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Contamination of the flesh of *Scrobularia plana* and *Solen marginatus* (Lamellibranch molluscs), hosting the estuary of Sebou (Morocco), by iron, zinc, copper and lead

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Abstract
The location of estuaries downstream of rivers favors their contamination by many polluting products, especially heavy metals. In the present work, we evaluated Fe, Zn, Cu and Pb concentrations in the sediment and in the flesh of two mollusc species living in the Sebou estuary (Morocco), *i.e. Solen marginatus* and *Scrobularia plana* (Lamellibranches molluscs).
The results have shown that the concentrations of the four metal elements were lower in the sediment than in the flesh of both species suggesting a bioaccumulation of some metal elements by these organisms. However, the concentrations of these elements varied between the two species and depended on the season for both materials (sediment and mollusc flesh). For *S. plana*, flesh Zn concentrations were relatively high and contrasted to those of Cu. As for lead, flesh concentrations were higher than those noted in the sediment.
On the other hand, an important bioaccumulation of Fe was observed in *S. marginatus*. This species behaved contrastively to *S. plana* with regards to Zn concentrations and showed a good ability to avoid lead excess in the medium.

Keywords: Molluscs - sediment - Pollution - Metallic elements - Estuary Sebou - Morocco

1. Introduction
Many species traded and consumed can cause health problems for the consumer [1,2]. Indeed, some species bioaccumulate metal elements by concentrating the low content of these elements in the biotope to reach high levels in their bodies [3]. Then, by the phenomenon of increasing concentration along trophic chains, the concentration of these substances in many species exceed the threshold harmful to the consumer, especially that occupying the top of the chain [4]. Thus, the evaluation of the content of these so-called bioaccumulative substances has proved an important ecological role. As reported by Kourradi [5] and by other researchers, the use of bioaccumulative species is of great benefit in the assessment of metallic pollution of ecosystems particularly aquatic or marine [6]. These living beings integrate palpable contamination rates during a considered period. This gives them a greater interest in evaluation of the abiotic constituents in which the presence of the contaminant can be fleeting. The metallic analysis of these organisms therefore measures the actual availability of pollutants for biomass. This gives this type of evaluation more meaning than the knowledge of the concentration of a pollutant in the inert medium [7].
Thus, estimating the ratio of pollutant concentrations and their toxic effects measured in benthic organisms has attracted more attention from several researchers in recent years [8].
Moreover, in Morocco, because of the often raw discharges of domestic and industrial wastewater into rivers and the marine environment, estuaries are often in a critical state of pollution; the estuary of Sebou is one [9]. Indeed, the river Sebou along its course and at its estuary large amounts of alluvial materials and pollutants brought by domestic and industrial wastewater discharged by several urban effluents [9]. Among the most harmful compounds spilled are heavy metals. It can thus be deduced that a monitoring of the degree of pollution of the environment by these polluting substances is necessary. In the present work we have been interested in this type of evaluation by estimating the content of many metallic elements in the pulpit of two lamellibranch molluscs: *Scrobularia plana* and *Solen marginatus*.

These two species were chosen to contribute to the determination of biological quality of the Sebou estuary because, in their alimentation, they could ingest some polluting substances. Indeed, molluscs, especially bivalves, are excellent biological indicators for quantifying the availability of heavy metals in their aquatic environment [2].

2. Materials and Methods

For both species studied, sampling of the specimens analyzed was conducted at a station located 4 km from the mouth, which we consider to be located on average in the fluvial estuary along the axis of the cities of Kenitra -Mehdia.

For the harvest of the individuals analyzed, we have followed the same technique that was used for the same purpose by Kourradi [5].

For *S. plana*, with a 1/16 m^2 spade, seasonal samples were taken at low tide. Only adults with a size of 25 to 30 cm were selected.

For *S. marginatus*, seasonal samples, the harvest of the individuals is carried out according to a traditional methodology, often used by local fishermen, which consists in identifying at low tide the external openings of the galleries in *Solen*. Then, a certain quantity of coarse salt is poured into these orifices so as to create momentarily at the level of the gallery a salt concentration so high that the individual is obliged to leave his refuge. The individual was then removed by non-metallic hooks. The size of the individuals chosen for the study was between 8 and 10 cm long.

In the laboratory, for both species, the samples were placed for 48 hours in cooler containing seawater to allow harvested individuals to purge their digestive system. Then, the individuals were rinsed with distilled water, measured using a vernier caliper, cleaned of impurities and freed of their shells using a sterile plastic scalpel. The extracted flesh containing all the vital organs were washed, dried in an oven at 80 ° C for 48 hours to reach a constant weight, then ground with a porcelain mortar before being mineralized. The determination of heavy metals was carried out by atomic absorption spectrophotometry (Zeeman spectrophotometry equipped with a HGA600 graphite furnace, an Air / acetylene flame, a hydride system for the determination of mercury and a smugger sample S.60).

It should also be noted that, in order to compare the contents of the metals studied in the pulpit of the studied species and the content of these same elements in the biotope, we have carried out an analysis of the contents of these elements in the sediment.

3. Results and Discussion

1. Metallic variations in the sediment

The seasonal contents of the heavy metals noted in the sediment of the studied station are grouped in Table II. These results show that:

Iron levels were 26531 mg / kg in Autumn, 18622 mg / kg in winter, 17934 mg / kg in spring and 22327 mg / kg in spring.

For zinc, the lowest value (69.67 mg / kg) was noted in winter, the highest value (114.05 mg / kg) in the spring; 109.22 mg / kg in autumn and 112.61 mg / kg in summer.

Regarding, copper fall content was 46.52 mg / kg, winter is 19.84 mg / kg, spring was 18.93 mg / kg and summer is 17.02 mg / kg.

For lead, the highest value (12.41 mg / kg) was summer and the lowest value (8.21 mg / kg) was winter. Autumn and spring showed intermediate levels (10.05 mg / kg and 11.03 mg / kg, respectively).

2. Variation of the content of the metal elements in the flesh of *S. plana*

The results (Table 1) show that the iron content varies between 1212 mg / kg, noted in winters, and 29658 mg / kg noted in autumn. Spring (20874 mg / kg) is lower than fall, while summer (9628 mg / kg) is intermediate between winter and spring.

Compared with iron concentrations in the sediment, the winter and summer levels of this metal in the flesh are lower than those of the sediment noted during the same period. In contrast, the concentrations of the two compartments are opposite but not too much higher than those noted in the sediment.

Table 1: Overall seasonal levels (in mg / kg) of heavy metals in sediment, flesh of *S. plana* and flesh of *S. marginatus.*

	Contents in the sediment				**Content in the flesh of *S. plana***				**Content in the flesh of *S. marginatus***			
	Fe	**Zn**	**Cu**	**Pb**	**Fe**	**Zn**	**Cu**	**Pb**	**Fe**	**Zn**	**Cu**	**Pb**
Autumn	**26531**	**109.22**	**20.92**	**10.05**	**29658**	**173.6**	**31.21**	**86.24**	**32022**	**28.78**	**13.43**	**1.99**
Winter	18622	69.67	19.84	8.21	1242	144.2	16.93	25.82	1791	27.15	16.31	1.67
Spring	17934	114.05	18.93	11.03	20874	151.2	21.7	79.8	25871	22.35	14.19	2.20
Summer	22327	112.61	17.02	12.41	9628	267	12.5	32	15988	29.64	22.23	1.82
Average	16915	101.83	19.17	10.42	10137.21	184	20.58	55.96	18918	26.98	16.54	1.92

For the zinc, the lowest value (144.2 mg / kg) was winter, the highest value (267 mg / kg) was summer. Spring and autumn show intermediate values (respectively 151.2 mg / kg and 173.6 mg / kg)

Moreover, during all seasons the contents of this metallic element in the flesh were higher than those noted in the sediment. *S. plana* could therefore accumulate the zinc in its flesh with a coefficient of bioaccumulation of 1.5 to 2.

For the copper, autumn and spring had the highest levels (respectively 31.21 mg / kg and 21.7 mg / kg). Winter and summer showed much lower levels (respectively 16.93 mg / kg and 12.7 mg / kg).

As regards the comparison of the copper concentrations noted in the two compartments studied, copper behaves inversely to that of zinc. Thus, it seems that *S. plana* could block the excess entry into his body of this element.

For the lead the autumn and winter showed low values; 26.24 mg / kg in summer and 25.82 mg / kg in winter. The values of spring (79.8 mg / kg) and summer (32 mg / kg) were higher.

Moreover, during all seasons, the concentration of lead in the flesh of *S. plana* was higher than that noted in the sediment.

3. Variation of the content of the metal elements in the flesh of *S. marginatus*

In *S. marginatus* the concentration of iron in the flesh varies seasonally. 32022 mg / kg is noted in autumn, 1791 mg / kg in winter, and 25871 mg / kg in spring and 15988 mg / kg in summer. Except in summer, all the seasonal concentrations

noted in the pulpit are higher than those noted in the sediment. It seems that this species bioacumulates iron in its pulpit.

For the zinc, the levels in the muscle tissues of this species were low compared to those recorded in the sediment. In addition, the seasonal variation of these grades is important. It ranges from 28.78 mg / kg in autumn to 22.35 mg / kg in spring.

Thus, concerning the zinc, *S. marginatus* behaves quite contrary to that of *S. plana*: for all seasons, contrary to *S. plana*, for *S. marginatus*; the zinc concentrations noted in the flesh are lower than those noted at the level of the sediment.

For copper, as for the zinc, grades do not vary much from one season to another. These levels are around 15 mg / kg with a value of 22.23 mg / kg in summer and a value of 13.43 mg / kg in autumn. Thus, in *S. marginatus*, for copper, the same phenomenon that occurs for zinc is for the copper.

It should also be noted that for lead, for all seasons, the concentrations of this metal in the flesh are weak in *S. marginatus* and vary little from one season to the next and are too much lower than those noted in the sediment. They are around 1.9 mg / kg. Thus, it seems that this species has a good ability to avoid the excess of lead that the medium presents.

Moreover, by their position at the end of the continental watercourse, direct terrestrial inputs from runoff and leaching of the emerged lands in the areas crossed estuaries accumulate many pollutants [10, 11]. Often, waste produced by industries along the shoreline as well as waste from human activities around the watercourse and watershed causes high concentrations of metallic elements in the sediments, which are often downstream [12]. Some of these trace elements are essential for living organisms, but increasing their concentration can lead to toxicity phenomena [13]. With regard to living organisms the toxicity of these metallic elements depends on their nature, concentration, mode of action, speciation and bioavailability. In the estuary, the contamination of different living species by heavy metals is done either by ingestion or skin contact. For several species, these metallic elements, once their amount in the body exceeds a certain threshold, they are concentrated in parts of the body. In the long term, the consequences of these concentrations vary depending on the species. Toxic elements present in the estuarine environment can cause, by bioaccumulation, anomalies such as shell deformation in molluscs [14], inhibition of the ciliary activity of their gills [15, 16] etc. It should be noted that the contamination of humans by heavy metals is generally done through the consumption of contaminated food and water.

Furthermore, the results of our present study have shown that the sediment has seasonally variable grades of iron, zinc, Cu and Pb. The variation of the physicochemical conditions of the watershed of the Sebou river under the effect of the climate variation, the flow of the river and the hydrodynamic waters of the estuary are the main causes. For the flesh of the two molluscs studied, the levels in the muscle of the metal elements studied vary from one season to another. As has been reported in some molluscs, by Rodriguez-Rua *et al*. [17], Basraoui *et al*. [18] and Gamain [19] these variations appear to be related to differences in the temperature of the environment and the importance of the availability of the metallic element in the sediment [18].

Similarly, in the flesh of both molluscs, the iron content is high. In *S. plana* the Zn content is much lower than that of the sediment, which is not the case of *S. marginatus*. For copper, with the exception of autumn, there is no great difference between the concentration in the flesh of *S. plana* and that in the sediment. Similarly, copper concentration is less concentrated in the flesh of *S. marginatus* than in sediment. For Lead, it seems that *S. plana* bioaccumulates this element in its flesh and the phenomenon is contrary for *S. marginatus*.

It should be noted that this seasonal variation in the concentration of metals in the sediment, the flesh of *S. marginatus* and the flesh of *S. plana* has been reported by Kourradi *et al*. [20] in the Bouregreg estuary, which is about 35 km south of the Sebou estuary, but sometimes with maximum or minimum concentrations in seasons that differ. These observations are mentioned in other bivalve molluscs as well in Pecten and Chlamys by Bryan [21]. In *S. marginatus*, these variations in seasonality are related to variations in the physico-chemical parameters of the environment and the biological cycle of the natural population studied [20]. It should also be noted that the bioaccumulation of a metallic element in the body of a living being could be related to the stage of development of the individual. For example, the increase in copper content is related to the metabolic activity of molluscs which is in turn dependent on the environmental mesological parameters [19, 22]. In addition, copper is an essential metal that is a vital element associated with the respiratory function of all living organisms. Its requirement is more remarkable when the environment is polluted as is the case of the estuary of Bou Regreg. Similarly, under the influence of the physico-chemical, climatic and hydrodynamic conditions of the estuary, the amount of organic matter dissolved in the water varies, hence its influence on the amount of bioaccumulated copper in the flesh of the studied molluscs [19, 23, 24]. Salinity, for example, for several molluscs, the accumulated contents of zinc, lead and copper are inversely proportional. salinity [25] In turn, pH can intervene in the bioaccumulation of heavy metals by influencing the availability of these metals in the aquatic environment [19,26] and by acting on the physiology of the exposed organisms [27]. The influence of physicochemical factors on the ability of some molluscs to bioaccumulate heavy metals could explain the difference in zinc concentrations, copper and lead in the flesh of *S. plana* and *S. marginbatus* that have been reported in earlier work, namely those of Cheggour [28], Kaimoussi [29], Cheggour *et al*. [30] and Kourradi [5].

4. Conclusion

In the sediment, the iron, zinc, copper and lead contents vary from one season to another. The same phenomenon is noted in the two natural populations of *S. marginatus* and *S. plana* living in the Bouregreg estuary. But, the comparison of the contents noted in the two compartments studied we can say that there is a phenomenon of bioaccumulation of the metallic elements studied. Almost everywhere the concentration of iron is high.

For *S. plana*, in all seasons the zinc levels in the flesh are higher than those noted in the sediment. *S. plana* could therefore concentrate the zinc in its flesh. For the copper behaves inversely to that of the zinc. Regarding lead in all seasons, its concentration in the flesh is higher than that noted in the sediment.

In *S. marginatus* iron concentration, it seems that this species bioaccumulates iron in its flesh but with a lesser degree in summer. Concerning the zinc *S. marginatus* behaves quite contrary to that of *S. plana*. For the lead, it seems that this species has a good capacity to avoid the excess of lead that the medium presents.

It should be noted that this seasonal variability in the concentrations of metallic elements in the flesh of the two molluscs studied and in the sediment is related to the variation of the physicochemical conditions of the environment, the availability of food and the biology of these species.

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