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Trace Metals (Cu, Cd, Pb, Zn, and Ni,) Concentrations in the Sea Cucumber *Holothuria tubulosa* (Gmelin, 1791) of the Algerian West Coast

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ABSTRACT

The present work was organized to evaluate the trace metal contaminations of Cu, Cd, Pb, Zn, and Ni in the organs of *Holothuria tubulosa* (Gmelin, 1791), at two sites on the Algerian west coast; Ain Franine (AF) and Sidi Lakhdar (SL). The obtained concentrations revealed the presence of heterogeneous metal contents and showed that the most important concentrations were relatively detected in zinc, with values of the male gonads at the level of AF (16.39 ± 2.38 mg / kg PF) for winter and SL (4.65 ± 0.59mg / kg PF) for summer. The lowest zinc values were recorded on the integument for the two sites. The highest Cu values were recorded in spring on the integument of two groups of *Holothuria tubulosa*. Nickel concentrations showed a high value in the integuments in males (1.08 ± 0.03mg / kgP.F) at AF during summer and at SL in spring (0.93 ± 0.02mg / kg PF). While they recorded the lowest values in winter for sea cucumbers of FA and in spring for those of SL for the same organ. The lead concentrations revealed high values on the integument with (1.77 ± 0.56 mg / kg PF) in winter for SL sea cucumbers and (1.36 ± 0.04 mg / kg PF) in summer for those from AF. Moreover, cadmium targeted the same organ as lead where high values were noticed on the integuments during fall for the two communities of sea cucumbers of the order of (0.51 ± 0.03 mg / kg PF) to AF and from (0.43 ± 0.01mg / kg PF) to SL. The analysis of the metal concentrations obtained revealed that, despite the difference in biotope, the two communities of *Holothuria tubulosa* did not present a significant difference (P> 0.005) for all the metals considered.

INTRODUCTION

Recently, environmental bio monitoring strategies are more than ever at the heart of the concerns of the studying groups from international bodies. The use of chosen bio-indicators within the affected marine communities represents one of the means of assessing the impact of human development on marine ecosystems to prevent critical situations by setting up restoration and conservation tools.

The species most often frequenting Algerian waters are *Holothuria forskali*, *Holothuria stellati*, *Holothuria* (Roweothuria) *poli*, *Holothuria* (Platyperona) *sanctori* and *Holothuria tubulosa* **(Mezali, 2008**), which contribute to the bioturbation of sediments, and stabilize the bacterial community in sediment and convert detritus into nitrogen compounds, thereby improving primary productivity **(Işgören-Meysman *et al.*, 2006; Emiroğlu & Günay, 2007; MacTavish et *al.*, 2012, Xing *et al.*, 2012)**. The disappearance of sea cucumbers in some areas has led to a hardening of the sediments, thus reducing the habitat of some benthic organisms.

The current study is related to a species of *aspidochirotida holothurians* that is massively widespread on the Algerian coasts, considering that *Holothuria tubulosa* is not exploited and is dependent on the *Posidonia herbarium* of the coast. The present work relatively monitored marine pollution, and more specifically, metal xenobiotics on the Oran coast to possibly define its status as a bio-indicator species at the second site on the Oran coast or on the mostaganem coast.

MATERIALS AND METHODS

1. Sampling sites

The Ain Franin site is located in the east of Oran with geographical position of 35° 46 '49.78 N and 0 ° 31' 01.51 W (Fig. 1). It is situated between two capes forming the Gulf of Oran, Cap Ferrat in the north and Cap Falcon in the south-east, about 28 km from Kristel. Eminently, it is considered a little impacted area because human activities are very limited **(Belkhedim *et al*., 2014; Chahrour *et al.*, 2014)**. The second site is located in the east of Mostaganem (SL: Sidi Lakhdar), with a geographical position of (36° 12'40"63 ° N) and (0° 23'20"78 ° E). It is an area characterized by a loose substrate rich in photophilic algae and the presence of Posidonia meadows (*Posidonia oceanica*) (**Dermeche *et al.*, 2012**).

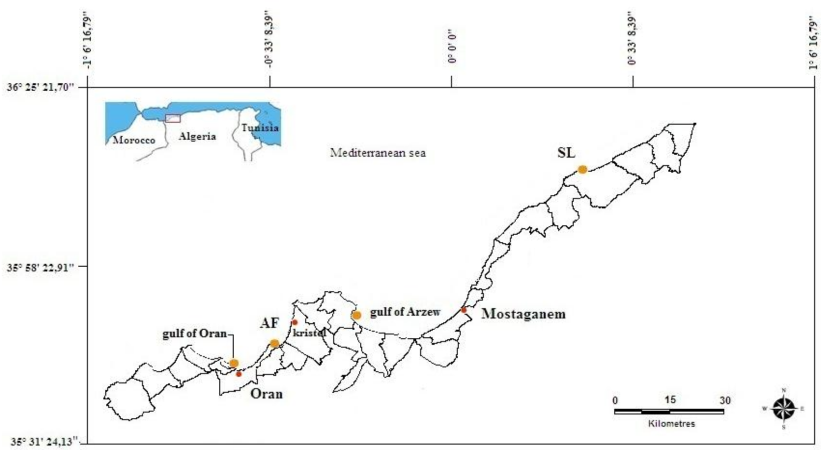


Fig. 1 : A map of the study area; Ain Franine (AF) and Sidi Lakhdar (SL), West Algerian Coast.

2. Collection of specimens

Sea cucumber (n = 20) were monthly collected randomly by hand by scuba diving from October 2018 to October 2019, in the shallow water (6 meters) of the two study sites. Specimens were placed in a plastic bag containing water from the surrounding environment. Then, they were stored in functioning aquariums for at least 4 hours before being measured, weighed and dissected **(Coulon, 1994)**. To determine heavy metals, four compartments were taken into consideration; namely, the male and female gonads separately taken (G♂, G♀), the intestine without its digestive content (Int), as well as the integument (Teg). They were placed in 4 labeled pillboxes for subsequent mineralization and analysis using S.AA according to the protocol of **Saniye Turk Culha (2016, 2018)** in the Red Sea, Jordan.

RESULTS AND DISCUSSION

The calculation of the various means of concentrations ± the standard deviations of the trace metals was carried out to express the finest possible reflection of the variation at contamination levels (Tables 1 & 2).

Table 1: Average (n = 20) seasonal concentrations of Cu, Cd, Pb, Zn, and Ni in *Holothuria tubulosa*, expressed in ppm F.W (Ain Franine site).

Trace Metals	***Organs***	***Autumn***	***Winter***	***Spring***	***Summer***
Cu	G♂	0,23±0,014	0,52±0,1	0,30±016	0,25±0,015
	G♀	0,2±0,01	0,19±0,01	0,36± 0,04	0,3±0,11
	Int	0,19±0,01	0,37±0,04	0,30±0,11	0,2±0,01
	Teg	0,66±0,018	0,76±0,03	0,96±0,01	0,63±0,026
Zn	G♂	1,38±0,37	16,39±2,38	4,21±0,48	2,09±0,88
	G♀	2,68±0,82	2,12±0,26	14,46±3,1	4,41±3,39
	Int	2,12±0,36	10,46±2,1	4,41±0,39	2,68 ±0,82
	Teg	2,25±0,03	4,08±0,17	3,3±0,68	4,25±0,39
Pb	G♂	0,01±0,001	0,45 ±0,023	0,11±0,023	0,05±0,021
	G♀	0,12±0,05	0,04±0,006	0,18±0,02	0,13±0,05
	Int	0,04±0,005	0,28±0,03	0,13±0,05	0,12±0,05
	Teg	0,92±0,007	0,99±0,04	0,69±0,031	1,36±0,045
Cd	G♂	0,05±0,004	0,1±0,02	0,09±0,003	0,03±0,004
	G♀	0,03±0,05	0,06±0,005	0,15±0,01	0,03±0,005
	Int	0,06±0,005	0,20±0,02	0,09±0,005	0,03±0,005
	Teg	0,51±0,032	0,48±0,04	0,35±0,014	0,43±0,018
Ni	G♂	0,04±0,016	0,43±0,014	0,19±0,017	0,08±0,017
	G♀	0,05±0,023	0,02±0,032	0,6±0,03	0,22±0,024
	Int	0,02±0,023	0,7±0,03	0,22±0,024	0,05±0,024
	Teg	0,56±0,032	0,47±0,02	0,84±0,029	1,08±0,035

Table 2: Average seasonal concentrations of the various pollutants (Cu, Cd, Pb, Zn, Ni,) in Holothuria tubulosa expressed in ppm F.W. (Sidi Lakhdar site).

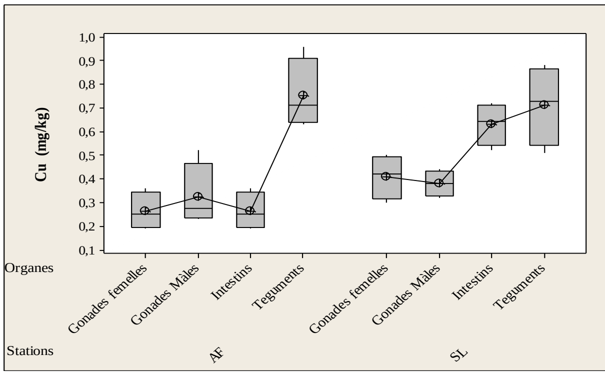
Metals	***Organs***	***Autumn***	***Winter***	***Spring***	***Summer***
Cu	G♂	0,35±0,09	0,44±0,011	0,32±0,013	0,41±0,013
	G♀	0,48±0,02	0,3±0,017	0,5±0,016	0,36±0,054
	Int	*0,72 ±0,028*	*0,52±0,025*	*0,6±0,024*	*0,69±0,17*
	Teg	0,88±0,027	0,63 0,026	0,51±0,025	0,83±0,08
Zn	*G♂*	*2,35±0,86*	*2,66±0,78*	*2,1±0,42*	*2,58±0,14*
	G♀	4,41±0,22	4,23 ±0,031	3,3±0,78	4,65±0,59
	Int	*0,93±0,053*	*0,74±0,048*	*3,77±0,71*	*0,78±0,034*
	Teg	1,11±0,044	1,08±0,041	1,12±0,042	0,72±0,012
Pb	*G♂*	*0,09±0,007*	*0,1±0,06*	*0,3±0,006*	*0,15±0,08*
	G♀	0,03±0,021	0,04±0,003	0,06±0,03	0,04±0,021
	Int	*0,12±0,018*	*0,28±0,015*	*0,22±0,016*	*0,39±0,19*
	Teg	1,62±0,048	1,77±0,56	1,09±0,054	1,39±0,036
Cd	*G♂*	*0,08±0,03*	*0,12±0,04*	*0,06±0,004*	*0,08±0,004*
	G♀	0,12±0,005	0,06±0,004	0,09±0,011	0,06±0,029
	Int	*0,12±0,018*	*0,28±0,015*	*0,08±0,026*	*0,15±0,02*
	Teg	0,43±0,013	0,39±0,09	0,31±0,008	0,37±0,04
Ni	*G♂*	*0,08±0,002*	*0,09±0,05*	*0,18±0,09*	*0,46±0,017*
	G♀	0,03±0,018	0,16±0,015	0,43±0,18	0,2±0,025
	Int	*0,08±0,018*	*0,05±0,016*	*0,47±0,019*	*0,26±0,025*
	Teg	0,5±0,023	0,64±0,022	0,93±0,026	0,73±0,011

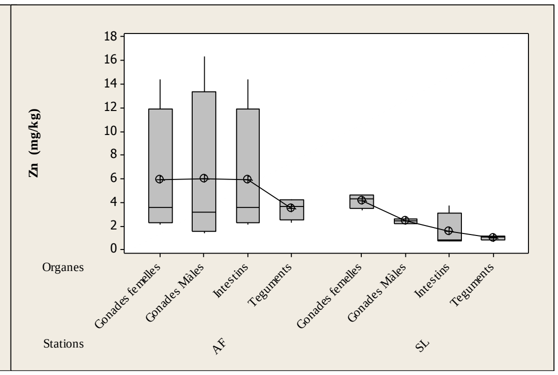
The results revealed the presence of heterogeneous metal contents, and showed that the highest concentrations were relatively recorded in zinc, with gonadal values in males of Ain Franine in winter (16.39 ± 2.38 mg / kg F.W) and in males from Sidi Lakhdar in summer (4.65 ± 0.59 mg / kg F.W.). While, the lowest values were recorded on the integuments of the two considered sites. Notably, Zinc is an essential metal **(Turk Culha *et al.*, 2016)**; however, a high concentration can cause physiological disturbances in the body **(Dermeche *et al.*, 2012; Al-Najjar *et al.*, 2018)** The Cu concentrations in the gonads of males of Ain Franine were 0.52 ± 0.1 mg / kg F.W. during winter; whereas, Cu conentrations in females of Sidi Lakhdar during spring were 0.50 ± 0, 01mg / kg F.W. It is worth mentioning that, the highest values were found in the spring in the integuments of the two groups of Holothuria tubulosa. It was noticed that, Nickel concentrations showed a high value in the integuments of males (1.08 ± 0.03mg / kg) at Ain Franin during the summer and 0.93 ± 0.02mg / kg in Sidi Lakhdar during the spring. Nevertheless, they recorded the lowest values in winter in Ain Franine and in spring for those of Sidi Lakhdar for the same organ. Furthermore, the lead concentrations revealed high values in the integuments with 1.77 ± 0.56 mg / kg in winter in Sidi Lakhdar and 1.36 ± 0.04 mg / kg PF in summer for those in Ain Franine. Additionally, cadmium targeted the same organ as lead where high values were noticed in the integuments during the fall for the two populations of sea cucumbers of the order of 0.51 ± 0.03 mg / kg to Ain Franine and 0.43 ± 0.01mg / kg in Sidi Lakhdar. The study and the 2-factor analysis of variance (station and organs) revealed a significant difference for Cu, Ni, Pb, Cd (P 0.005), while for Zn no significant difference was recorded (Table 3).

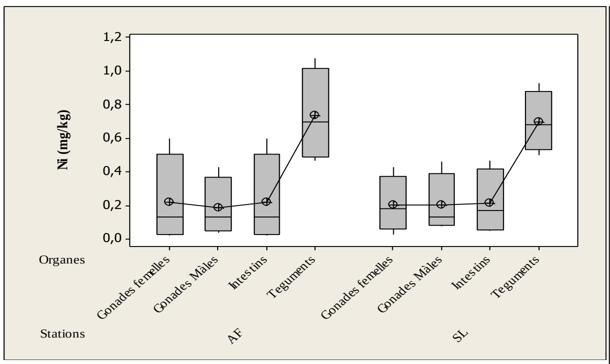
Table 3: Results of two factors Anova for metal concentrations at the two sites and different *Holothuria tubulosa* organs (organ-station) .0.000 ** (Highly significant difference).

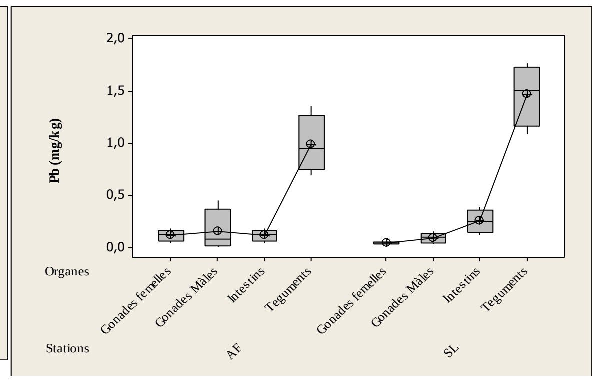
	Source	***ddl***	***Sum of squares***	***Means of squares***	***F value***	***P value***
	Stations	1	0,1417	0,1417	10,94	0,003
Cu	Organes	3	0,8086	0,2695	20,80	**0,000****
	Interactions	3	0,1847	0,0615	4,75	0,010
	Stations	1	74,298	74,2980	4,97	0,035
Zn	Organes	3	33,095	11,0316	0,74	0,539
	Interactions	3	8,006	2,6688	0,18	0,910
	Stations	1	0,0010	0,0010	0,02	0,885
Ni	Organes	3	1,5634	0,5211	11,00	**0,000****
	Interactions	3	0,0031	0,0010	0,02	0,995
	Stations	1	0,1128	0,1128	3,97	0,058
Pb	Organes	3	7,2935	2,4311	85,51	**0,000****
	Interactions	3	0,3987	0,1329	4,67	0,010
Cd	Stations	1	0,0196	0,0196	1,88	0,183
	Organes	3	0,4231	0,1410	13,55	**0,000****
	Interactions	3	0,0151	0,0050	0,49	0,695

The analysis of all the metal concentrations revealed that, despite the difference in the biotope of the two populations of *Holothuria tubulosa* with respect to the geographical position and nature of the substrate as well as the anthropogenic contribution. The latter showed no significant difference (P> 0.005) for all the considered metals, while the organs showed a significant difference (P> 0.005) (Fig. 2 & 3).









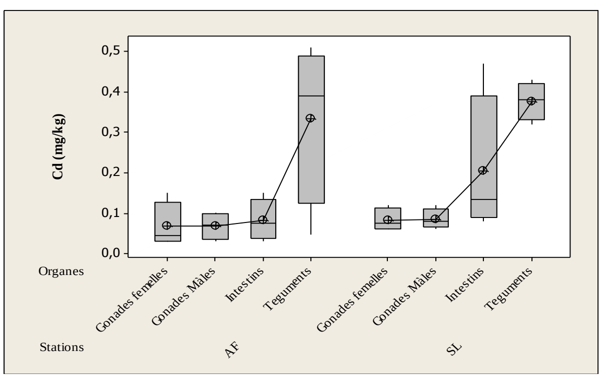
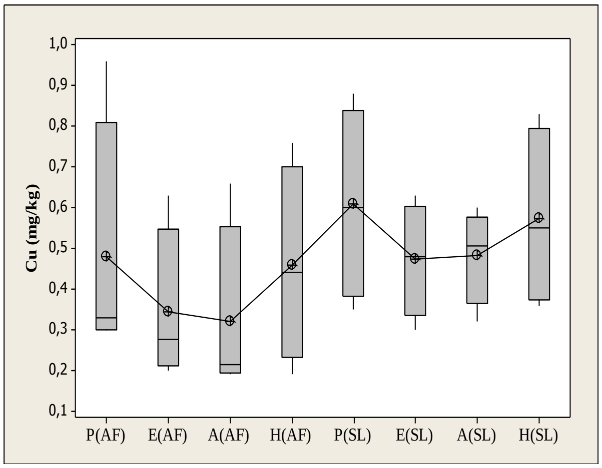


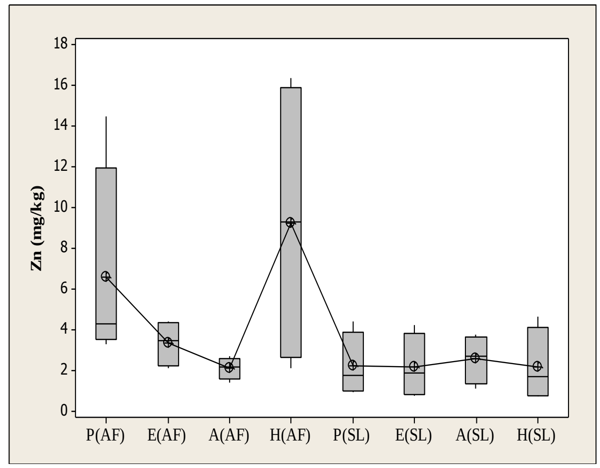
Fig. 2: Boxplots represent the metal concentrations in *Holothuria tubulosa* in each study site (Sidi Lakhdar and Ain Franine) and each organ. Those boxplots are bounded by quartiles 1 and 3 and have a line showing the median. Standard deviations are shown in solid lines.

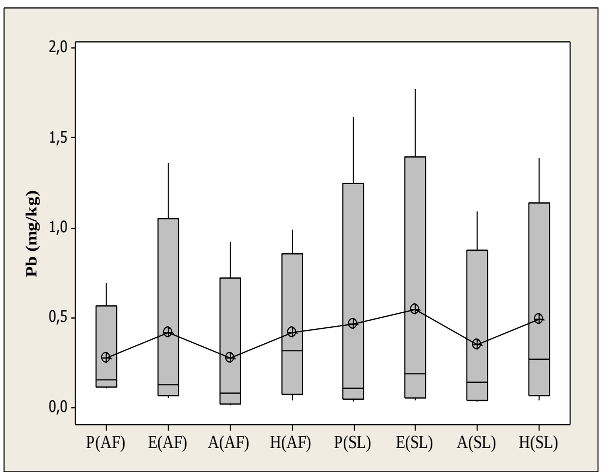
The comparison of two-way ANOVA (season, trace metals), represented in the boxplot graph (Fig. 2), represents a seasonal variation of the metal contamination at two sites which showed that the sea cucumber community of the two sites displayed similar concentrations in the four seasons for Cu, Ni, Pb and Cd (Fig. 2). However, the distribution was not symmetrical in all the corresponding graphs, which translates the non-existence of a seasonal variability in concentrations (no significant difference) with the exception of Zinc where a slight difference (P = 0.044) was detected for the two sites for the spring and winter seasons. This significant difference in Zn concentrations is related to the role of this essential element during gonad maturity, which coincides with the activation of secondary metabolism (Saponins) during this physiological activity **(Louiz *et al.*, 2003 ; Turk Culha *et al.*, 2016 Al-Najjar *et al*., 2018 ; Rdiansyah *et al.*, 2020).**

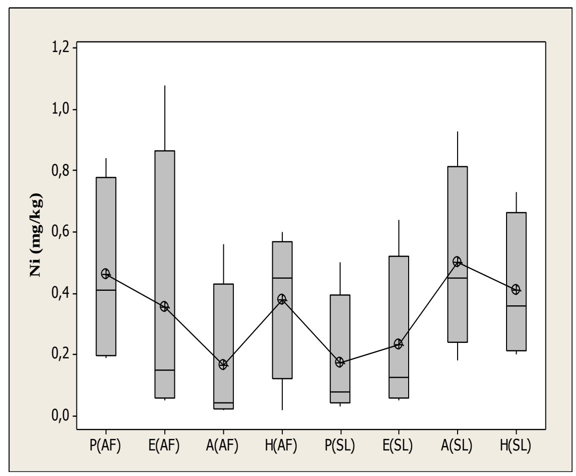
During this study, the geographic variability of the distribution of metals in the communities of *Holothuria tubulosa* was not significant (P 0.005), with the exception of Zn that marked a bioaccumulation which fluctuated according to the sex where there is a clear demarcation for females reaching a much higher rate than males **(Ueda *et al*., 1991; San Martin, 1995; Sicuro *et al*., 2012).**This could be explained by the same phenomenon that occurs in the edible sea urchin *Paracentrotus lividus* where the environment of males is more acidic than females .However, the activity of sperm is repressed; the concentration of certain compounds is therefore different **(Dermeche *et al.*, 2009**). Zinc trace is an essential element for the development of life since it is not very toxic for humans, but at high concentrations can disrupt larval growth of marine invertebrates **(Asso, 1982; RNO, 2006)**. Remarkably, a concentration between 10 and 40µg / l is found to be harmful on the life of marine organisms such as an inhibitory effect on the growth of the sea cucumber crown and on the reproduction of the isopod *Idothea baltica*. On the other hand, lead and cadmium have no known metabolic role, and are considered highly dangerous and non-biodegradable pollutants **(C.E.E, 1982; E.E.A, 1997)** that cause effects on the larval development of certain organisms, particularly crustaceans **(Chiffoleau *et al*., 2003)**, and generate a wide variety of harmful effects in the cellular, tissue and organ level: carcinigenicity **(Costa, 1998; Rojas *et al*., 1999);** neurotoxicity **(Waalkes *et al*., 2000) or** teratogenicity **(Belles *et al.*, 2002).**

Furthermore, in the marine environment, nickel is much less toxic to organisms than cadmium, and does not bioaccumulate much. This element is necessary at low concentrations for the physiological functions of certain organisms, but effects on the embryonic development of marine invertebrates were, however, observed at higher concentration. Echinoderms tend to store cadmium and copper in a non-toxic form **(Bremner, 1979)**, as *Holothuria tubulosa* behaves in this way.









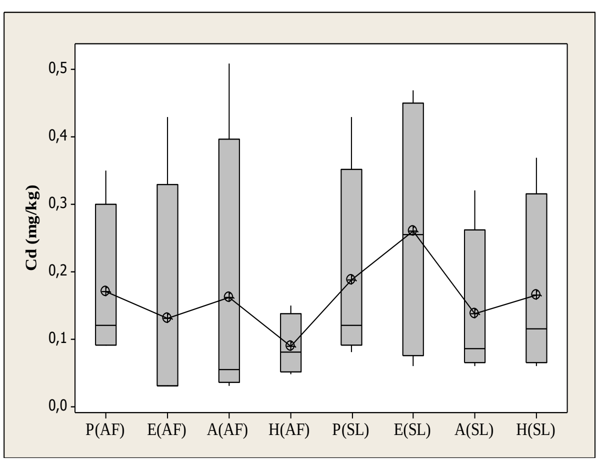


Fig. 3: Boxplots represent the metal concentrations in *Holothuria tubulosa* in each study site (Sidi Lakhdar and Ain Franin) for each season.

These diagrams (boxplots) are limited by quartiles 1 and 3 and have a line showing the median. The standard deviations are shown in solid lines.

The resistance to these contaminants could be due to the secondary metabolisms of several types of saponin and these are glycosides of triterpene **(Bruno *et al.*, 1993; Van Dyck *et al.*, 2009, Van Dyck *et al.*, 2010)** which present different concentrations depending on the season, the period of sexual maturity and environmental conditions

(Louiz *et al*., 2003). These are released in stressful situations as means of defense, and / or during gonadal maturity. According to **Van Dyck *et al.* (2010, 2011)** and **Caulier *et al.* (2011)** the saponin richness of these secondary metabolites is found in Cuvier's tubes and sea cucumber teguments. The period of sexual rest is a phase of gametogenesis characterized by an increased accumulation of nutrient reserves and a synthesis and storage of carbohydrate, lipid and protein materials **(Webb, 1997)** and even different types of saponins in sea cucumbers **(Louiz *et al.*, 2003).** At laying, the nutrient reserves and the saponins run out automatically, the heavy metal concentrations drop and release metals at this time (spring and winter period for Holothuria tubulosa at the two study sites), and the accumulation reserves will not resume slowly until the beginning of the period of sexual rest **(Webb, 1997)**. An important decontamination factor in sea cucumber community is observed from their first reproduction in spring season is related to the decrease in metal concentrations. Numerous studies have shown that the metal concentrations measured in marine species vary seasonally; an observed phenomenon reported in several branches including Echinoderms **(Wright & Mason, 1999; Kaimoussi *et al.*, 2000; Orban *et al.*, 2002; Michel *et al.*, 2006; Warnau *et al.*, 2006; Dermeche *et al.*, 2012)**. The evaluation of the seasonal distribution of the metallic concentration revealed that the maximum accumulation was observed in the spring for Zn, Mg, and Cu. While for Pb it was in the winter and in autumn for Cd. Moreover.
Warnau *et al.* (2006) reported that, the maximum concentrations of Zn and Cu were observed in spring, while the maximum concentrations of Pb were recorded in winter and Cd in autumn. The concentrations obtained in the current study, concerning the fluctuations of the metal concentrations according to the seasons, result from the changes in the quality, the food strategy and the ecological niche. But, the reproduction and the reproduction time remain the most important factors that have an effect on the accumulation of metals in marine organisms **(Adami *et al.*, 2002; Türkmen *et al*., 2005; Mubiana *et al*., 2006; Cardellicchio *et al*., 2008; Sicuro *et al*., 2012; Turk Culha *et al*., 2016; Al-Najjar *et al*., 2018 ; Rdiansyah *et al.*, 2020).** Due to the absence of a study on metal contamination concerning this species of sea cucumber frequenting the Algerian marine waters, the researchers are content to compare the heavy metal concentrations obtained in *Holothuria tubulosa* during this study with respect to the tolerated threshold (Table 3). It seems that the trace element of Zn contents do not constitute a danger for the consumer because they are clearly lower than the D.M.A.

The average doses of heavy metals recorded in Holothuria tubulosa at the two study sites compared to those in the literature relating to AMD are worrying for Cd, Pb as well as Cu compared to the obtained results for the same species **(Tyrrell, 2005).** This observation highlights the potential risk for these macrobenthics given their ecological importance and their exposure to non-essential and dangerous metallic elements found in significant quantities in Algerian coastal marine waters. Consequently, their transfer to the various links of the chain tropho-dynamics by the phenomenon of bio-magnification in the medium or long term is dangerous.

Table 3: Comparison of the heavy metal contents (ppm) in *Holothuria tubulosa* compared to the maximum admissible doses (D.M.A) ppm.

Species	*Cd*	*Pb*	*Zn*	*Cu*	*References*
H. tubulosa	0,07	1,16	17,40	2,5	Sicuro *et al.*, 2012 ; Turk Culha *et al*., 2016
	1,27	4 ,71	15,37	1,48	Michel *et al.*, 2006 ; Al-Najjar *et al*., 2018 ; Rdiansyah *et al.*, 2020
	1,02(a) 0,77(b)	3,23(a) 4,66(b)	5,54(a) 12,08(b)	3,65(a) 4,03(b)	Present study (a) Site A.F (b) Site S.L

CONCLUSION

The analysis of all the obtained metal concentrations revealed that, despite the difference in biotope (nature of the substrate and anthropization), the two communities of *Holothuria tubulosa* did not show a significant difference ($P > 0.005$) for all the studied heavy (traces) metals. The two-way Anova comparison (season, metal), showed that the sea cucumber community of SL and AF had similar concentrations in the four-seasons for Cu, Ni, Pb and Cd. The distribution was not symmetrical, which reflects the non-existence of a variability in seasonal concentrations (no significant difference) with the exception of Zinc where a slight difference was noticed for the two sites for both spring and winter. This significant difference in Zn concentrations is related to the role of this essential element during gonad maturity. Further researches are recommended such as the inventory of the different species living in the western coastal fringe and widening the range of dosage of heavy metals by taking numerous study sites on the fringe coastal west. Thus, a good correlation between the different communities of sea cucumbers would be attained to highlight the role of saponins (secondary metabolism).

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