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Heavy metals (copper, zinc, iron and cadmium) in sediments and the small clam *(Chamelea gallina)* of the coastal area north-east of Morocco

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ABSTRACT

The Moulouya estuary located at the north-east of Morocco between 33° and 35° 30'north latitude and between 2° and 4°30' west longitude receives the water from Moulouya River. This river can drain various pollutants from the backcountry who knows a strong agricultural activity marked by the massive use of fertilizers and pesticides. These pollutants might disturb the balance of the local marine environment. To measure the extent of this impact, the marine sediments in this area were analyzed to determine the content of heavy metals (Cd, Cu, Zn, Fe) in sediment fraction less than 63microns. We also studied the impact of sediment contamination on the small clam (Chamelea gallina) by the use of sequential extraction method of metal species. The results showed that the content of heavy metals (Cd, Cu, Zn, Fe) in sediments at both stations studied did not exceed the recommended guidelines from the U.S. National Oceanic and Atmospheric Administration levels (NOAA) and the Canadians on the Sediment Quality guidelines. The study of correlation between sediment contamination and that of bivalve showed the possible involvement of sediment contamination in the small clam by Cd, Cu and Fe. This correlation does not seem to be verified in the case of zinc. In order to better understand and assess the impact of sediment contamination on heavy metal levels recorded in the small clam, we expected to study the bioavailability of different metals by using different extraction methods sequentially. The results showed that although the contamination of sediment by iron, zinc and copper is sometimes marked, the forms present are mobilized only in certain percentage. So contrary to what can be concluded from the total digestion, sequential extraction suggests that the risk of this contamination on the small clam (Chamelea gallina) may be less noticeable.

Keywords: bivalve mollusks, Microbiological Quality, Mediterranean, Morocco.

INTRODUCTION

Coastal fringes are under increasing pressure from industrial and human activities whose effects are quickly felt. These activities are often the source of anthropogenic releases that end up more or less rapidly in aquatic environments, particularly coastal and estuarine, where they may have adverse short and long term effects [1].

In these aquatic environments, a large part of anthropogenic or natural compounds are adsorbed on suspended particles and then accumulate in the sediments. Thus, the sedimentary deposits at the interface between oceanic and continental areas constitute real filters and tanks for heavy metals and are an important source of contamination [2]. However, the overall behavior of these heavy metals in the aquatic environment is strongly influenced by the metal associations with different geochemical phases in sediments [3].

Bivalve molluscs (clams, mussels, oysters) are then directly exposed to contaminants that are adsorbed on the particulate phase, but also contaminants that are dissolved in the interstitial water at the water/sediment interface. These species can, sometimes, accumulate heavy metals at very high concentrations and are used as bio-indicators for monitoring environmental conditions in coastal aquatic environments [4,10]. These bivalves can then constitute a danger to the health of people who consume these aquatic resources [11,12].

The transfer of these heavy metals in the aquatic receiving environment to organisms depends on the concentrations present in these sources and their bioavailability [13,14].

However, in order to assess the environmental impact of contaminated sediments, information on total concentrations are not sufficient. A more particular interest is the fraction of the total content of heavy metals that may participate in other biological processes [15,16].

It gets essential to evaluate the concentrations of heavy metals and their bioavailability in the sediment fraction of these aquatic environments.

It is with this objective that fits our research which aims to establish the level of contamination by heavy metals (Fe, Cu, Cd, Zn) in the three compartments of the ecosystem (water, sediment and bivalve molluscs) in the estuarine area of the Moulouya located in the north-east of Morocco located between 33 ° and 35 ° 30 'north latitude and between 2 ° and 4 ° 30' west longitude (Fig. 1)

This area of the Mediterranean coast knows a significant growth of tourism projects through the installation of a new tourist city (Marina Saidia) and new tourist harbor. These new projects alongside existing settlements (city of Saidia, port of Ras Kebdana) and the contribution of Oued Moulouya that drains all the watershed of the plain Triffa with intensive agricultural activity and inputs from urban areas developed on its surroundings may contribute to the contamination of the coastal zone.

To better understand and evaluate the impact of the receiving environment on the levels of heavy metals recorded, we are also interested to study the bioavailability of these metals by using different sequential extraction procedures.

EXPERIMENTAL SECTION

The small clam (*Chamelea gallina*) was selected as bioindicator in this study for its wide commercial exploitation in this coastal area [17]. Individuals of *Chamelea gallina* and sediment samples were taken at two stations S_1 and S_2 (Fig. 1).

Figure 1: Location of sampling stations

Station S_1: located at the mouth of the Moulouya, it undergoes direct influences of the river (Input of organic matter and nutrient salt especially during floods)

Station S_2: located at the sea about 4km from the mouth of the Moulouya and 5 km of Saidia, this place remains far from the direct influence of the river is considered as the control station.

Clam samples were performed with a monthly frequency from a boat equipped with a dredge clam. Individuals were separated on site, stored in plastic bags and stored at 4°C. To avoid environmental contamination or equipment, sampling methods were performed according to the precautions in the manual of Aminot [18].

In the laboratory, the soft parts were extracted from the shell and dried at 800°C until reaching constant weight. They were then finely ground using an agate mortar. An amount of 0.2 g dry weight (MS) of biological material was placed in Teflon container and then mineralized with 3 ml of HCl and 1 ml of pure HNO_3 using a microwave oven. The mineral deposit is then filtered on Whatman paper No. 541, completed to 25 ml with distilled water and then stored at 4 ° C until assayed [19].

The sediment samples were performed simultaneously with those of bivalve molluscs. Sediment samples were dried in an oven for 4 hours at 80 ° C and then sieved. Only the fraction of less than 63 microns diameter was chosen. An amount of 0.2 g dry weight of sediment was used for metal analysis. The total extraction was performed in Teflon containers menus screw caps, using a mixture of strong acids (HCl, 3 ml of HNO_3 and 1 ml) pure supra using a microwave oven (table I).

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Metallic elements studied were determined by atomic absorption spectrometry (Varian AA 20) flame (air / acetylene) for iron and zinc and graphite furnace for cadmium and copper. To take account of the matrix effect that can sometimes induce significant analytical errors, reference material (SD-M-2/TM for marine sediment and NIST 1566: oyster tissue) were used for calibration measurements. These reference materials were treated in the same conditions as the samples. Blank samples were used in side.

The results of the recovery percentages of the four metal elements in the reference materials used are shown in Table I.

Table I: Recovery of heavy metals from different certified reference materials (CRMs).

	SD-M-2/TM		NIST 1566 (CRM : Oyster tissue)	
	Target	% Recovery.	Target	% Recovery.
Cadmium	0.2 ± 0.0	90	4.2 ± 0.4	107
Copper	10 ± 0.2	94	66 ± 4	97
Zinc	49 ± 3	90	830 ± 57	95
Iron	56 ± 5	88	921 ± 59	89

SD-M-2/TM (MRC: marine sediment), NIST 1566 (MRC: oyster tissue), (Mg / g dry weight).

For the study of the bioavailability of heavy metals, we have used the sequential extraction of these metals in four steps [20]. The different steps of this method are shown in Table II.

After each step, the solution was filtered by suction through a 0.45 microns Millipore filter and the filtrate was collected in a container made of Teflon (PTFE) with screw cap. Then, the solutions for each step were prepared for measuring atomic absorption. Each sediment sample was subjected to analysis in triplicate.

RESULTS AND DISCUSSION

The analysis results of the metal contents in the sediment compartment and bivalve mollusc showed an average of 133 μgg^{-1}_{dw} and 184 $\mu g\ g^{-1}_{dw}$ respectively for zinc and iron in sediment of the S_1 station and 108 $\mu g\ g^{-1}_{dw}$ and 131 $\mu g\ g^{-1}_{dw}$ in sediment of the S_2 station.

In the bivalve mollusc (*Chamelea gallina*) the respective average content of zinc and iron is 115$\mu g\ g^{-1}_{dw}$ and 142$\mu g\ g^{-1}_{dw}$ at S_1 station and 98$\mu g\ g^{-1}_{dw}$ and 89$\mu g\ g^{-1}_{dw}$ at S_2 station. Copper and cadmium are at respective average concentrations of 1.9 $\mu g\ g^{-1}_{dw}$ and 0.62 $\mu g\ g^{-1}_{dw}$ in sediment of the S_1 station and 1.2 $\mu g\ g^{-1}_{dw}$ and 0.43 $\mu g\ g^{-1}_{dw}$ in sediment of the S_2 station. In *Chamelea gallina*, the mean levels of copper and cadmium are in the order of 4.1 $\mu g\ g^{-1}_{dw}$ and 1.17 $\mu g\ g^{-1}_{dw}$ at S_1 station and 2.5 $\mu g\ g^{-1}_{dw}$ and 0.62 $\mu g\ g^{-1}_{dw}$ at the S_2 station (Table III).

Table II: Total and sequential extraction of heavy metals [22].

Total extraction	**Sequential extraction of heavy metals**			
	step 1	**step 2**	**step 3**	**step 4**
0.2 g (DM<63µm) ↓ 0.5 ml distilled water ↓ 3 ml HCI + 1 ml $HN0_3$ ↓ Heating in micro-waves 850 W for 50 s. cool at room temperature ↓ filter (Whatman N° 541) ↓ Adjusted to 25 ml with distilled water ↓ Measure in Atomic Absorption spectrometry (AAS) with flame or graphite furnace.	1 g (DM<63um) ↓ 40 ml acetic acid (0.11M) ↓ Shake for 16 hours at room temperature ↓ centrifuge (4000 rpm) ↓ The supernatant was then stored in polypropylene vials at 4 ° C for analysis.	The residue from the first step ↓ 40ml Hydroxylamonium in HC1 (0.1 M) ↓ Shake for 16 hours at room temperature ↓ centrifuge (4000 rpm) ↓ The supernatant was then stored in polypropylene vials at 4 ° C for analysis.	Residue of the 2nd stage ↓ 10 ml hydrogen peroxide (8.8 M) ↓ Cover the tube with watch glass. Shake at ambient temperature for 1 hour ↓ Heat to 85 ° C in a Waterbath for 1 hour. ↓ Remove the glass watch and reduce up to 1 to 2 ml. ↓ 10 ml of hydrogen peroxide (8.8 M) Heat to 85 ° C for 1 hour Remove the glass watch and reduce up to 1 to 2 ml. ↓ 50 ml Ammonium acetate (1 M) ↓ Shake for 16 hour at room temperature ↓ Centrifuge (4000 rpm) ↓ The supernatant was then Stored in polypropylene vials at 4 ° C for analysis.	Residue of the 3rd stage ↓ 3 ml of HC1 + 1 ml of $HN0_3$ ↓ Digestion with microwave
total metals	Exchangeable metal Fraction of metals bound to carbonates or specifically adsorbed	Reducible metal Fraction of metal bound to iron / Manganese oxides	Oxidizable metal Fraction of metal linked to the organic matter	Fraction of residual metal

Table III: Heavy metal content in the two compartments: sediment and bivalve mollusc ($\mu g\ g^{-1}_{dw}$)

		Zinc		Iron		Copper		Cadmium	
		S_1	**S_2**	**S_1**	**S_2**	**S_1**	**S_2**	**S_1**	**S_2**
Sediment n=12	x	133	108	184	131	1.9	1.2	0.62	0.43
	s	31.2	15.7	29.5	28.8	0.9	0.9	0.30	0.27
	cv	23 %	14 %	16 %	22 %	47 %	35 %	32 %	42 %
Bivalves Molluscs n=12	x	115	98	142	89	4.1	2.5	1.17	0.62
	s	30.85	21.54	45.8	19.5	1.4	1.5	0.39	0.27
	cv	27 %	22 %	32 %	22 %	34 %	60 %	33 %	43 %

n: number of samples; s = standard deviation; cv: coefficient of variation; x: arithmetic mean.

To ensure the safety of the small clam *Chamelea gallina*, we compared our results with Regulation (EC) 1881/2006 which sets the maximum content of cadmium in bivalve molluscs to 1 ppm wet weight [21].

The results we obtained for the small clam have shown that zinc levels recorded are below the permissible value of 50 ppm wet weight which is equivalent to 250 $\mu g\ g^{-1}_{dw}$. The copper and cadmium are also below the recommended levels (20 ppm wet weight corresponding to100 $\mu g\ g^{-1}_{dw}$ for copper and 1 ppm wet weight which is equivalent to 5 $\mu g\ g^{-1}_{dw}$for cadmium). The treatment of bivalves in purification center or relaying area is not needed before placing it on the market.

For sediment, quality criteria based on ecotoxicological data allow to estimate the risk of toxicity on benthic organisms.TEC ("Threshold Effect Concentration") and PEC values ("Probable Effect Concentration") proposed by MacDonald [23] seem best suited to the objective of protection set by the l'OEaux [24]. The TEC is the concentration below which it does not expect to observe the effects. PEC is the concentration at which a high probability of having effects is expected.

In the absence of guidelines in Morocco on sediment quality currently, we used the U.S. National Oceanic and Atmospheric Administration (NOAA) and Canadian guidelines to determine if the concentrations of heavy metals in sediments may have adverse biological effects (Table IV).

The results we obtained showed that no elements have incurred over the TEC values of Canadians guidelines and the ERL of NOAA (U.S. National Oceanic and Atmospheric Administration). This indicates that current levels of metals in these sediments are not high enough to cause adverse biological effects.

Table IV: Guiding values for heavy metals according to the guidelines of the U.S. National Oceanic and Atmospheric Administration (NOAA) and Canadian guidelines Sediment Quality.

Metal	NOAA guidance		Canadian guidance	
	ERL	ERM	TEC	PEC
Cadmium	1.2	9.6	0.7	4.2
Copper	34	270	18.7	108
Zinc	150	410	124	271

ERL : effects range-low ; ERM : effects range-Median; TEC : threshold effect concentration; PEC : probable effect concentration (µg g^{-1}Poids sec).

Comparison of results between the two stations (student test) showed that fluctuations concentrations at both stations showed no significant difference ($p> 0.05$), although the average is slightly higher in the case of the S_1 station.

Except for the copper in *Chamelea gallina*, the variability of the measurements in the two compartments (sediment and bivalve) measured by the coefficient of variation (CV) for each set of results (Table III) showed no significant difference between the two compartments ($p> 0.05$).

Also the average values of the coefficients of variation of these two compartments (sediment and bivalve) showed that they can integrate in part the variations detected in water.

To verify if the metal levels determined in the small clam (*Chamelea gallina*) depend on the levels present in the sediment, correlations between pairs of concentrations in the compartment (Bivalve / Sediment) were performed (linear regression).

The correlation coefficients between the content of heavy metals in the tissues of the small clam (*Chamelea gallina*) and sediment were statistically significant for iron, copper and cadmium ($p <0.05$) (Table V). In the case of zinc this relationship is not significant.

Table V: Correlation between the concentration of heavy metals in clams (*Chamelea gallina*) and sediment.

	Bivalve/Sediment
Iron	0.70 *
Zinc	0.31
Copper	0.72 *
Cadmium	0.67 *

**: significant at 5%.*

This correlation implies that the sediments are an important source in the transfer of cadmium, copper and iron to the small clam (*Chamelea gallina*). However, and although the sediments can be an important source of these elements, they may also come from other sources. It has been demonstrated in an earlier study [14] that the absorption of dissolved components from the water and food intake may contribute to the accumulation of metals in *Perna viridis*. These results suggest that the particles of suspended sediments can be a source of metals for filter feeders. However, the relationship between sediment metal partitioning and bioavailability of this metal is not obvious, because the organic matter content of the sediment particles and intestinal acidity influences the metal absorption in mussels [25,26].

In order to better understand the contribution of sediment in the metal contamination of bivalve mollusc, we proceeded to study the bioavailability of these metals in the sediment of the two stations by performing a sequential extraction as an additional means for study of metal speciation (Table II).

The results we obtained showed that the amount of zinc, iron and copper bound to the residual fraction [Fr_4] represents the major fraction with respective means percentages of 60%, 49% and 85% at S_1 station and 63 %, 46% and 80% at S_2 station (Fig. 2).

Cadmium was present at a nearly similar level in the four fractions with a preference for the reducible fraction. Copper is weakly present in fractions [Fr_2 and Fr_3] (Fig. 2).

The proportion of heavy metals in the exchangeable fraction [Fr_1] is low compared to all other fractions and represents respectively for cadmium, iron, copper and zinc 20%, 9%, 7% and 5% at the S_1 station and 18%, 10%, 10% and 4% at the S_2 station (Fig. 2).

The high proportion of heavy metals in the residual fraction (metals which cannot be mobilized) and their low presence in the exchangeable fraction shows that these metals are weakly available to aquatic organisms.

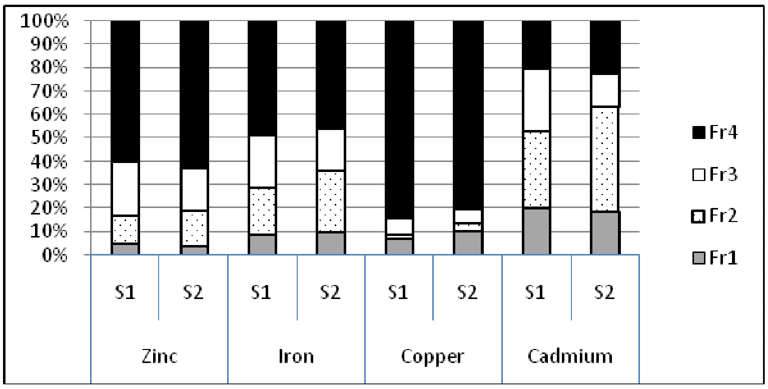

Figure 2: heavy metal content in the different Fractions of the sequential extraction
Fr_1: exchangeable metals (fraction of metals bound to carbonates or specifically adsorbed); Fr_2: reducible metals (fraction of metals bound to Iron / Manganese oxides); Fr_3: oxidizable metals (fraction of metals linked to the organic material); Fr_4: residual metals.

By calculating the affinity (Formula I) of the heavy metals studied towards the three mobile fractions (Fr_1, Fr_2, Fr_3), we find that the majority of heavy metals have more affinity with organic matter (Fr_3) and iron / manganese oxides (Fr_2) (Fig. 3). Copper has more affinity for the fraction Fr_1 (exchangeable metals) and Fr_3 fraction (oxidizable metals) (Fig. 3).

Formula I: heavy metals affinity to the three mobile fractions:

$$\text{Affinity} = \frac{\text{metal content bound to each fraction}}{\text{total content of exchangeable metal (Fr1+Fr2+Fr3)}} \text{ X } 100$$

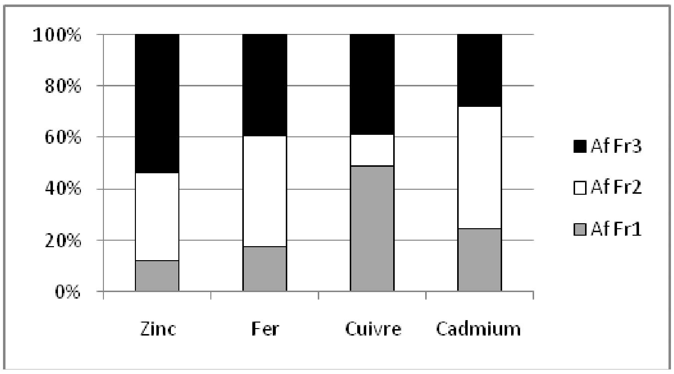

Figure 3: affinity of heavy metals towards the three mobile fractions
Fr_1: exchangeable metals (fraction of metals bound to carbonates or specifically adsorbed); Fr_2: reducible metals (fraction of metals bound to Iron / Manganese oxides); Fr_3: oxidizable metals (fraction of metals linked to the organic material);

In this way, Ramos et al. [27] also found a high affinity between the organic material and the copper and cobalt. Other authors have also shown that the copper extracted from the mobile phase is mainly associated with the organic matter, where it is likely in organometallic complex form [28,29,30,31].

For iron, the results are in agreement with those obtained by the previous work of Tessier et al, [32], where the majority of the mobile iron is obtained from fraction 2 (Fr_2: oxidizable). Some part of Iron remains associated with the fraction 3 (Fr_3 reducible: metals related to organic matter).

The zinc has more affinity for the mobile fraction Fr_2 and Fr_3 as has been reported by others [30].

Cadmium has an affinity towards the three fractions with much more affinity to Fr_2 fraction (fraction of reducible metal: metal related to Iron / Manganese oxides) (Fig. 3). The presence of this element in the mobile fraction may constitute a hazard to aquatic life. But the total content of cadmium we recorded remains low compared to polluted stations. Also the distribution of the element between the various fractions indicates that its presence in natural environment may be related to that of zinc.

Therefor and based on their affinity towards different fractions, we can rank these heavy metals by affinity decreasing order as follows:

Fraction 1	exchangeable metals (fraction of metals bound to carbonates or specifically adsorbed)	Copper >Cadmium>Iron>Zinc
Fraction 2	reducible metals (fraction of metals bound to Iron / Manganese oxides)	Cadmium>Iron>Zinc> Copper
Fraction 3	oxidizable metals (fraction of metals linked to the organic material);	Zinc> Copper =Iron>Cadmium

The comparison between the total extraction and sequential extraction of heavy metals was performed by calculating the percentage of recovery as follows:

Formula II: percentage recovery of the sequential extraction with respect to the total extraction of heavy metals

$$\text{recovery} = \frac{\text{metal content (Fr1+Fr2+Fr3+Fr4)}}{\text{total metal content}} \text{ X } 100$$

The results obtained show that the recovery percentages vary from 77% for copper to 112% for cadmium. The amount of the metal content in the four fractions obtained by sequential extraction is in most cases, except for copper, slightly higher than that obtained by total digestion (Table VI). However this difference is not significant ($p > 0.05$).

Table VI: Comparison between the metal content obtained by the sequential extraction and that obtained by the complete digestion of the sediment

		Zinc	Iron	Copper	Cadmium
mobile Fraction	Fr_1	5.7 (5%)	15.1 (9%)	0.10 (8%)	0.12 (19%)
	Fr_2	16.7 (13%)	36.9 (23%)	0.03 (2%)	0.23 (38%)
	Fr_3	26.0 (21%)	33.6 (21%)	0.08 (7%)	0.13 (22%)
résiduel Fraction	Fr_4	76.7 (61%)	77.4 (47%)	0.99 (83%)	0.13 (21%)
Somme		125	162	1.19	0.60
total digestion		120	157	1.55	0.53
Recovery %		104%	104%	77%	112%

In previous studies on river sediment, recovery percentages ranging from 89% to 111% were obtained for chromium, copper, lead, manganese, vanadium and zinc and 82% for nickel. [20] Other studies have reported recovery percentages ranging from 80% to 120% [33].

The proportion of heavy metals present in the mobile fraction, 79% for cadmium, 53% for iron, 39% for zinc and 17% for copper (Table VI), shows that these metals may be available to aquatic organisms and therefore contribute to their contamination. These percentages explain a contribution of anthropogenic sources and therefore the Backcountry of the mouth of the Moulouya River. In this back country strong agricultural activity is developed and is marked by a massive use of phytosanitary products. In this way Horowitz [34] and Förstner et al. [28] showed that these heavy metals reach the aquatic environment in the inorganic complexes or hydrated ions form and are readily adsorbed by the surface of the sediment particles through a weak physicochemical bond.

These anthropogenic metals are usually found in mobile form or easily extractable from sediment [28,35]. The Alteration products of the bedrock which are generally rich in heavy metals contain these metals in less available form [28,31,36].

We can deduce that although sediment contamination by iron, zinc and copper is sometimes marked, the forms present are mobilized at a certain percentage. So contrary to what can be concluded from the total digestion, sequential extraction suggests that the risk of such contamination may be lower.

CONCLUSION

In general, the results we found are important since they allow knowing the proportion of heavy metals that can be easily mobilized in response to changes in environmental conditions. This would allow us to better assess the impact of metal contamination on aquatic organisms.

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