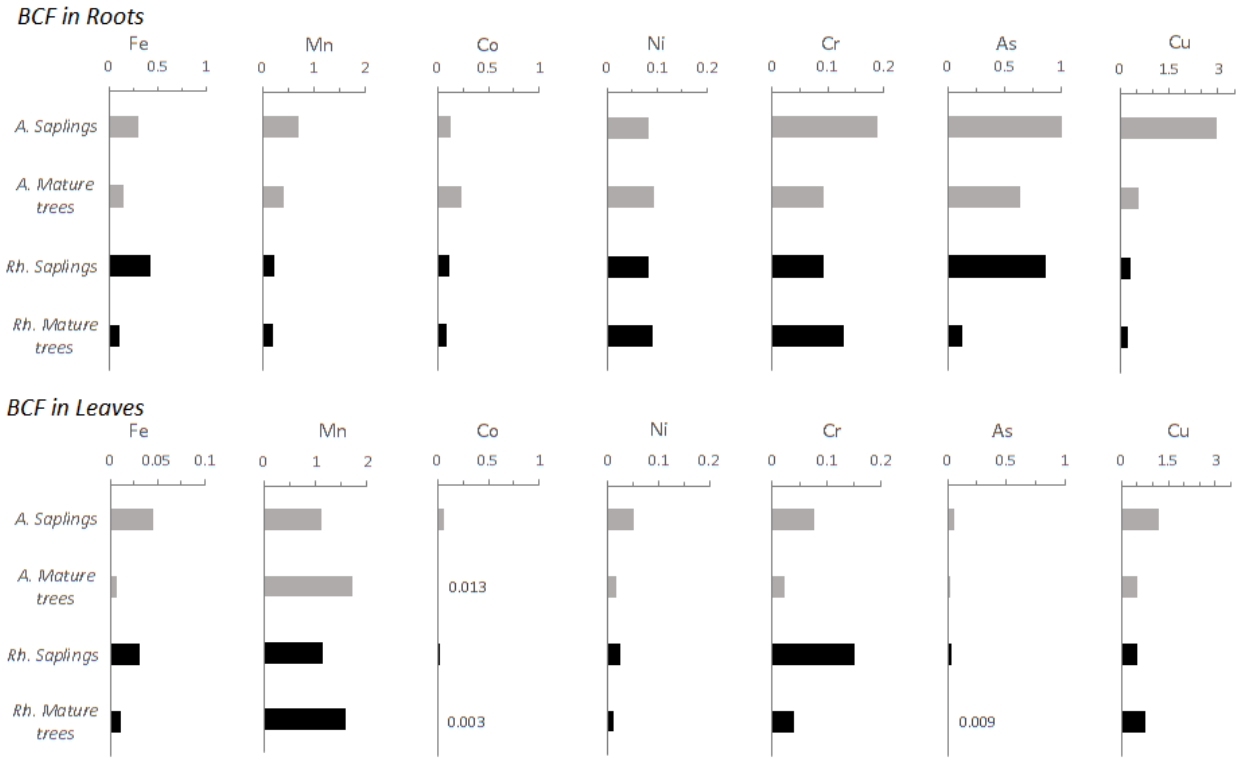

Fig. 2. Bioconcentration factors (BCF = concentration of trace element in tissues/ concentration of trace element in sediment) of the Avicennia alba (gray column) and the Rhizophora apiculata (black column).

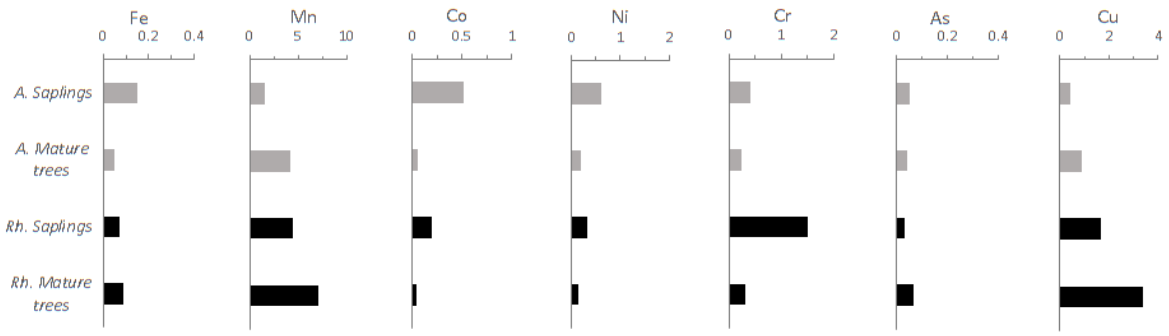

Fig. 3. Translocation factors (TF = concentration of trace element in leaves/ concentration of trace element in roots) of the Avicennia alba (gray column) and the Rhizophora apiculata (black column).

3.2. Trace elements in snails

Mean concentrations of Fe, Mn, Co, Ni, Cr, As, and Cu (µg g^{-1}) in snails' soft tissues are given in Table 2. The *C.capucinus* and *N.articulata* presented similar trace elements concentrations beneath two mangrove stands: Fe > Cu > Mn > As > Ni ~ Cr ~ Co for *C.capucinus* and Fe > Mn > Cu > As ~ Ni ~ Cr > Co for *N.articulata*. *L.melanostoma* and *C.obtusa* exhibited higher concentrations in Fe, Mn and Cu than As, Ni, Cr, and Co, and concentrations differed between mangrove stands (*Avicennia alba* and *Rhizophora apiculata)*. Bioconcentration factors of all trace elements are presented in Fig. 4. The BCF of each trace element varied between snail species, ranging from 0.02 to 1.58; 0.6 to 1.13; 0.1 to 5.09; 0.56 to 2.9; 0.04 to 0.3; 0.85 to 20.1 and 7.7 to 42.8 for Fe, Mn, Co, Ni, Cr, As, and Cu respectively.

Table 2. Concentrations of trace elements in soft tissues of various snails (expressed in µg g^{-1}): C.capucinus (predator), L.melanostoma (leaves eater), C.obtusa (sediment eater) and N.articulata (algae eater).

Mangrove stand	Species	Length (cm)	Width (cm)	Fe	Mn	Co	Ni	Cr	As	Cu
Avicennia	*C.capucinus*	2.5 - 4	1 - 1.5	536 ± 40	139 ± 10	0.88 ± 0.11	1.52 ± 0.16	1.11 ± 0.17	**10.9 ± 1.1**	**481 ± 38**
	L.melanostoma	1.5 - 2.5	1 - 2	**714 ± 95**	**766 ± 29**	1.04 ± 0.03	2.14 ± 0.08	0.61 ± 0.12	3.64 ± 0.17	120.6 ± 9.1
	C.obtusa	2 -4	1 - 1.5	360 ± 44	**780 ± 81**	2.64 ± 0.26	5.93 ± 0.44	0.70 ± 0.11	4.32 ± 0.88	113.9 ± 5.7
	N.articulata	2 - 2.6	1.5 - 2	271 ± 26	42.5 ± 2.6	0.20 ± 0.13	1.16 ± 0.15	0.90 ± 0.25	2.83 ± 0.22	7.84 ± 0.65
Rhizophora	*C.capucinus*	3 - 4	1.5 - 2.5	885 ± 80	119 ± 11	1.14 ± 0.03	2.07 ± 0.12	1.27 ± 0.31	**14.3 ± 2.2**	**389 ± 13**
	L.melanostoma	1.5 - 3	1 - 2	**1,517 ± 120**	**201 ± 20**	1.41 ± 0.02	3.34 ± 0.30	2.5 ± 1.3	5.39 ± 0.25	163 ± 17
	C.obtusa	3 - 4	1 - 1.2	338 ± 29	**158 ± 16**	0.99 ± 0.09	6.15 ± 0.74	1.58 ± 0.41	6.95 ± 1.15	120 ± 20
	N.articulata	1.8 - 2.5	1.2 - 1.5	795 ± 82	96 ± 14	0.88 ± 0.27	3.58 ± 1.74	5.99 ± 1.43	6.45 ± 1.69	16.6 ± 0.9

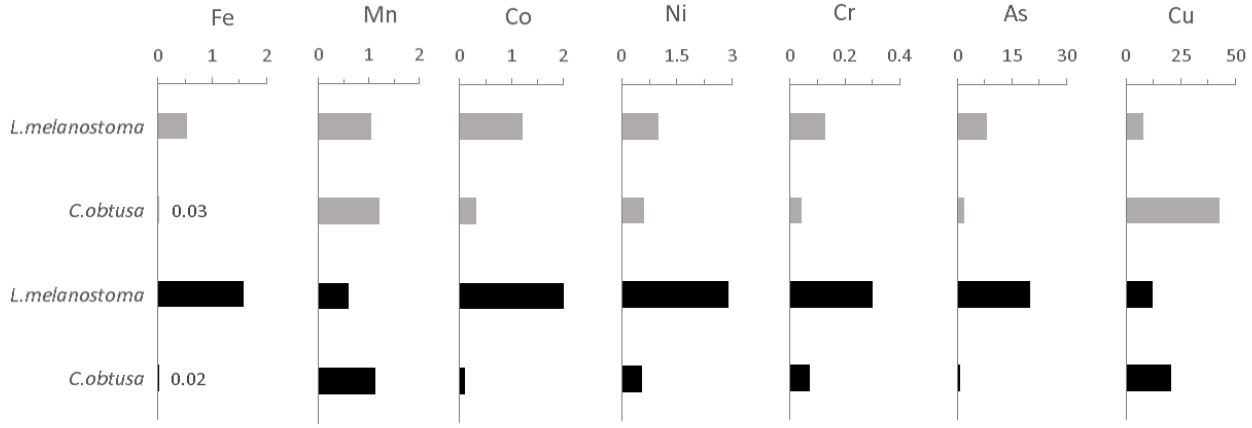

Fig. 4. Bioconcentration factors (BCF = concentration of trace element in soft tissues/ concentration of trace element correspond to its concentration in feeding of individual species) of various snails living in the Avicennia alba (gray collum) and the Rhizophora apiculata (black column).

4. Discussions

4.1. Bioaccumulation of trace elements in roots and leaves of Avicennia alba and Rhizophora apiculata

4.1.1. Essential elements: Fe, Mn, Cu

Iron is an important component of chlorophyll, being useful for protein synthesis and root growth (Jones Jr et al. 1991). In the present study, despite low bioconcentration factors (BCF), Fe was the most abundant element in the roots whatever the mangrove species, reaching up to 19,897 µg g^{-1} (Table 1). These mean concentrations of Fe in the plants' roots of the Can Gio mangrove were substantially higher than in most mangroves, like for instance in Indian mangroves (Kathiresan et al. 2014). We suggest that the high concentrations of measured Fe in the roots resulted from the high iron concentrations in the sediments (Table SD2) and in pore-waters (Thanh-Nho et al. 2019) (e.g. where it can be highly bioavailable (Abohassan 2013)), as observed for *Avicennia marina* and *Rhizophora stylosa* in New Caledonian mangroves, where the mangrove sediments are rich in iron because these ecosystems develop downstream lateritic soils (Marchand

et al. 2016). However, if the Fe concentrations were high in the roots, the translocation factors to the leaves were low for this element (Fig. 3), suggesting that the root acted as a barrier preventing iron translocation to aerial parts. Machado et al. (2005) suggested that the seedlings of mangrove species can exclude some trace elements through iron plaque formation on the roots. The oxygen released by the roots of some mangrove species may promote oxidizing conditions within the rhizosphere, which results in trace elements precipitation at the root surface, creating iron-rich root coatings, generally called iron plaques (Chaudhuri et al. 2014, Koch and Mendelssohn 1989, Zhou et al. 2011). Machado et al. (2005) also demonstrated that the washing of the roots prior to analysis influences the fixation of the iron plaque on the roots: in contrary to distilled water, the dithionite–citrate–bicarbonate (DCB) solution is able to extract the iron plaque from the roots. Considering that we only washed the roots with deionized water, the presence of iron plaques at the root surface may be a possible explanation for the elevated iron concentrations in the plants' roots in the Can Gio mangrove. For the mature trees, iron concentrations were higher in the *Avicennia* roots than the *Rhizophora* ones, which may be the results of the higher iron concentrations both in the solid and the dissolved phases (Table SD2) in the sediment of the *Avicennia* stand (Thanh-Nho et al. 2019). Also, the *Avicennia* root system is known to release oxygen (Marchand et al. 2004, Scholander et al. 1955), which may increase iron plaque precipitation. Notably, whatever the mangrove species, iron concentrations in saplings leaves presented higher values than the mature ones as a result of higher iron concentrations in saplings roots (Table 1). We suggest that iron plaque may be a source of Fe for plants uptake by bacterial reductive dissolution of plaque during organic matter decay processes on dead roots surface. Additionally, the root system of the saplings may develop in the upper layer, and not as deep as the one of the mature trees. This upper layer is more subject to variations in the redox conditions, and possibly to enhanced alternation of

precipitations/dissolution processes. Consequently, elements, specifically iron, may be more available at the depth of the saplings' roots. Eventually, it is possible that the adaptation strategy of the mangrove trees was not fully developed at the sapling stage, as a result, the role in barrier of the root system may be limited.

Manganese is a major contributor to various biological processes, including photosynthesis (Millaleo et al. 2010). In Can Gio mangrove, whatever the mangrove species, Mn was the second abundant element in their tissues, with average concentrations in the range of those measured worldwide in mangroves (see review of Lewis et al. (2011) and Bayen (2012)). Conversely to Fe, Mn accumulated more in the leaves than in the roots (Table 1), as previously observed in New Caledonia by Marchand et al. (2016). TF values (i.e. maximum TF: 4.08) and Mn concentrations in roots and leaves of mangrove plants measured in New Caledonia were lower than in the present study (e.g. TF reached up to 7.07). We suggest that the high dissolved Mn concentrations measured in the pore-waters and the difference of Mn partitioning in the mangrove sediments may be responsible for the elevated values measured in the present study. Within the top 50 cm of the sediments in the Can Gio mangrove, approximately 60 % of Mn was associated with the bioavailable fractions (Table SD2) whereas in New Caledonia, 90 % of Mn was bound to the refractory fraction, preventing its transfer to mangrove plants (Marchand et al. 2016). In the Can Gio mangrove, Mn was only the element to potentially present an ecological risk due to its high bioavailability (ratio in concentrations of trace element in the bioavailable fractions (exchangeable/carbonate bound) to the total concentrations of trace element) (Thanh-Nho et al. 2019). Since high TF of Mn was already reported for *Rhizophora* species, we suggest that it may be related to metabolic requirements, this element playing an important role in enzymes reactions

is also needed for water splitting at photosystemII (McEvoy and Brudvig 2006). Consequently, *Rhizophora* trees have a high potential for Mn phytoextraction.

Regarding copper, it is known as an essential element for plants growth, being required in enzyme system related to photosystemII electron transport, mitochondria and chloroplast reaction, carbohydrate metabolism, cell wall lignification, and protein synthesis (Yruela 2009). Cu concentrations in the roots and leaves of mangrove plants measured in the present study were in the range of those in other mangroves (Bayen 2012) such as in China (He et al. 2014), in southern Brazil (Madi et al. 2015), or in Indonesia (Martuti et al. 2016). It was reported that the Cu partitioning in sediments of the Can Gio mangrove was mainly related to sulphide precipitation and to its complexes with organic compounds (Thanh-Nho et al. 2019). Thus, we suggest that the low Cu concentrations measured in the mangrove roots may be related to Cu association with sulphide, which limit its bioavailability. However, this element presented the second highest values of TF, beside Mn, with TF reaching 1.69 for *Avicennia* mature trees and 3.37 for *Rhizophor*a mature ones. MacFarlane and Burchett (2002) showed that Cu can be toxic to plants growth if its concentration in the sediments exceeds 400 µg g^{-1}, which is almost 20 fold higher than the concentrations measured in the Can Gio mangrove sediments. Consequently, we suggest that the high values in TF of Cu resulted from specific metabolic requirements (Baker 1981, Dudani et al. 2017) as Cu has a direct impact on photosynthesis I, being a constituent of plastocyanin, which is involved in the photosynthetic electron transport chain (Maksymiec 1998).

4.1.2. Non-essential elements: Co, Ni, Cr, As

Non-essential elements such as Co, Ni, Cr, and As mainly accumulated in the roots (Table 1), with low translocation to the leaves (Fig. 3). This phenomenon may result from different mechanisms developed by mangrove plants to prevent the uptake of toxic elements and to limit

their transport within the components of plants (Almeida et al. 2006), notably through cell wall immobilization and/or sequestration in the epidermal layers (MacFarlane et al. 2007). They, thus, accumulate in the perennial tissues, especially the roots (Carbonell et al. 1998, Zhou et al. 2011).

Concerning Arsenic, some forms can be subject to plant uptakes, involving arsenites-As(III) and arsenates-As(V) (Asher and Reay 1979). However, arsenites can be toxic for radicular membranes because of As reaction with sulfhydryl groups in proteins, causing disruption of roots functions and cellular death (Speer 1973, Wu et al. 2015). In the present study, arsenic concentrations in the roots and leaves of mangrove plants were higher than those of other mangroves like in the Indian Sundarban (Chowdhury et al. 2015), or in the Chinese Futian (He et al. 2014). Arsenic was also characterized by higher BCF in the roots, specifically for the *Avicennia*. These results may be related to the high As concentrations in bioavailable fractions in the Can Gio mangrove sediments (Table SD2). In Vietnam, soils are naturally rich in As (Gustafsson and Tin 1994, Nguyen et al. 2016), which may explain its accumulation in mangrove sediments and then its transfer to mangrove roots. We suggest that As may be incorporated into iron plaque at the roots surface, which restricted As transfer to aerial parts. This hypothesis may be supported by a positive correlation between the concentrations of Fe and As in the roots (i.e. $r = 0.92$, Pearson correlation). Like iron, As was more bioavailable beneath the *Rhizophora* stand, where the sediment was more enrich in organic matter and the oxidizable fraction represented up to 36 % of total As concentrations. However, BCF of As was higher for the *Avicennia* than *Rhizophora* roots, which comforts our hypothesis in enhanced plaque formation beneath the *Avicennia* rhizosphere due to specific redox conditions resulting from oxygen release via the roots system. Similar to Fe, As concentrations in sapling's leaves of both *Avicennia* and *Rhizophora* species were higher than mature ones. We suggest that the As incorporated in iron plaque could be a As source for plant

uptake from plaque reductive dissolution by bacteria during organic matter decay processes of death roots tissues. Arsenic uptake by mangrove leaves may concomitantly occur to Fe (i.e. a positive correlation was observed between concentration of As and Fe in the leaves, r = 0.91, Pearson correlation). Again, the extension of the root system in the upper layer, and possibly the less developed adaptation strategy of saplings may also be responsible for the As transfer to saplings' leaves.

Regarding Ni and Cr, their concentrations in the roots and leaves of *Avicennia* and *Rhizophora* in the Can Gio mangrove were in the range of those presented in the review of Lewis et al. (2011) but lower than those measured in the Indian Sundarban mangroves (Chowdhury et al. 2017) or in New Caledonia (Marchand et al. 2016). In the later environments, the authors reported higher Ni concentrations in plants tissues, with values up to 700 µg g^{-1} due to high Ni concentrations in mangrove sediments resulting from the proximity of lateritic soils enriched in Ni. Thus, we suggest that the moderate Ni and Cr concentrations in the Can Gio mangrove plants may result from their association to the refractory fraction (i.e. more than 80 %, Table SD2). For plants, Ni is a micronutrient that is required at very low concentrations (Gajewska and Skłodowska 2007), and which can inhibit plant growth at high concentrations (Rao and Sresty 2000). The effect of Ni on plants varies according to plant species as well as the Ni concentration in sediment; one example of its toxic symptom is chlorosis or yellowing of the leaves (Mishra and Kar 1974). Cr is also toxic for plant growth (Shanker et al. 2005). When mangrove seedlings are exposed to excessive Cr concentrations, their roots would be shortened, and their height and biomass would be limited (Fang et al. 2008). However, considering the low BCF and the low concentrations of those trace elements in the Can Gio mangrove plants, their impacts on plant growth are probably limited. Cr concentrations in the roots of *Rhizophora* trees were lower although its concentrations in

bioavailable forms were higher than in the *Avicennia* (Table SD2). This phenomenon, supported by higher TF values in the *Rhizophora* trees, may be related to a more intense phytoextraction of *Rhizophora* species. In addition, translocation factor of Cr in the saplings of both *Avicennia* and *Rhizophora* trees were higher than in the mature ones, which may be due to higher allocation of this element to leaf (Weis and Weis 2004) in the saplings. Similar result was also observed for Ni (Fig. 3).

Co is a micronutrient, which can be used in redox processes to stabilize molecules through electrostatic interactions as components of various plant enzymes. However in excess, it can be toxic, inducing enzyme modification, disturbing cellular function (Palit et al. 1994). In the present study, Co concentrations in the roots and leaves of the different mangrove species were higher than in Australian mangroves (Nath et al. 2014), in French Guianan mangroves (Marchand et al. 2006), or New Caledonian mangroves (Marchand et al. 2016). We suggest that high Co concentrations in the Can Gio mangrove plants may result from its high concentrations in the bioavailable fractions in the sediments, reaching up to 44 % (Table SD2). However, if Co concentrations in the roots were relatively high, TF were lower than 0.5 (Fig. 3), which suggest its limited physiological role and/or the fact that the roots acted as a physical barrier. As a consequence, the present study showed a narrow range of Co level in the leaves (i.e. 0.07 to 1.43 $\mu g\ g^{-1}$). The BCF values of Co in the tissues of *Avicennia* were much higher than in the *Rhizophora*, which may reflect the specific physiological requirements of the trees, considering that its bioavailability was higher beneath *Rhizophora* stand (Table SD2).

4.2. Bioaccumulation of trace elements in snails

In the Can Gio mangrove, whatever the snails' species and their habitat, the concentrations of Fe, Mn and Cu were the most abundant in their soft tissues (Table 2). Previous studies showed that

soft tissues of mollusks accumulated higher concentrations of Cu and Fe than in the shells because of specific physiological requirements (Szefer et al. 1999, Vukašinović-Pešić et al. 2017, Yap and Cheng 2009). In fact, Fe, Mn, and Cu play important roles in metabolic biomolecules like enzymes or metalloenzymes (Langston et al. 1998, Rainbow 1997). Snails also need elevated amount of Cu, being a constituent of hemocyanin (Dallinger et al. 2005). Conversely, the other elements studied can be toxic, inducing growth retardation, edema and thinning of the shell (Factor and de Chavez 2012). Similar results were reported concerning snails and different mollusks in mangroves in Malaysia (Yap and Cheng 2013), in India (Palpandi and Kesavan 2012), in Costa Rica (Vargas et al. 2015), or in Senegal (Sidoumou et al. 2006).

We also suggest that the different amounts of trace elements in the food sources and feeding habits of individual snail affect the degree and extent accumulation of trace elemental in their tissues. The snail *C.obtusa*, a sediment eater, exhibited higher trace elements concentrations in the *Rhizophora* stand than in the *Avicennia* one, except for Mn and Co. This could result from higher concentrations of trace elements in the available fractions in sediments beneath the *Rhizophora* stand (Table SD2). We evidenced that because of the enhanced organic enriched-sediment beneath the *Rhizophora* stand, reductive dissolution of Fe-Mn oxihydroxides by bacteria during organic matter decay processes is a source of dissolved trace elements in pore-waters, these elements being more bioavailable (Thanh-Nho et al. 2019). As a consequence, concentrations of trace elements in *C.obtusa* tissues in the present study were far higher than those measured in other mangroves (Joseph and Ramesh 2016) or estuary with strong anthropogenic pressure (Kesavan et al. 2013). Concerning *L.melanostoma*, a leaf eater, we suggest that the higher concentrations of trace elements (except Mn), combined to higher BCF (Fig. 4), in snails tissues living beneath the *Rhizophora* stand compared with the *Avicennia* one (Table 2) is likely related to the higher trace

elements concentrations in the *Rhizophora* leaves than the *Avicennia* ones (Table 1). In addition, *L,melanostoma* presented the highest concentrations of Fe and Mn in the tissues compare to other snail species in the Can Gio mangrove. This result suggest that leaf eater is more sensitive to accumulation of trace elements or that trace elements in leaves are more bioavailable. Furthermore, *L.melanostoma* in the present study showed higher Fe and Mn concentrations than those in *L.scabra* collected from a polluted mangrove (De Wolf and Rashid 2008). *C.capucinus*, a predator of barnacles, bivalves and other mollusks (Berandah et al. 2010), presented the highest Cu concentrations, which may result from copper biomagnification via their food chains because Cu is known to be very compatible with protein binding in organisms (Jiang and Qiu 2009). Cu biomagnification in snails body is usually observed, and they are considered as "macroconcentrators" species for Cu (Nica et al. 2012). However, concerning *N.articulata*, the microalgae eater, Mn and Cu concentration in their tissues were lower than those in other species whatever the mangrove stands, and were lower than those measured in *N.articulata* living in other mangroves like in Malaysia (Yap and Cheng 2013) or Southeast coast of India (Palpandi and Kesavan 2012). This result may be related to low Mn and Cu accumulation in microalgae in the Can Gio mangrove. Unfortunately, in the present study, we were not able to measure concentrations of trace elements in microalgae. Considering the key role of microphytobenthos in mangrove trophic food chain (Lee 2008, Raw et al. 2017), we suggest that further investigation should be carried out on accumulation of trace elements in these microalgae.

Eventually, among non-essential elements, all snails contained high quantity of As, which can result from its availability in their diet, and snails' ability to metabolize As and retain it (Kirby et al. 2002, Zhang et al. 2013). Arsenic also presented the highest BCF whatever the snail species (Fig. 4). In the Can Gio mangrove, dissolved As concentrations in pore-waters reached up to almost

20 µg L^{-1}. In addition at the *Rhizophora* stand, characterized by higher organic content, the organic fraction, being bioavailable, represented up to 36 % of total As concentrations (Table SD2). Khokiattiwong et al. (2009) studied on two mollusks species (*C.capucinus* and *Telescopium telescopium*), and they showed that *C.capucinus*, as a predator, contained more As than the detritus and algae eater species, *T.telescopium*. We also observed that *C.capucinus* contained the highest As concentrations in their body (2 to 4 fold higher level tissues than other snail species) in the Can Gio mangrove, which could be related to their predator diet and magnification along food chain (Goessler et al. 1997).

According to Dallinger (1993), snail tissues can be classified in macroconcentrators (BCF > 2), microconcentrators (1< BCF < 2) or deconcentrators (BCF < 1). Thus, in the present study, the *L.melanostoma* and *C.obtusa* can be classified as macroconcentrators for Cu. The *L.melanostoma* was macroconcentrators for As, Co and Ni. Conversely, all snails were deconcentrators to microconcentrators for Mn and Fe. Due to negative effects of toxic trace elements on community structure, gender, size of snails (Amin et al. 2009, Yap and Cheng 2013), the obtained results in the present study provided a further claim that these snails can be used as good biomonitors for environmental quality (Samsi et al. 2017).

5. Conclusions

Trace elements accumulation in the tissues of mangrove plants and snails studied in the Can Gio mangrove reflected their concentrations in the sediment, their bioavailability, and specific adaptation strategies or physiological processes of the biota. Clear differences between the two stands were evidenced, notably because of different sediment geochemistry. The main conclusions concerning mangrove trees can be summarized as follow:

- Fe and As were characterized by low translocation to the leaves of the *Avicennia* and *Rhizophora* trees. We suggest that the formation of iron plaque on roots by oxygen release in the rhizosphere may be a key factor preventing this translocation. Consequently, mangroves roots can be considered as “phytostabilizators”, immobilizing trace elements and limiting their transfer in the environment.
- Co exhibited higher concentrations in roots and leaves of the Can Gio mangrove trees than the world average. However, this element was characterized by low BCF and TF, which may be related to its high concentrations in available forms in the sediment
- High translocation factors for Mn and Cu may possibly result from physiological requirements, both elements being useful in photosynthetic processes, but also from elevated bioavailability in the sediment. We also suggest that the *Rhizophora* trees have a high potential for Mn “phytoextraction”.
- Ni and Cr were characterized by low BCF and TF whatever the mangrove plants, which may result from their low bioavailability in the sediment and from the limited physiological roles of those elements.
- Consequently, we suggest that the mangrove trees species studied can act as sinks when elements are stored in their root systems, or sources for the trophic chain when they accumulate in the leaves. Therefore, studies regarding the turnover of trace elements from decomposing of leaf litter should be developed to get a better understanding of the cycling of trace elements in mangroves ecosystem.

The main conclusions concerning mangrove snails can be summarized as follow:

- The great variability in concentrations of studied trace elements in the various snails indicated that bioaccumulation of trace elements depended not only on characteristics of

trace elements (i.e. physiological properties and biological functions), and metabolic requirements of each species but also on available concentrations of these trace elements in their feeding sources.

- Fe, Mn and Cu, which are essential elements, presented higher concentrations in all snails tissues than As, Ni, Cr and Co, which can be toxic.
- Most of the snails living in the *Rhizophora* stand presented higher levels of trace elements concentrations (except Mn and Co) than those living in the *Avicennia* stand, specifically for *L.melanostoma* and *C.obtusa*, due to higher concentrations of trace elements in the leaves or in the bioavailable fractions in the sediment.
- Concerning the predators, *C.capucinus*, possible biomagnification of Cu via the food chain induced elevated Cu concentrations in their tissues.
- In this study, we also measured that all snails contained high amount of As, most probably because it is highly bioavailable in mangrove sediments.
- Eventually, most snails can be classified as “maroconcentrators” of some trace elements such as Cu, As, Co and Ni. Because of their potential toxic effects, a further study should be performed to assess the possibility of using snails as bioindicators of environmental quality. In addition, it would be relevant to compare trace elements dynamics between seasons. We suggest that during the dry season, lower elements inputs and lower sediment reactivity may induce lower accumulation in mangrove plants and snails tissues.

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