



# Journal of Applied Sciences

ISSN 1812-5654

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## Research Article

# Heavy Metals in Sediments and Their Transfer to Edible Mollusc

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## Abstract

**Background and Objective:** Vridi canal is subjected to intense human activities because it represents the only way to entry and exit from the largest harbour of Côte d'Ivoire. However, no study has been conducted on the metal contamination in this zone. Therefore, the objectives of this study were to assess metals concentrations, to determine the mobility and bioavailability of cadmium, copper and zinc in the sediments and their influence on bioaccumulation in edible mollusc. **Materials and Methods:** Sediments and gastropod (*Purpurea haemastostoma*) samples from Vridi canal were seasonally collected between February, 2014 and September, 2015. Sequential extraction was used to determine the mobility and bioavailability of cadmium, copper and zinc. Analysis of variance carried out to observe the effect of season and Pearson correlation to show the relationship between these metals in *Purpurea haemastostoma* and those of bioavailable fractions in sediments. **Results:** The results showed that the Vridi canal sediments had the high contents of cadmium (1.95-8.35 mg kg<sup>-1</sup>) and zinc (19.48-242.09 mg kg<sup>-1</sup>). The results of the sequential extraction showed that copper (62.21-67.84%) and zinc (54.48-55.19) were mainly found in the residual fraction in sediments during dry season, rainy season and flooding season. Concerning the cadmium (24.45-36.81%), it prevailed in the exchangeable fraction during the three seasons. Total concentrations of cadmium and zinc in *Purpurea haemastostoma* exceeded the maximum permissible levels, according to Food and Agriculture Organization (FAO). Pearson correlation showed that the concentration of cadmium and zinc in the bioavailable fractions in sediments influenced the bioaccumulation in edible mollusc. **Conclusion:** These results suggest that copper and zinc were much less mobile, bioavailable and toxic to organisms in the aquatic environment while cadmium was highly mobile, bioavailable and toxic. However, the bioavailable fractions of cadmium and zinc are bioaccumulated in *Purpurea haemastostoma*. There is a risk for human consumption of this gastropod.

**Key words:** Heavy metals, sediment, *Purpurea haemastostoma*, bioaccumulation, fractionation

**Received:** July 01, 2016

**Accepted:** August 25, 2016

**Published:** October 15, 2016

**Citation:** Kouakou Adjoumani Rodrigue, N'Guessan Louis Berenger Kouassi, Benjamin Kouassi Yao, Albert Trokourey and Kopoin Adouby, 2016. Heavy metals in sediments and their transfer to edible mollusc. J. Applied Sci., 16: 534-541.

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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

The environmental contamination by heavy metals has interested many researchers because of its inherent toxicity, non-degradability, persistence and vast sources<sup>1</sup>. The major sources of heavy metals in environment are industrial mining, domestic and agricultural activities<sup>2-4</sup>. In aquatic environment, these metals can be accumulated in sediments and biomagnified along the aquatic food chains<sup>5</sup>.

Sediments are the main receptacle of heavy metals in marine and estuarine environments, but also act as a source of metals during changes in environmental conditions such as redox potential, pH, desorption and ligands<sup>6</sup>. Thus, the only determination of the concentration of metals in sediments is not sufficient to predict its mobility, bioavailability and toxicity, since the environmental behavior of metal depends strongly upon its specific chemical forms and binding states<sup>7,8</sup>. Hence, it is crucial to distinguish and quantify metal species in sediments in order to predict its mobility, bioavailability and potential environmental toxicity. Sequential extraction is an important and ubiquitous method that provides information about the strength of metal binding to particulates and the phase associations of metals in solid matrix<sup>1,9</sup>. Among the metal fractions, the exchangeable fraction and the fraction bound to carbonates are considered to be bioavailable and it determines the bioaccumulation and biomagnification in aquatic organisms<sup>10</sup>. It is recognized that bioaccumulation of metals in the soft tissue of gastropod molluscs reflects the metal levels in the aquatic environment<sup>11,12</sup>. Therefore, it is crucial to determine heavy metal concentrations in widely consumed gastropod species. Several researchers have reported the bioaccumulation of heavy metals in some species of gastropod<sup>11,13,14</sup>. For example, Liang *et al.*<sup>11</sup> reported that Cu, Cd and Zn contents in *Rapana venosa* and *Neverita didyma* species in Bohai Sea (China) exceeded the maximum permissible levels established by WHO. Berto *et al.*<sup>14</sup> mentioned that the Biota Sediment Accumulation Factor (BSAF) and correlation analyses pointed out a significant mobilization of Cu from sediments and its accumulation in the gastropod *Nassarius reticulatus* in Venice lagoon (Italy). According to El-Sorogy and Youssef<sup>13</sup>, in the Saudi Arabian Gulf coast, *Diodora rueppellii* and *Lunella coronata* are good accumulators for Cu than Zn. However, these studies did not include the seasonal accumulation of heavy metals in the gastropod species. In this study, the effect of season on metal accumulation in gastropods has been examined, specifically in *Purpurea haemastostoma*. This gastropod mollusc is found along Vridi canal and is also consumed by the surrounding populations of Vridi canal. This canal is the unique way to

enter and exit of the harbour area of Abidjan. It is also the unique way of communication between Ebrie lagoon and Atlantic ocean. Every day, a huge amount of untreated industrial waste is being discharged into the water body of Vridi canal. To date, no data on total metal concentration in Vridi canal is available in the literature.

Several studies conducted in Côte d'Ivoire reported high level of metal contamination in water, sediments and aquatic organisms<sup>4,15-18</sup>. However, these studies have not shown the relation on the metal bioaccumulation in aquatic organisms from the bioavailable fractions in sediments. Therefore, the objectives of this study were to assess metals concentrations, to determine the mobility and bioavailability of cadmium, copper and zinc in the sediments and their influence on bioaccumulation in edible gastropod: *Purpurea haemastostoma*. Zn, Cu and Cd were selected in this study because they are toxic to gastropods and human health when they are submitted to high concentrations.

The relevance of this study is that determining metal concentration in biological matrices and metal mobility in sediments simultaneously could provide accurate information about the risks associated with metal contamination. Most notably, this study represents the first metal analysis of sediments and aquatic organisms from Vridi canal and could prevent an environmental catastrophe in this area.

## MATERIALS AND METHODS

**Description of the study area:** This study focused on an important canal of the harbour of Abidjan (the economic capital of Côte d'Ivoire). Vridi canal is an artificial canal that was created in 1954 and it is located in the Southern part of Abidjan. It was selected for its proximity to most of the industrial and maritime activities taking place, such as maritime transport and fishing and the company of oil Refinery. This canal is 2.7 km long, 370 m wide and has a navigable depth of 15 m. It receives domestic raw sewage, household waste and industrial wastes from its neighbourhood.

### Sampling and analysis

**Sampling and treatment of sediment:** The sediment samples were collected between February, 2014 and September, 2015 (dry season, rainy season and flooding season) from different sites (Fig. 1). Sediment samples were collected by using plastic container. Then sediment samples were put into small plastic bags and kept in a cooler at 4°C. All samples were transported to laboratory and subsequently stored in a freezer at -20°C.

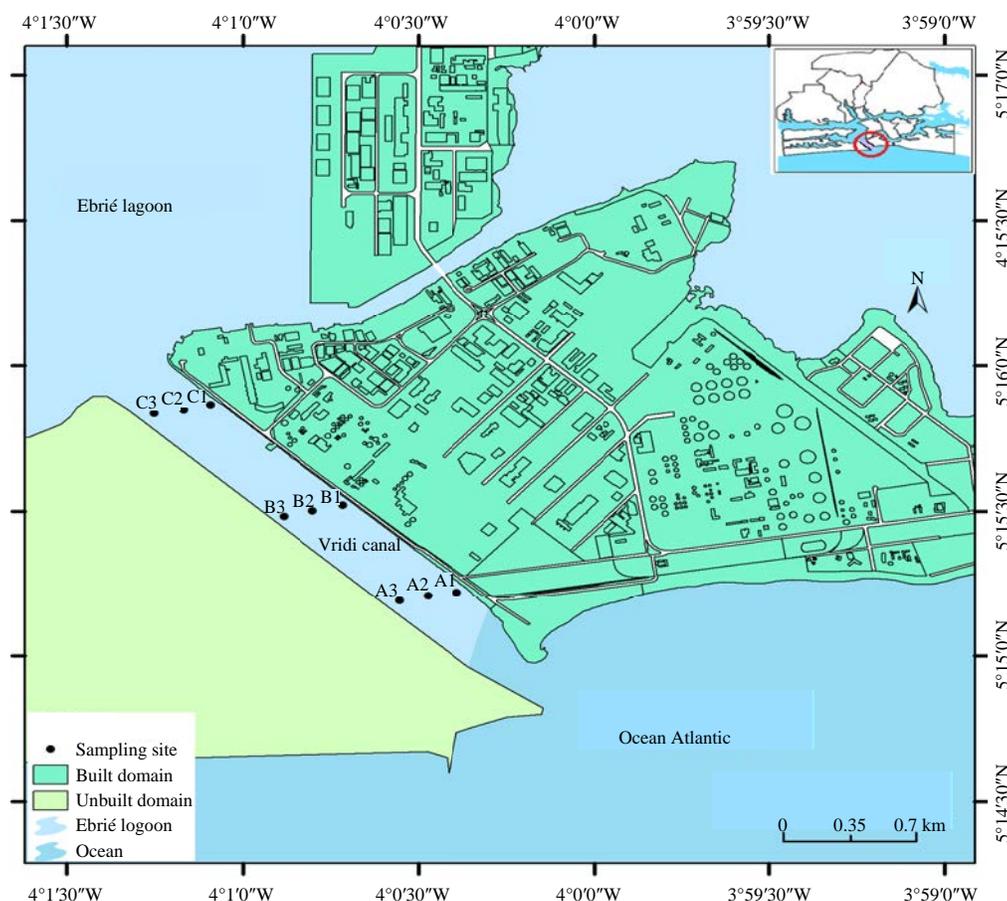


Fig. 1: Map of the study area showing sampling sites

The sediments samples were treated according to the treatment method described by Saleem *et al.*<sup>8</sup> and Radojevic and Bashkin<sup>19</sup>.

A mass of 0.2 g of each of the homogenized sediment was digested in closed teflon bomb with a mixture of 1 mL of aqua regia (HNO<sub>3</sub>: HCl; 1:3, v/v) and 3 mL of HF, heated to 100°C for 3 h in a water bath. After cooling, a volume of 20 mL of H<sub>3</sub>BO<sub>4</sub> (140 g L<sup>-1</sup>) was added to each teflon bomb to mask free fluoride ions in solution and re-dissolve fluoride precipitates<sup>3</sup>. The final volume was made up to 50 mL with 2% ultrapure HNO<sub>3</sub>. The solutions were filtered and stored in polyethylene flasks for later determination of metal content.

**Sequential extraction procedure:** The sediment samples were sequentially extracted according to Tessier *et al.*<sup>20</sup> and Zerbe *et al.*<sup>21</sup>. The detailed procedures employed in this process are presented in Table 1. The reliability of sequential extraction protocols was checked by comparing the sum of the five fractions (F1+F2+F3+F4+F5) with the total metal concentration. The recovery rates for heavy metals ranged from 97.50-101.50%.

**Sampling and treatment of gastropod:** *Purpurea haemastostoma* was collected from the same sites and in the same seasons as sediments. The individuals were selected for the following sizes of shells: 4 cm for *P. haemastostoma*. Approximately 144 of gastropod samples were collected between February, 2014 and September, 2015. After sampling, the samples were treated according to Chiffolleau<sup>22</sup> before analysis.

**Total metal digestion of the soft tissues of gastropod:** The digestion of soft tissues was made according to the method described by Chiffolleau<sup>22</sup>. A mass of 0.2 g of each of the homogenized soft tissues was placed in a teflon bomb, then a volume of 4 mL of concentrated HNO<sub>3</sub> (65%) was added and was allowed to stand all night for a pre-digestion and digested on a water bath (above 100°C) for 3 h. After cooling, the solution was filtered by using filter, stored in polyethylene flasks and made the filtrate up to 50 mL with deionized water. Blank digestion was also made to quantify possible contamination.

Table 1: Sequential extraction procedure

| Chemical fractions                          | Procedure of each step  |
|---|---|
| Exchangeable fraction (F1)                  | 0.5 g of sample was extracted with 5 mL of 1 M CH <sub>3</sub> COONH <sub>4</sub> , pH 7.0 at room temperature with frequent agitation for 1 h  |
| Fraction bound to carbonates (F2)           | The residue from step 1 was extracted with 10 mL of 1 M CH <sub>3</sub> COONH <sub>4</sub> adjusted to pH 5.0 with acetic acid periodically agitated for 5 h at room temperature  |
| Fraction bound to Fe-Mn oxides (F3)         | The residue from step 2 was extracted with 10 mL of 0.04 M NH <sub>2</sub> OH·HCl in 25% (v/v) CH <sub>3</sub> COOH at 96±3°C with occasional agitation for 5 h   |
| Fraction bound organic matter/sulfides (F4) | The residue from step 3 was digested first for 2 h with 2.5 mL of 30% H <sub>2</sub> O <sub>2</sub> and 2.5 mL of 0.02 M HNO <sub>3</sub> adjusted to pH 2 with HNO <sub>3</sub> at 85±2°C, 2 h and then again with 2.5 mL of 30% H <sub>2</sub> O <sub>2</sub> (pH 2 with HNO <sub>3</sub> ) for 3 h at 85±2°C with intermittent agitation. After cooling, a volume of 5 mL of 3.2 M CH <sub>3</sub> COONH <sub>4</sub> in 20% (v/v) CH <sub>3</sub> COOH, HNO <sub>3</sub> was added and agitated continuously for 30 min |
| Residual fraction (F5)                      | The residue from step 4 was washed with deionised water and was transferred to teflon beakers and was digested totally with the concentrated mixture of HF and aqua regia   |

After each step, samples were centrifuged, decanted and 10 mL of deionized water was added to the residue (sample-centrifuged and water-removed) before proceeding with the next step

**Determination of metal concentration:** Metals were determined by using an air-acetylene flame atomic adsorption spectrometer (Varian SpectrAA 20). The detection limit was 0.003 mg kg<sup>-1</sup> for Cd, Cu and Zn. In addition, accuracy and precision of analysis was checked by replicate measurements of standard reference materials (BCSS-1, National Research Council Canada, DORM-2 dogfish muscle). The measured concentrations fell within the range of certified values and the recoveries varied between 94 and 108%.

**Statistical analysis:** The analysis of variance (ANOVA one-way) was used to evaluate the difference between the seasons. Then, Tukey test (Honest significant difference) was performed if significant difference was found in ANOVA. Differences were considered significant at p-values <0.05. Statistical analyses (mean value, minimum, maximum and correlation) were carried out with Statistica 7.1 software.

## RESULTS AND DISCUSSION

**Total concentrations of heavy metals in sediments:** The mean concentrations of metals in the Vridi canal sediments are given in Table 2. The Cd, Cu and Zn in sediments ranged from 1.95±1.02 to 8.35±1.82 mg kg<sup>-1</sup>, from 4.06±1.26 to 8.21±1.01 mg kg<sup>-1</sup> and from 19.48±14.40 to 242.09±233.48 mg kg<sup>-1</sup>, respectively. The levels of Cu concentrations in all sediments were lower than the mean value in the Upper Continental Crust (UCC)<sup>23</sup>. On the opposite, concentrations of Cd and Zn were higher than their corresponding values in UCC (except for Zn in the rainy season). This suggests that sediments were most enriched in Cd and Zn. The high contents of Cd and Zn could be explained by anthropogenic activities, these include phosphate fertilizers, industrial effluents, waste disposal related to the demographic pressure in the vicinity of Vridi canal and maritime activities. Although the determination of the total

Table 2: Total concentration of metals in sediments of Vridi canal (mg kg<sup>-1</sup>)

| Seasons  | Cd        | Cu        | Zn            |
|----------|-----------|-----------|---------------|
| Dry      | 4.01±2.25 | 8.21±1.01 | 242.09±233.48 |
| Rainy    | 1.95±1.02 | 4.36±1.46 | 19.48±14.400  |
| Flooding | 8.35±1.82 | 4.06±1.26 | 238.28±239.79 |
| UCC      | 0.10      | 14.3      | 52.00         |

UCC: UPPER continental crust, Mean value±Standard Deviation

concentration of metals is essential, it provides no information on concentrations of metals bioavailability in nature system. Thus, chemical fractionation of Zn, Cu and Cd in the Vridi canal sediments was further studied.

### Chemical fractionation of Cd, Cu and Zn in the sediments:

Table 3 illustrates the chemical fractionation of Cd, Cu and Zn based on the Tessier sequential extraction for the Vridi canal sediments.

The mean values of Cd in the exchangeable fraction during the dry season, the rainy season and the flooding season were 36.81, 29.80 and 24.45%, respectively. The analysis of variance (p<0.05) showed a significant difference between the dry season and the flooding season for Cd in the exchangeable fraction. The highest value was obtained in the dry season. It is widely accepted that the metals contained in the exchangeable fraction is labile, highly toxic and represents the most bioavailable fraction in sediments<sup>24,25</sup>. The Cd contents in this fraction are very elevated and represent a threat to the biota. The results of this study also showed that the fraction bound to carbonates, for Cd, accounted for 19.87, 11.45 and 20.34% of total Cd content in the sediments during the dry season, the rainy season and the flooding season, respectively. The concentrations of Cd in the fraction bound to carbonates showed a significant difference (p<0.05) between the dry season and the rainy season and between the rainy season and the flooding season. The residual fraction containing 11.89, 19.74 and 15.69% of Cd were extracted in the sediments during the dry season, the rainy season and the

Table 3: Chemical fractionation of heavy metals in sediments of Vridi canal (%)

| Fractions | Cd (%) |       |          | Cu (%) |       |          | Zn (%) |       |          |
|-----------|--------|-------|----------|--------|-------|----------|--------|-------|----------|
|           | Dry    | Rainy | Flooding | Dry    | Rainy | Flooding | Dry    | Rainy | Flooding |
| F1        | 36.81  | 29.80 | 24.45    | 2.55   | 1.73  | 2.40     | 2.27   | 2.01  | 1.95     |
| F2        | 19.87  | 11.45 | 20.34    | 7.10   | 5.69  | 7.50     | 12.66  | 12.39 | 13.24    |
| F3        | 16.12  | 19.54 | 22.64    | 10.10  | 10.24 | 9.90     | 12.50  | 13.39 | 11.22    |
| F4        | 15.31  | 19.47 | 16.87    | 18.05  | 15.11 | 12.37    | 18.07  | 17.02 | 18.55    |
| F5        | 11.89  | 19.74 | 15.69    | 62.21  | 67.23 | 67.84    | 54.48  | 55.19 | 55.03    |

flooding season, respectively. A significant difference ( $p < 0.05$ ) was observed between the dry and rainy seasons.

In this study, Zn was mainly associated with the residual fraction, with percentages of 54.48, 55.19 and 55.03% during the dry season, the rainy season and the flooding season, respectively. At the same time, the levels of the exchangeable fraction of Zn were low during the three seasons: 2.27% in the dry season, 2.01% in the rainy season and 1.95% in the flooding season. The results of the sequential extraction indicate that the residual fraction for Cu was 62.21, 67.23 and 67.84% during the dry season, the rainy season and the flooding season, respectively. This result suggested that Cu had the strongest associations with crystalline sedimentary components. The results obtained are in agreement with those reported in literature, where Cu was also mainly found in the residual fraction<sup>26,27</sup>. The residual fractions of metals were generally much less mobile, bioavailable and toxic to organisms in the aquatic environment<sup>24</sup>. The seasonal distribution of Cu and Zn in surface sediments collected in Vridi canal was similar in all seasons: Residual (F5) > organic matter (F4) > Fe/Mn oxides (F3) > carbonates (F2) > exchangeable (F1). The Cd partitioning in the dry season was exchangeable (F1) > carbonates (F2) > Fe/Mn oxides (F3) > organic matter (F4) > residual (F5), while that of the rainy season was exchangeable (F1) > residual (F5) > Fe/Mn oxides (F3) > organic matter (F4) > carbonates (F2). Finally, the following pattern exchangeable (F1) > Fe/Mn oxides (F3) > carbonates (F2) > organic matter (F4) > residual (F5) was found during the flooding season.

The Risk Assessment Code (RAC) was used to determine the risk of each metal and it is based on the percentage of the total concentration of heavy metals in the exchangeable fraction and the fraction bound to carbonates<sup>24</sup>. The evaluation of RAC levels in sediments at various seasons are given in Table 4. According to RAC classification introduced by Perin *et al.*<sup>28</sup> (Table 5), the RAC results revealed low risk for Cu (<10%) during the three seasons and medium risk for Zn during the three seasons. For Cd, the high risk was indicated during the rainy and flooding seasons. Moreover, a very high risk was shown for Cd, during the dry season and was considered very dangerous for the biota. Overall, RAC

Table 4: Evaluation of RAC levels in sediments of Vridi canal (%)

| Seasons  | Cd              | Cu   | Zn    |
|----------|-----------------|------|-------|
|          | ----- (%) ----- |      |       |
| Dry      | 56.68           | 9.65 | 14.95 |
| Rainy    | 41.25           | 7.41 | 14.40 |
| Flooding | 44.80           | 9.90 | 15.20 |

Table 5: Classification of risk assessment (%)

| RAC (%)        | Criteria |
|----------------|----------|
| No risk        | <1       |
| Low risk       | 1-10     |
| Median risk    | 11-30    |
| High risk      | 31-50    |
| Very high risk | >50      |

indicated that Cd was highly mobile and bioavailable in the aquatic environment. The results obtained for Cd is similar to those conducted by Kouassi *et al.*<sup>29</sup> in Ebrie lagoon, in the vicinity of Vridi canal. However, bioavailability and toxicity of Cd were influenced by the seasonal variations.

#### **Bioaccumulation of heavy metals in *P. haemastostoma*:**

Concentrations of Cu, Cd and Zn in the soft tissues of *P. haemastostoma* are given in Table 6. The Cd concentrations varied from  $4.59 \pm 4.23$  to  $10.84 \pm 6.23$  mg kg<sup>-1</sup>. The Zn concentrations varied of  $145.11 \pm 83.11$  to  $215.55 \pm 149.06$  mg kg<sup>-1</sup>. The Cu concentrations fluctuated within the range of  $3.30 \pm 1.78$  to  $5.38 \pm 4.84$  mg kg<sup>-1</sup>. No significant differences were found between the seasons for Cu and Zn ( $p > 0.05$ ), while a significant difference was observed for Cd between the dry and rainy seasons ( $p < 0.05$ ). The *P. haemastostoma* have accumulated Cd, Cu and Zn throughout the year with higher values during the dry season. The total concentrations of Cd, Cu and Zn showed similar seasonal patterns, because the total concentrations of these metals were higher in the dry season, lower in the rainy season and medium in the flooding season. However, seasonal variations of the accumulation of Cd were not different ( $p > 0.05$ ). On the contrary, seasonal variations of the accumulation of Cu and Zn were different ( $p < 0.05$ ). The seasonal difference noted in the metal accumulation could be due to the seasonal pollution in sediments. The metal accumulation in could be influenced by the age, size, growth cycle and feeding habits and also the capacities for

accumulating metals<sup>30</sup>. The difference in metal distribution could be attributed to the differences in tissue physiology and metal handling, storage and detoxification strategies<sup>31</sup>. The *P. haemastostoma* is consumed by the surrounding population of Vridi canal. Table 6 shows that the levels of Cd and Zn except for Cu, exceeded the maximum permissible levels of WHO<sup>32</sup>, indicating risk for human consumption. The high concentrations of these metals in most of the gastropod samples may be attributed to the industrial activities, the activities of the oil refineries company which is taking place in the vicinity of Vridi canal and the ships transiting through this canal.

**Correlation between the bioaccumulation in *P. haemastostoma* and the bioavailable fractions of metals in sediments:**

In order to establish the relationship between the bioaccumulation of Cd, Cu and Zn in *P. haemastostoma* and the bioavailable fractions in sediments, a correlation matrix was calculated for the concentration of these metals in the bioavailable fractions of sediments (F1 and F2) and in the soft tissues of *P. haemastostoma*. According to the values of Pearson coefficients (Table 7), a significant positive correlation existed between the bioavailable fractions in sediments (F1 and F2) for Cd and Zn with their respective total concentrations, indicating that the concentrations of Cd and Zn associated with exchangeable fraction (F1) and fraction bound to carbonates (F2) in the sediments increased with the concentrations of Zn and Cd in the gastropod. It suggests that the exchangeable fraction (F1) and the fraction bound to carbonates (F2) for Zn and Cd can be good indicators for the bioavailability of Cd and Zn in the Vridi canal sediments. Furthermore, these results indicated that the concentration of Cd and Zn in the bioavailable fractions (F1 and F2) influenced

the bioaccumulation in *P. haemastostoma*. The high concentration of Cd in the bioavailable fractions (F1 and F2) may facilitate the high accumulation in the biota<sup>33</sup>. Other studies such as those of Fan *et al.*<sup>34</sup> and Baumann and Fisher<sup>35</sup> found significant correlation between Cd bioaccumulation and easily mobile fractions in the sediments. The results also showed that the concentrations of Cu associated with the fraction bound to carbonates (F2) in the sediments and the concentrations of Cu in *P. haemastostoma* were not correlated ( $r = -0.13, p > 0.05$ ). These results showed that the concentrations of Cu in the bioavailable fractions have, weakly or not influenced the bioaccumulation of Cu in *P. haemastostoma*.

**CONCLUSION**

This study showed that Vridi canal sediments had high contents of Cd and Zn. The results of the sequential extraction showed that Cu and Zn were mainly found in the residual fraction in sediments during the three seasons. This suggests that Cu and Zinc were much less mobile, bioavailable and toxic to organisms in the aquatic environment. Concerning the Cd, it prevailed in the exchangeable fraction during the three seasons and it was highly mobile, bioavailable and toxic to organisms in the aquatic environment. Total concentrations of Cd and Zn in *P. haemastostoma* exceeded the maximum permissible levels, according to Food and Agriculture Organization (FAO), indicating a risk for human consumption of this gastropod. The values of Pearson coefficients showed that the concentration of Cd and Zn in the bioavailable fractions (F1 and F2) in sediments influenced the bioaccumulation in *P. haemastostoma*.

**ACKNOWLEDGMENTS**

The authors thank Centre de Recherches Océanologiques (CRO) of Côte d'Ivoire for sampling. Thanks to Dr. Djedou Adaman Etrin-Ehoussou in Institut National Polytechnique Felix Houphouët-Boigny (INP-HB) of Yamoussoukro and Mr. Boli-Bi in Antenne de la pédagogie et de la formation continue (AFPC) of Gagnoa for their help and suggestions to the draft manuscript.

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Table 6: Distribution of heavy metals in *P. haemastostoma* (mg kg<sup>-1</sup>)

| Seasons  | Cd         | Cu        | Zn            |
|----------|------------|-----------|---------------|
| Dry      | 10.84±6.23 | 5.38±4.84 | 215.55±149.06 |
| Rainy    | 4.59±4.23  | 3.30±1.78 | 145.11±83.11  |
| Flooding | 8.06±2.45  | 4.27±3.23 | 195.30±63.92  |
| MPLs     | 2          | 10        | 100           |

MPLs: Maximum permissible levels of FAO/WHO

Table 7: Correlation between metal concentrations in *P. haemastostoma* and bioavailable fractions in sediments

| Metals | Bioavailable fractions | <i>P. haemastostoma</i> |
|--------|------------------------|-------------------------|
| Zn     | F1                     | 0.38*                   |
|        | F2                     | 0.49*                   |
| Cu     | F1                     | 0.18                    |
|        | F2                     | -0.13                   |
| Cd     | F1                     | 0.41*                   |
|        | F2                     | 0.29*                   |

\*Significant at the 0.05 level

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