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Evaluation of the concentrations of heavy metals (Cu, Zn Cd and Pb) in *Octopus vulgaris* Cuvier, 1797 (Cephalopoda Octopoda Octopodidae) fished in the port of Ghazaouet (western Algeria)

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ABSTRACT This study aims to determine the accumulation and the seasonal variation of the average concentrations of some heavy metals in various organs (branchial hearts, digestive gland, mantle) in a cephalopod mollusc (the common octopus: *Octopus vulgaris* Cuvier, 1797) in the port of Ghazaouet (extreme western Algeria). The elements Cu, Zn, Cd and Pb were determined using flame atomic absorption spectrophotometry. The study was monitored seasonally in the site, from January 2018 to December 2018. The results obtained revealed that the levels of contamination by heavy metals obey an organotropism between the metal and the targeted organ. Statistical processing (ANOVA) revealed highly significant variations for the average concentrations of heavy metals between octopus organs throughout the sampling campaign, with the exception of zinc ($P > 0.05$). The sample is marked only for cadmium: The summer season and the printed season show the highest levels, while the fall season and winter season show the lowest levels.

KEY WORDS Branchial hearts; Digestive gland; Heavy metals; Mantle; *Octopus vulgaris*.

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INTRODUCTION

The pollution of the aquatic environment by toxic substances of anthropic origin, in particular metallic trace elements (MTE), is one of the major problems facing today's societies. These pollutants contaminate aquatic systems from various point as drainage, wastewater, industrial, agricultural effluents (Ciutat, 2003; Abdel Khalek et al., 2016; Dahri et al., 2018). Thus, MTEs are present in all compartments of the environment both because they are present in natural sources and multiple anthropogenic activities favor their dispersion (Amadi, 2012; Swarnalatha & Nair, 2017). In aquatic environments, these trace elements are distributed throughout the water column, are deposited in bottom sediments and can be consumed by biota (Wu et al., 2014). Coastal cities have often been identified as hotspots for several types of pollution. In the Algerian coastal zone, untreated industrial and domestic wastewater effluents represent a major source of chemical contamination for the local aquatic environment (Inal et al., 2018). Marine resources are an obligatory part of the alimentation of many people around the world. As such, regular monitoring of trace element concentrations in aquatic environments and related food chains is important to prevent toxic effects in humans consuming aquatic organisms. Between these, the octopus and similar species (Cephalopoda) feed on these organisms and bioaccumulate higher concentrations of some of these elements in their tissues (Napoleão et al., 2005; Rjeibi et al., 2014; Roldán-Wong et al., 2018).

Due to their territorial nature and small area of activity (Arechavala-Lopez et al., 2018), the octopus (*Octopus vulgaris* Cuvier, 1797: Cephalopoda Octopoda Octopodidae) can provide useful information on adaptations to coastal habitats and serve as bioindicators on the quality of coastal environments (Boyle et Knobloch, 1982; Miramand et al., 2006). In Algeria, we did not find enough studies on the content of heavy metals in *Octopus vulgaris*. Therefore, we considered it necessary to provide data on the concentrations of heavy metals in the organs in these species in order to establish an inferred contamination gradient between these organs and a seasonal assessment of the quality of the marine environment. To this end, the bioavailability and bioaccumulation of heavy metals (Zn, Cu, Pb and Cd) in the organs of *O. vulgaris* (branchial hearts, digestive gland, mantle) were evaluated on the port of the far west of Algeria Ghazaouet. Also, we proceed to the comparison of the heavy metal contents between the different organs studied and sampling seasons.

MATERIAL AND METHODS

The study area is located in the extreme northwest of Algeria (35°06' N - 1°52' W). It is a fishing and commercial port located about thirty kilometers, as the crow flies, east of the Algerian-Moroccan border, and 45 km from Messali El Hadj airport in Tlemcen, 130 km northwest of the city of Oran (Fig. 1). For this study, octopuses sampling was carried out at the port of Ghazaouet(western Algeria), as soon as the trawlers arrived, with the help of local fishermen, monthly between January and December 2018. The samples were immediately kept in plastic bags, sealed and stored in a cooler for transport to the laboratory. These samples were stored separately in these individual plastic bags and frozen at low temperature to minimize the mobilization of metals between organs and tissues (Martin & Flegal, 1975) until further use. Total lengths and weights were measured for each octopus. The specimens were then immediately dissected and the digestive gland, gill hearts, and mantle of each octopus were completely removed without rupturing the outer membrane. A total of one hundred and eighty (180) sub-samples were taken during the annual sampling campaign. The weight of the removed organs is noted before their conservation in Petri dishes, these samples are then frozen at low temperature. All samples underwent mineralization. Mineralization by dry voice was used and the protocol described is that adopted by Kjeldalh-F.A.O. (1960). After thawing, the samples are weighed: 1 gram of digestive glands, branchial hearts, and mantle are placed in a crucible which are then placed in the oven at a temperature of 110 °C for 3 hours. They are then placed in a muffle oven for 15 min at 450 °C then they are moistened with nitric acid ($HNO3$) and placed back in the oven at 350 °C for 1h and 30 min.

The solutions obtained from the different mineralizations were filtered using filter paper with a porosity of 0.45 µm. Filtration is done with a 1%

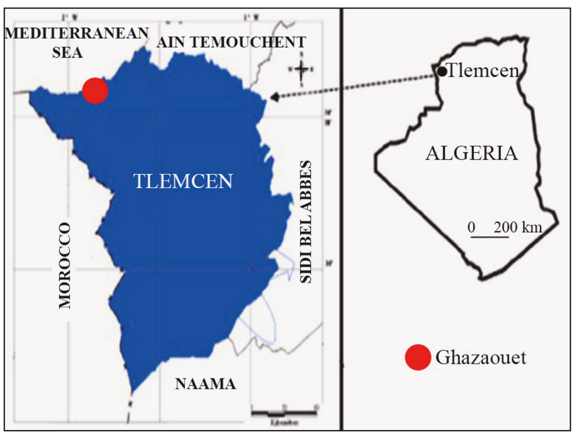

Figure 1. The study area located on the extreme western coast of Algeria.

acidulated water solution; must be adjusted to 25 ml of a distilled water/nitric acid mixture then they were put in buckets and kept cool until analysis by atomic absorption spectrophotometer with flame of the Perkin Elmer AA100 type. The calibration was carried out with a relative range with the concentration of the samples, for each metal to be analyzed, the samples were taken from the stock solutions. The concentrations of the biological samples must be within the range of the concentrations of the standard solutions (Pinta et al., 1979). As a result, a standard curve relating to the concentration of our samples was drawn from the standard solutions. A blank has been introduced in each series of analysis, so that we can correctly read our flame AAS solutions. The quantified elements are zinc, copper, lead and cadmium. Also, the emission lines and the detection limits of the device are grouped in Table 1. The average metal concentrations were calculated with their standard deviations. The statistical processing of the data is carried out using the Minitab program (version 16). ANOVA was applied to the results obtained to quantify and test the effect of organs and the effect of sampling seasons on metal levels.

RESULTS

The average heavy metal contents in the organs of *O. vulgaris* (branchial hearts, digestive glands and mantle) are recorded for the three organs, and presented in Table 2 and illustrated by Figs. 2 and 3.

The Cu contents recorded in the gill hearts (2.40 mg/kg of dry weight) exceed those noted in the digestive gland and the mantle (1.894 and 1.005 mg/kg, respectively).

The mean Zn contents do not vary much between the organs of *O. vulgaris* ($p > 0.05$), they are 1.666 mg/kg of dry weight noted in the digestive gland and 1.58 and 1.465 mg/kg of dry weight recorded respectively in the mantle and the branchial hearts.

The distribution behavior of cadmium indicates that the highest level of this metal was recorded in the digestive gland (3.116 mg/kg of dry weight) compared to those noted in the branchial hearts and the mantle (2.283 and 0.111 mg/ kg of dry weight, respectively).

Regarding lead, a very low content (0.091 mg/kg dry weight) was found in the mantle, compared to that reported in the digestive gland and branchial hearts (2.121 and 1.842 mg/kg dry weight, respectively).

The average heavy metal contents in the organs of *O. vulgaris* are recorded according to the seasons, and presented in Table 3 and illustrated in figure 4.

Figure 4 summarizes the results of the analyzes of the metallic elements (Cd, Pb, Cu, and Zn) obtained from a large number of samples analyzed by atomic absorption spectrophotometry (AAS) over a period of one year (2018). We compare the seasonal metal concentrations in the organs of *O. vulgaris* in the port of Ghazaouet.

Taking into account the essential elements, the average copper and zinc contents noted in the different organs of *O. vulgaris* according to the seasons show significant contents with an almost identical maximum average concentration ($p > 0.05$) recorded in summer and in spring with a maximum in summer (2.353 ± 1.024 mg/kg dry weight; 2.32 ± 0.813 mg/kg dry weight), and in spring with

Elements	Wavelength (nm)	Limits of detection (ppm)
Copper	324.7	0.05
Zinc	213.9	0.05
Cadmium	228.8	0.03
Lead	283.3	0.10

Table 1. Wavelengths and detection limits of metals assayed by flame ASS.

	Branchial hearts	Digestive glands	Mantle
Cu	2.40 ± 0.871	1.894 ± 0.852	1.005 ± 0.572
Zn	1.465 ± 0.629	1.666 ± 0.761	1.58 ± 0.554
Cd	2.283 ± 0.765	3.116 ± 0.72	0.111 ± 0.046
Pb	1.842 ± 0.942	2.121 ± 0.774	0.091 ± 0.053

Table 2. Mean concentrations of heavy metals (Cu, Zn, Cd, Pb) in the organs of *Octopus vulgaris* (means ± standard deviation) from January to December 2018 expressed in mg/kg dry weight.

(1.795 ± 0.723 mg/kg dry weight; 1.915 ± 0.675 mg/kg dry weight, respectively). Considering non-essential metals, cadmium stands out by revealing significant levels. The highest values are recorded in summer and spring, while they decrease in autumn and winter ($p < 0.05$). It is noted that the most important values are of the order of 2.688 ± 0.566 mg/kg of dry weight and 2.178 ± 0.569 mg/kg of dry weight obtained respectively in the summer season and in the spring season. In the case of lead, an almost identical maximum average concentration noted in the summer season and in the spring season (1.946 ± 0.791 mg/kg dry weight; 1.863 ± 0.799) respectively ($p > 0.05$).

DISCUSSION

Most studies on heavy metals in octopus have been performed with *Octopus vulgaris* from the coasts of Portugal and the Mediterranean Sea (Miramand & Guary, 1980; Raimundo et al., 2004; Napoleão et al., 2005; Raimundo et Vale, 2008; Raimundo et al., 2010, b; Semedo et al., 2012). The digestive gland, the branchial hearts and the mantle are the most primordial organs from a toxicological point of view, because of their key role in the phenomena of metabolism and accumulation. The mantle is usually analyzed because it is the main part of octopus consumed by humans and is implicated in health risks. Our present study carried out in the port of Ghazaouet made it possible to formulate correlations between the concentrations of metals and the order of their bioaccumulation in the accumulating organs (Fig. 2). Statistical analysis using ANOVA 1 shows highly significant variability between octopus organs ($p < 0.01$) for all the metals studied, except for Zn ($p > 0.05$). As a result, the following accumulation gradients have been established (Fig. 2):

	Autumn	Winter	Spring	Summer
Cu	1.532±0.622	1.384±0.691	1.795±0.723	2.353±1.024
Zn	1.001 ± 0.565	1.045 ± 0.539	1.915 ± 0.675	2.32 ± 0.813
Cd	1.491 ± 0.399	0.988 ± 0.507	2.178 ± 0.569	2.688 ± 0.566
Pb	0.944±0.342	0.654±0.426	1.863±0.799	1.946±0.791

Table 3. Average concentrations of heavy metals (Cu, Zn, Cd, Pb) according to the seasons (averages ± standard deviation expressed in mg/kg dry weight) from January to December 2018 expressed in mg/kg dry weight.

Cu: Branchial hearts > Digestive gland > Mantle ($p < 0.01$).
Zn: Digestive gland ≈ Mantle ≈ Branchial hearts ($p > 0.05$).
Cd: Digestive gland > Branchial hearts > Mantle ($p < 0.01$).
Pb: Digestive gland > Branchial hearts > Mantle ($p < 0.01$).

Similarly, it was possible to establish orders of accumulation of heavy metals for each organ, which is presented as follows (Fig. 3):

Branchial hearts: Cu > Cd > Pb > Zn.
Digestive gland: Cd > Pb > Cu > Zn.
Mantle: Zn > Cu > Cd ≈ Pb.

The metals sought (Cd, Pb, Cu, Zn) showed organotropism for the sampled organs. We found a highly significant difference between *Octopus vulgaris* organs in mean copper, cadmium and lead contents ($p < 0.01$); whereas for zinc, this variability does not exist ($p > 0.05$). Metal concentrations differ considerably among the three octopus organs, apparently due to the role of metals in metabolic functions (Raimundo Pimenta, 2010). Given the ability of the digestive gland to accumulate higher Cd levels than the mantle, as reported (Roldán-Wong et al., 2018). The highest levels of Cd are found in the digestive gland (Fig. 2) confirming the main role of this organ in the processes of bioaccumulation and detoxification of Cd (Bustamante et al., 2002; Storelli et al., 2010). Cd and Pb favor the digestive gland, followed by the gill hearts and the mantle. The presence of Cd and Pb at a higher concentration in the digestive gland and the branchial hearts throughout the sampling campaign were interpreted as being due to pollution sources (Raimundo et al., 2004). In contrast, copper shows a preference for gill hearts (Fig. 2). This is not surprising in which Cu is one of the main components of respiratory pigment hemocyanin (Craig & Overnell, 2003; Villanueva & Bustamante, 2006). Although this organ represents only 0.2% of the total weight, it is nevertheless an important site for the storage and release of elements (Mangold et al., 1989; Miramand & Fowler, 1998).

Branchial hearts also exhibited increased levels of non-essential elements (Cd and Pb) (Fig. 3) that may be related to specific ligands or excretion and detoxification mechanisms (Raimundo Pimenta, 2010). Zinc is an essential element for most cephalopods, significant differences in the concentrations of Cu, Cd and Pb existed in the mantle and the two other organs throughout the sampling campaign, compared to those obtained for Zn , where the contents between the organs vary little (Fig. 2).

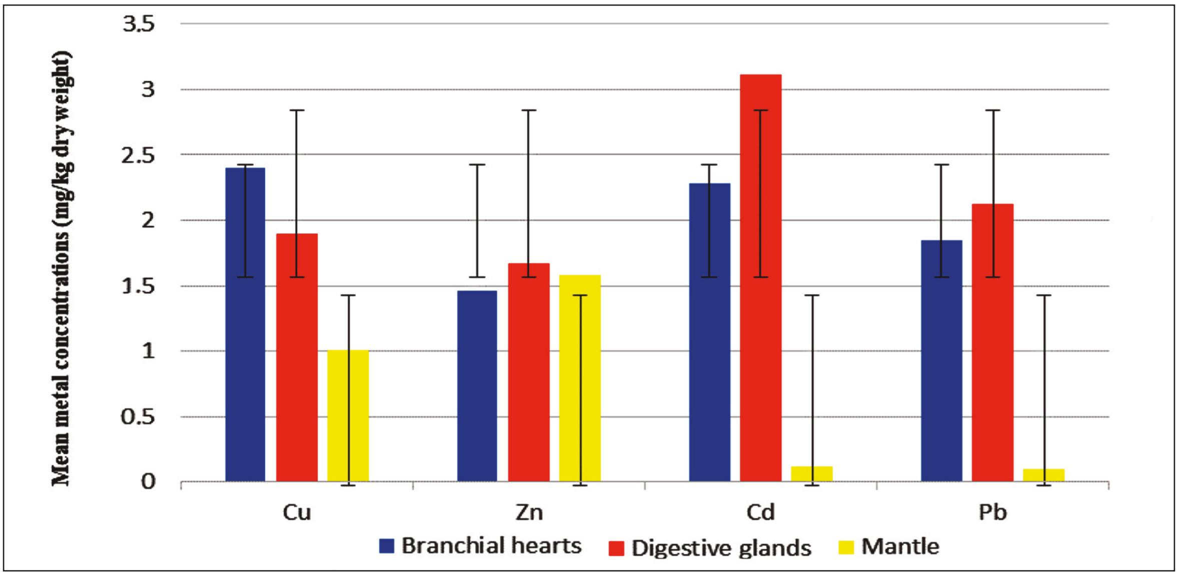

Figure 2. Partitioning of mean heavy metal (Cu, Zn, Cd, Pb) levels (means ± standard deviation expressed in mg/kg of dry weight) in the organs of *Octopus vulgaris* (branchial hearts, digestive glands, mantle) during the annual sampling campaign.

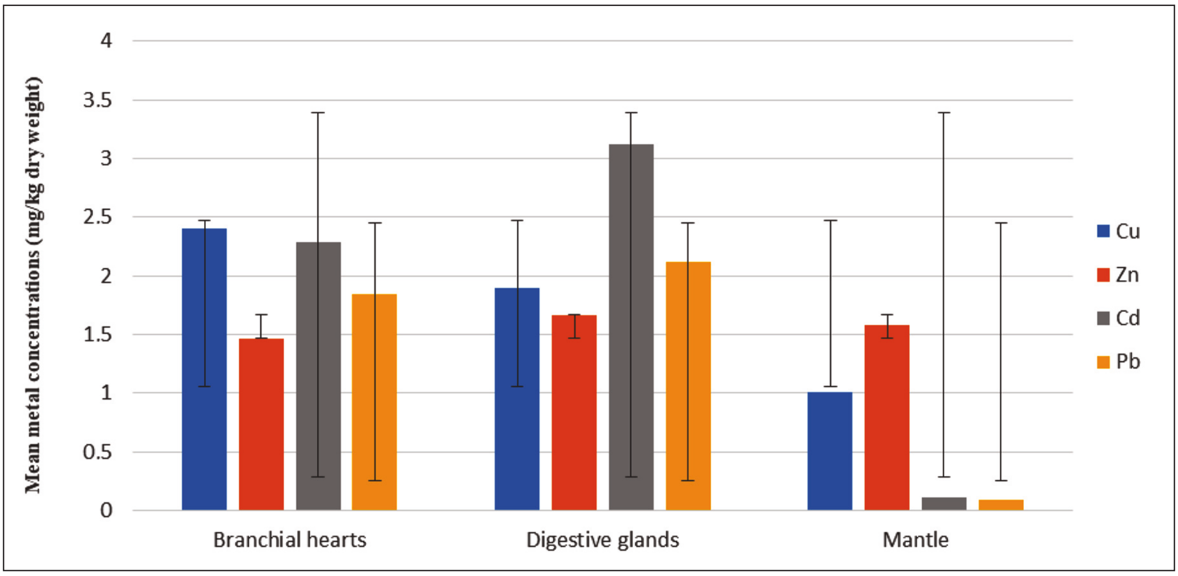

Figure 3. Orders of accumulation of heavy metals (Cu, Zn, Cd, Pb) (means ± standard deviation expressed in mg/kg of dry weight) for each organ of *Octopus vulgaris* (branchial hearts, digestive glands, mantle) during the annual sampling campaign.

Moreover, high levels of Cu and Zn are linked to their role as essential elements involved in various metabolic processes, such as the formation of metal-dependent enzymes involved in digestion (Craig & Overnell, 2003). The diet of cephalopods represents a major exposure pathway for many elements including Cu and Zn (Bustamante et al., 2004, 2006a). This should explain the high values of the essential elements reported in this study. Metal levels in the present study were low in all mantle samples except for copper and zinc (Fig. 3). The same observation was retained in the majority of studies (Napoleão et al., 2005; Raimundo & Vale, 2008). This can be explained by the eco-toxicological behavior of this organ related to excretion towards bioaccumulation processes. However, some reports indicate that octopus mantle and arms exceed the maximum heavy metal content in international standards for human consumption, posing a risk to human health (Rossi et al., 1993; Grayson & Sekadende, 2014). Risk assessments carried out to date (local effects in the Adriatic Sea, South Korea and Tunisia) indicate the absence of risk of eating muscle tissue (the most commonly eaten) and the possible risk of eating internal organs (consumed in Japan, South Korea or Italy) (Shingaro, 2017). The bioaccumulation of heavy metals in octopus occurs differentially in the various organs and tissues, the digestive gland and the branchial hearts being the main sites of storage and detoxification (Nessim & Riad, 2003; Raimundo & Vale, 2008; Raimundo et al., 2010).

At the end of the results obtained, we found that seasonal variations do not seem to govern the distribution of Cu, Zn and Pb ($p > 0.05$) except for Cd ($p < 0.05$). An upward trend in average levels was noted during the summer and spring periods and more so in the autumn and winter periods (Fig. 4). Statistical analysis using ANOVA1 shows a significant difference ($p < 0.05$) noted only for Cd contents. As a result, the seasonal accumulation order is as follows (Fig. 4):

Cu: Summer season ≈ Spring season ≈ Autumn season ≈ Winter season ($p > 0.05$).

Zn: Summer season ≈ Spring season ≈ Winter season ≈ Autumn season ($p > 0.05$).

Cd: Summer season > Spring season > Autumn season > Winter season ($p < 0.05$).

Pb: Summer season ≈ spring season ≈ Autumn season ≈ Winter season ($p > 0.05$).

Overall, the analysis of variance has only one factor (ANOVA 1) carried out on the metal content in octopus *Octopus vulgaris* from the port of Ghazaouet and it reveals that the sampling season did not present any significant difference ($p > 0.05$) for Cu, Zn and Pb while only one significant difference between seasons is observed corresponding to the accumulation of Cd ($p < 0.05$), leading to a higher accumulation in the summer and spring season than in the autumn and winter season (Fig. 4). Following our present study, we found that seasonal variations seem to govern the distribution of cadmium which fluctuates considerably in the organs of *Octopus vulgaris* ($p < 0.05$). An upward trend in average levels was noted for most pollutants during the summer and spring periods, in particular for cadmium and moreover in the autumn and winter periods. The seasonal variation of cadmium concentrations in organs could be particularly attributed to physiological processes, including those of reproduction as well as the variation of certain physico-chemical parameters of the environment (pH, salinity, temperature) which have a role in the bioavailability of heavy metals, and the effect of biological parameters (i.e., size, mass, and sex) on metal accumulation in cephalopods is far from consensual (Miramand & Bentley, 1992; Bustamante et al., 1998; Bustamante et al., 2006b; Pierce et al., 2008).

CONCLUSIONS

In the present study, the digestive gland, the branchial hearts and the mantle of *O. vulgaris* are selected as the target organ for the evaluation of the accumulation of heavy metals (Cd, Pb, Cu, Zn). The results obtained revealed the following points:

- There is an influence of the "Organ" factor on the Cu, Cd, Pb contents in the *O. vulgaris* except for zinc (ANOVA 1). The digestive gland and the branchial hearts, metabolically active tissues, tend to accumulate cadmium, lead and copper to a greater degree than the mantle, which generally has a low potential for accumulation of these metals;
- Lead and cadmium are abundant in the branchial hearts and the digestive gland. These are non-essential metals which are not subject to regulation, their concentration increases in the organs according to the levels in the environment;

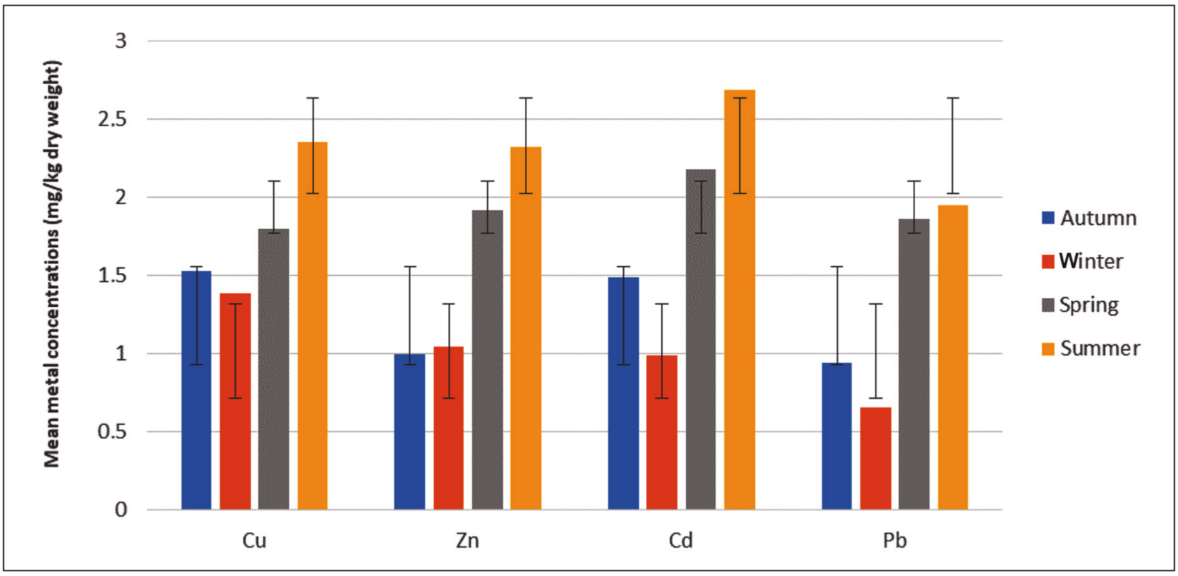

Figure 4. Seasonal variations in average concentrations of heavy metals (Cu, Zn, Cd, Pb), averages ± standard deviation expressed in mg/kg dry weight.

• Cadmium preferentially accumulates in internal organs such as the digestive gland, but is weakly concentrated in the external organs (mantle), where the concentrations are generally very low;

• High levels of Cu and Zn are related to their role as essential elements;

• Branchial hearts have priority for copper;

• The mantle preferentially concentrates zinc and copper;

• The importance of the metallic accumulation is done according to the following order:

Digestive gland > Branchial hearts > Mantle. For Cd and Pb;

Branchial hearts > Digestive gland > Mantle. For Cu;

• The organs of *O. vulgaris* seem to bioaccumulate heavy metals in the summer season and in the spring season, in particular for cadmium (ANOVA 1).

Therefore, the digestive gland appears to be a good indicator of octopus exposure to metal contaminants, especially cadmium, and could provide a new tool for monitoring the geographical distribution of this metal.

This work has as its logical continuation its extension to all Algerian ports. In addition, other chemical and organic contaminants should be included and the list of trace metal elements sought should be expanded.

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