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Trace Element Concentrations in Soft Tissue and Shell of the Mangrove Oyster (*Crassostrea Gasar* Dautzenberg, 1891) from the Lake Zowla-Aného Lagoon Hydro System (Southern Togo)

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Abstract

The Lake Zowla-Aného lagoon hydro system, located on the southeast coast of Togo, is very exposed to metal contamination as it receives a huge amount of untreated domestic and industrial effluents from Aného City and the phosphorite treatment plant at Kpémé. This study aims to assess the concentrations of seven heavy metals and their bioaccumulation status in soft tissues and shells of the mangrove oyster (*Crassostrea gasar*) collected from this aquatic environment. Samples were collected in January, April and July 2017 at two stations and analyzed using flame atomic absorption spectrophotometry. Results indicate that the waters are contaminated by Cd, Pb and Cr with average concentrations that are significantly above WHO standards. At both locations, the contents of the trace elements (except Cr) in oysters' soft tissues were significantly higher than those in the shells. In the soft tissues, the concentration of Cr, Cu, Zn, As, Cd, Hg, and Pb ranged (in $mg \cdot kg^{-1}$) from 4.33 to 23.14, 93.4 to 366.7, 455.68 to 1384.15, 0.11 to 1.34, 16.42 to 62.6, 0.05 to 0.473 and 0.39 to 21.08, respectively. Furthermore, statistical analyses showed marked differences in mean concentrations between sampling time on the one hand, and between sampling locations on the other hand ($p < 0.05$). The highest metal concentrations were recorded in oysters collected in June at Zalivé whereas the lowest values were recorded in April at Zowla. Based on BCF results in the lagoon system, the oyster *C. gasar* can be used as a biomonitor of Zn, Cd, Cu and Hg exposure. Finally, the Cd, Pb, As, Zn and Cu concentrations in soft tissues and were found to be significantly higher than the permissible limit for human consumption according to WHO and FAO and thus pose a threat to human health.

Keywords

Bioaccumulation, Oyster *Crassostrea Gasar*, Trace Elements, Anécho Lagoon, Lake Zowla, Togo

1. Introduction

Bivalve molluscs are key components of marine and estuarine environment because, as filter feeders, they play a critical role in maintaining water quality and ecosystem integrity. Besides, they have been rightfully regarded as "natural functional foods" and their consumption has been common since ancient times [1] [2]. Edible molluscs, including oysters are naturally low in carbohydrate, as well as total and saturated fat. With regard to omega-3 fatty acids, iron, selenium, and zinc, the nutrient value of some shellfish is superior to land-based protein sources, such as beef, chicken, and pork [3] [4]. Despite the above-mentioned nutritional properties, there are also several potential hazardous substances that have been found in edible molluscs, including pathogens, marine toxins, environmental pollutants, and trace elements [5].

Trace elements are constantly released into aquatic systems from natural and anthropic sources. Unfortunately, unlike organic chemicals, the majority of metals cannot be easily metabolized into less toxic compounds, because their characteristics are lack of biodegradability. Once introduced into the aquatic environment, metals are redistributed throughout the water column, accumulated in sediments or consumed by biota [6]. In spite of the fact that some of them are essential at low concentrations for living organisms, at higher concentrations, they could induce toxic effects disturbing organisms' growth, metabolism, or reproduction with consequences for the entire trophic chain, including humans [7] [8] [9] [10].

Lagoon and lake systems in coastal areas are key parts of agroecosystems and economic development in countries because of the diversity of habitats, dynamic interactions between water bodies, abundant fishery resources and contributions to biogeochemical cycles. They act as habitat and nursing ground for a great variety of fish and other shellfish species [11]. However, they are often simple systems that are easily disturbed both by natural processes and pollution from adjacent urban and industrial development activities [12] [13]. As a result, they are affected by an array of physical, chemical and biological factors. In addition, because of their generally shallow nature and relatively small sizes, environmental factors have a marked effect on the flora and fauna inhabiting the systems [14] [15].

In Togo, the Lake Zowla-Anécho lagoon complex is located in the Maritime

Region which supports 42% of the national population [16]. Moreover, a phosphate procession is taking place in the watershed with the discharge of liquid and solid waste without prior treatment [17] [18]. Several threats relating to the direct or indirect discharge of domestic sewage, mining waste and agricultural chemicals have been shown to affect this ecosystem [19] [20] [21] [22] [23].

In this study, we evaluated the Cu, Zn, Cd, Cr, Pb, Hg and As contents in the soft tissue and shell of the mangrove oyster *Crassostrea gasar* (Dautzenberg, 1891) which, in view of its commercial value and of the volumes landed, is the most important bivalve molluscs caught by the traditional fishery in the Zowla-Aného lagoon system. These results provide essential information to update the pollution status of the study area and to evaluate the impact of different human activities on the marine environment.

2. Material and Methods

2.1. Study Area

The Lake Zowla-Aného lagoon hydro system includes Lake Zowla (6.55 km^2), the Zalivé Channel and the Aného Lagoon in the south-east which consists of a narrow and shallow channel (4 to 11 m) network. This lagoon system belongs to the Togolese coastal zone which is located between latitudes 6˚17'37" and 6˚14'38" North and longitudes 1˚23'33" and 1˚37'38" East. The waters of the Lake Zowla-Aného lagoon hydro system communicate downstream with the sea through the Aného pass, which has been continuously open since 1989 (MERF, 2007). The hydrological regime of Lake Zowla-Aného lagoon hydro system depends mainly on the regime of the Zio, Haho, Boco and Mono Rivers [24] [25] [26] [27]. **Figure 1** shows the study area and sampling sites.

2.2. Oyster Sampling and Laboratory Analysis

Thirty (30) oyster individuals were randomly sampled in January, April and July 2017 in two localities (Zowla and Zalivé), labeled and transported to the laboratory. The choice of sampling site was based on previous studies in the area [23] [28], oyster harvesting activities and the position of the sites in relation to the sea at Aného. At the same time, the waters were sampled 30 cm from the surface in clean polypropylene bottles for analysis in order to determine bioconcentration factors.

In the laboratory, the oysters were washed and put in tap water for 72 hours for purging before shelling them using a clean stainless steel scalpel without losing the intra-tissue fluid. After drying in an oven at 70˚C, the shells were ground to obtain particles of 63 μm whereas all the soft tissues are preserved in its entirety. The samples were digested using nitric acid (3 ml of pure HNO_3 per 0.05 - 1 g of sample) in borosilicate glass tubes and in pressurized medium and at 90˚C, to near dryness of the reagent [29]. Then the residues were recovered in a test tube and then diluted to 15 ml with distilled water and filtered. Samples intended for the determination of mercury were mineralized without heating. The

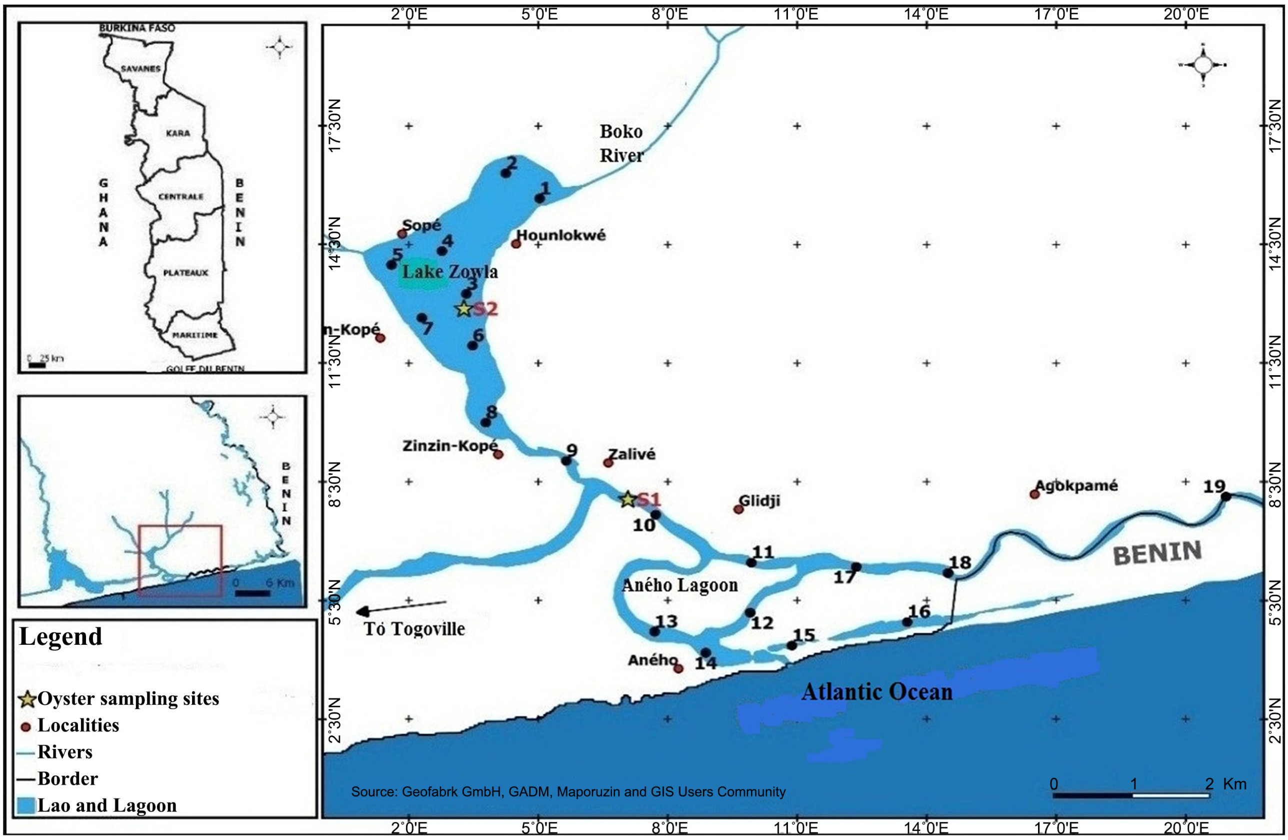

Figure 1. Map of the study area with oyster sampling sites.

water samples were filtered through a 0.45 µm filter, acidified to 1% with nitric acid (HNO_3). All the samples were then stored at room temperature until analysis [30] [31]. The Cd, Cu, Pb, Cr and Zn were analyzed using a Thermo Electron flame Atomic Absorption Spectrometer (AAS) while Hg and As were analyzed using AAS coupled to a Thermo Scientific hydride and cold vapour generator (VP-100) with flame for As and without flame for Hg.

The validity of the analytical methods was verified by internal control. A procedural blank was prepared simultaneously with the same acid (68% HNO_3) as for the other samples under the same experimental conditions and measured for each 10 sample batch. Standard solutions of each element were analyzed in the same condition as previous samples. This made it possible to highlight the possible contamination of the sample, to eliminate quantification errors and to verify the accuracy of the method. Also, in order to verify the repeatability of the results, multiple duplicates were incorporated into the analytical batch in a random manner.

2.3. Bioconcentration Factor

The Bioconcentration Factor (BCF) was determined using the following formula:

$$BCF = \frac{\text{Concentration of the trace element in the biological matrix}}{\text{Concentration of the trace element in the biotope}}$$

2.4. Metal Pollution Assessment

A Metal Pollution Index (MPI) equation was used by different authors to assess the degree of pollution of different tissues from different areas or different times [32] [33]. The equation is as shown below:

$$\text{MPI} = \left(\text{Cf}_1 \times \text{Cf}_2 \times \text{Cf}_3 \times \cdots \text{Cf}_n\right)1/n$$

where Cf_1 is concentration value of the first metal, Cf_2 = concentration value of the second metal and Cf_n = concentration value of the nth metal. The MPI index values are tabulated and interpreted as follows: MPI < 2 indicates not impacted, 2 ≤ MPI < 5 is very low contamination, 5 ≤ MPI < 10 is low contamination, 10 ≤ MPI < 20 is medium contamination, 20 ≤ CF < 50 is high contamination 50 ≤ MPI < 100 is very high contamination and MPI > 100 is extreme contamination.

2.5. Statistical Analysis

Analysis of Variances (ANOVA) was performed to assess the differences between the monthly average trace element concentrations. Student's t-test was used to compare average concentration at sampling sites. Also, Principal Component Analysis (PCA) was performed to highlight the relationships between the different trace elements on the one hand and between the different sampling sites on the other hand. Principal Component Analysis (PCA) defines a hypothetical relationship among a series of variables, and so the main objective of this technique is to reduce the number of main variables that define the existing variation among them [34] [35] [36]. The STATISTICA 6.0 software was used for the analysis.

3. Results

3.1. Concentrations of Trace Elements in Waters

According to the results shown in **Table 1**, the concentration pattern of trace elements in waters was Pb > Cu > Cr > Cd > Zn > As > Hg. In Zalivé, mean values in waters range from 8×10^{-3} mg/l (Hg) to 0.59 mg/l (Pb) while in Zowla these values were 3.3×10^{-4} and 0.33 mg/l respectively. As shown in **Table 2** above, trace element concentrations obtained in the waters during the dry season were lower than those of the rainy season. In addition, the concentrations recorded at Zalivé were higher than those obtained at Zowla (**Table 1**). Overall, as shown in the table, the elements that have exceeded the allowed levels under the WHO legislation were lead, cadmium and chromium from both sites.

3.2. Trace Elements Contents in Soft Tissues of *Crassostrea Gasar*

3.2.1. Trace Elements Contents in Soft Tissue

Among the trace metals investigated, zinc showed the highest value of 2488.1 mg/kg and mercury, the lowest value of 0.08 mg/kg in soft tissue (**Table 2**). It can also be seen from the results that the concentrations of studied elements varied temporally on the one hand and from one location to another on the other hand (**Table 2** and **Figure 2**). Overall, at both sites, oysters sampled in April

Table 1. Seasonal trace element concentrations (mg/l) in water samples taken from Lake Zowla-Aného lagoon system.

		Hg	As	Pb	Cd	Zn	Cu	Cr
Dry season	Zalivé	0.0008	0.0039	0.52	0.07	0.042	0.28	0.24
	Zowla	0.00033	0.0032	0.32	0.036	0.04	0.25	0.086
Rainy season	Zalivé	0.0009	0.0072	0.59	0.032	0.068	0.46	0.32
	Zowla	0.00051	0.0042	0.33	0.03	0.07	0.34	0.16
WHO		0.006	0.010	0.01	0.003	3	2	0.05

Table 2. Temporal metal concentrations in soft tissues of *C. gasar* of two selected sites in Lake Zowla-Aného lagoon hydro system in mg/kg.

TE			Hg	As	Pb	Cd	Zn	Cu	Cr
January	Zalivé	Av ± SD	0.09 ± 0.04	0.53 ± 0.22	4.53 ± 3.4	32.92 ± 9.7	859.72 ± 436.2	213.09 ± 127.3	9.45 ± 4
		min	0.035	0.2	0.96	11.66	339.35	57.5	4.07
		Max	0.23	1.13	15.17	57.92	1947.06	599.44	18.61
		CV	44.44	41.51	75.05	29.46	50.74	59.74	42.33
	Zowla	Av ± SD	0.046 ± 0.013	0.29 ± 0.18	3.16 ± 2.77	26.33 ± 6.02	721.27 ± 217.16	125.14 ± 31.74	7.4 ± 3.07
		Min	0.026	0.08	0.71	8.98	359.78	58.18	3.29
		Max	0.08	0.95	10.32	38.31	1253.11	233.64	18.79
		CV	28.26	62.07	87.66	22.86	30.11	25.36	41.49
April	Zalivé	Av ± SD	0.44 ± 0.07	0.88 ± 0.46	15.42 ± 5.66	52.26 ± 10.36	969.14 ± 415.01	271.08 ± 95.58	17.25 ± 5.89
		Min	0.34	0.48	6.03	31.03	572.99	150.32	9.38
		Max	1.02	2.24	23.72	71.2	2488.1	508.45	33.21
		CV	13.64	52.27	36.7	19.82	42.82	35.26	34.14
	Zowla	Av ± SD	0.32 ± 0.18	0.62 ± 0.47	8.99 ± 6.92	45.71 ± 22.1	811.32 ± 540.77	194.82 ± 152.98	13.03 ± 4.76
		Min	0.14	0.22	2.58	18.64	291.04	44.76	7.39
		Max	0.8	2.05	29.05	98.92	2317.05	746.71	25.32
		CV	56.25	75.81	76.97	48.35	66.65	78.52	36.53
July	Zalivé	Av ± SD	0.41 ± 0.09	0.67 ± 0.23	10.45 ± 3.73	35.76 ± 18.09	896.85 ± 260.72	255.25 ± 101.5	13.34 ± 3.42
		Min	0.24	0.35	5.96	9.97	432.43	111.46	9.09
		Max	0.61	1.19	10.04	79.22	1534.12	584.14	21.64
		CV	21.91	34.33	35.69	50.59	29.07	39.76	25.64
	Zowla	Av ± SD	0.24 ± 0.14	0.52 ± 0.28	7.15 ± 3.7	30.73 ± 14.31	766.3 ± 310.62	175.33 ± 56.68	10.77 ± 2.09
		Min	0.08	0.16	2.07	10.79	296.1	97.84	8.27
		Max	0.69	1.25	16.63	62.95	1487.32	297.15	16.46
		CV	62.5	53.85	51.75	46.57	40.53	32.33	19.4
		WHO*	0.5	0.1	2	1	100	30	50

Note: TE = Trace elements; Av = Mean; SD = Standard deviation; Min = Minimal; Max = maximum; CV = Coefficient of variation.

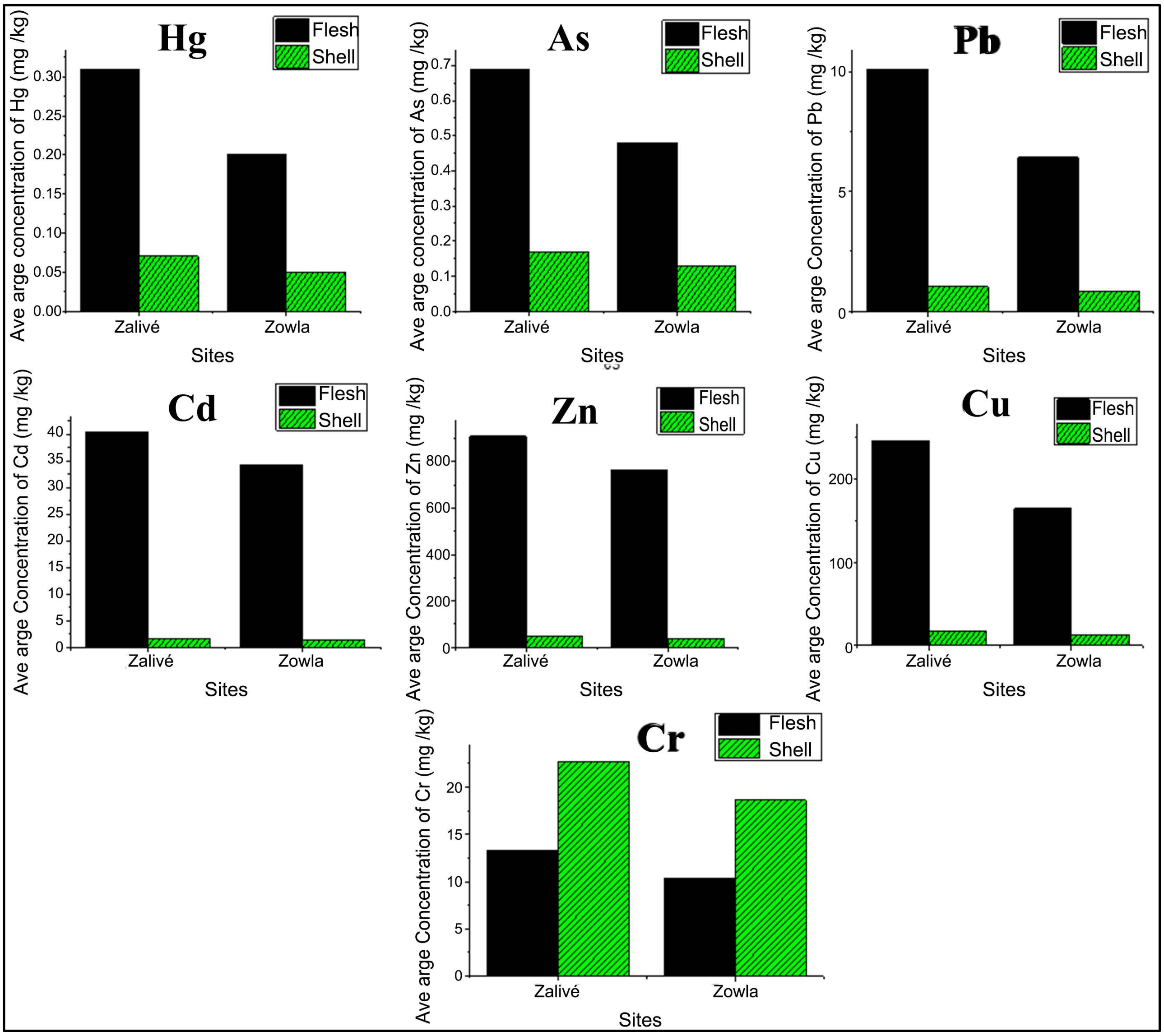

Figure 2. Variation of mean trace element concentrations in soft tissues and shells of *C. gasar* from Lake Zowla-Aného lagoon.

showed the highest concentrations for all elements, whereas the January samples contained the lowest concentrations.

The analysis of variance indicated a significant difference in the mean concentrations of Hg, As, Cd, Pb and Cr in the Zalivé oyster's soft tissues (Table 3). On the other hand, at Zowla, soft tissues showed significant differences only between the mean concentrations of Hg, As Pb, Cd, Cu and Cr (Table 4). Also, regarding the differences between the mean values for all the investigated metals in oyster tissue, a Student t-test was applied and the differences between the values obtained in the lagoon (Zalivé) were statistically different ($p < 0.05$) compared to those identified in the lake Zowla except for Cd and Zn (Table 5).

3.2.2. Trace Element Contents in Oysters' Shells

Large variations in metal concentration in oysters' shells were observed both

Table 3. Analysis of variance of the trace element concentrations in the soft tissues of *C. gasar*.

	F value and significance						
	Hg	As	Pb	Cd	Zn	Cu	Cr
Zalivé	251.02***	8.98**	46.41***	18.54***	0.65 NS	2.27 NS	21.99***
Zowla	31.74***	7.83**	11.54***	12.73***	0.42 NS	4.21*	19.82***

Note: *p < 0.05; **p < 0.001; ***p < 0.0001; NS = Not significant.

Table 4. Student's t test of the mean trace element concentrations in the soft tissues of *C. gasar* from Zalivé and Zowla.

	T value and significance						
	Hg	As	Pb	Cd	Zn	Cu	Cr
January	5.53***	4.55***	1.69 NS	3.15**	1.56 NS	3.67***	2.23*
April	3.44**	2.16*	3.94***	1.47 NS	1.27 NS	2.31*	3.05**
July	4.95***	2.22*	3.44**	1.19 NS	1.76 NS	3.76***	3.5***

Note: *p < 0.05; **p < 0.01; ***p < 0.001; NS = Not significant.

Table 5. Temporal changes in trace element concentrations in oysters' shells (dry and fresh weight, mg/kg; mean, standard deviation, maximum and minimum limits and coefficient of variation).

TE			Hg	As	Pb	Cd	Zn	Cu	Cr
January	Zalivé	Av ± SD	0.011 ± 0.002	0.095 ± 0.023	0.34 ± 0.075	0.67 ± 0.16	28.17 ± 8.15	6.65 ± 1.66	15.34 ± 2.98
		Min	0.0076	0.047	0.142	0.42	13.51	5.4	9.96
		Max	0.13	0.14	0.44	0.95	52.12	13.9	19.34
		CV	18.18	24.21	22.06	23.88	28.93	24.96	19.43
	Zowla	Av ± SD	0.004 ± 0.001	0.087 ± 0.02	0.3 ± 0.08	0.53 ± 0.15	15.46 ± 4.31	3.92 ± 0.83	12.97 ± 1.08
		Min	0.002	0.051	0.18	0.3	9.38	2.61	10.53
		Max	0.006	0.126	0.42	0.84	26.3	5.23	15.14
		CV	25	23	26.66	28.3	27.88	21.17	8.33
April	Zalivé	Av ± SD	0.09 ± 0.019	0.19 ± 0.023	1.18 ± 0.36	1.79 ± 0.32	46.6 ± 12.92	16.73 ± 2.2	20.68 ± 2.69
		Min	0.061	0.16	0.41	1.14	27.65	10.46	14.35
		Max	0.127	0.26	1.86	2.35	71.62	20.17	25.83
		CV	21.11	12.1	30.51	17.88	27.72	13.15	13.01
	Zowla	Av ± SD	0.07 ± 0.017	0.13 ± 0.023	0.96 ± 0.31	1.55 ± 0.26	38.67 ± 7.09	12.93 ± 1.99	16.51 ± 2.92
		Min	0.03	0.09	0.43	1.07	29.93	9.74	11.47
		Max	0.09	0.17	1.8	1.97	59.61	16.79	22.68
		CV	24.28	17.69	32.29	16.77	18.33	15.39	17.69
July	Zalivé	Av ± SD	0.11 ± 0.02	0.22 ± 0.03	1.56 ± 0.66	2.22 ± 0.37	62.25 ± 10.94	28.15 ± 2.77	31.98 ± 3.01
		Min	0.062	0.15	0.69	1.37	43.93	23.97	27.21
		Max	0.15	0.29	2.77	2.94	88.68	35.66	37.89
		CV	18.18	13.64	42.31	16.67	17.57	9.84	9.41

Continued

Zowla	Av ± SD	0.08 ± 0.015	0.18 ± 0.03	1.28 ± 0.23	2.03 ± 0.25	50.69 ± 9.48	23.7 ± 2.44	26.32 ± 1.98
	Min	0.05	0.13	0.95	1.3	34.46	20.36	23.09
	Max	0.1	0.22	1.96	2.47	61.96	29.05	30.56
	CV	18.75	16.67	17.97	12.31	18.7	10.29	7.52

Note: TE = Trace element; Av = Mean; SD = Standard deviation; Min = Minimal; Max = maximum; CV = Coefficient of variation.

temporally and spatially, for each of the studied elements. In general, the mean trace element concentrations increased from January to April and from April to July. Thus, the lowest contents were obtained in January while the highest concentrations were recorded in July (Table 5).

Furthermore, significant variations in mean trace element concentrations have been observed between months in Zalivé and in Zowla (ANOVA; $p < 0.05$) (Table 6).

At both sites, there were significant differences among trace metals concentrations in shells collected in April and July. However, no difference was observed for arsenic and copper in January (Table 7).

In addition, there was a significant correlation ($p < 0.05$) between the mean concentrations of trace elements in soft tissues and oyster' shells from Zalivé and Zowla (Table 8 and Table 9).

3.2.3. Comparison of Trace Element Concentrations between Soft Tissues and Shells of *C. gasar*

Student's t-test (Table 10) showed that the mean concentrations of As, Hg, Pb, Zn and Cu in the soft tissues were higher than those in the shell. On the other hand, mean Cr concentrations were higher in shell compared to soft tissues (Figure 2).

3.3. Principal Components Analysis

Two principal components (Eigenvalues > 1) emerged from the principal component analysis accounting for 96.92% of cumulative variance. The first principal component (F1) loading with 82.13% variance showed strong negative correlations between Hg (−0.89), As (−0.98), Pb (−0.96), Cd (−0.98); Zn (−0.96) and Cu (−0.98). On the contrary, the F2 axis is defined significantly by the Cr (−0.89) on the negative side (Figure 3(a)). The projection of individuals in Figure 3(b) shows individuals contributing to the significant correlations between Hg, As, Pb, Cd, Zn, Cu and factor F1 and the significant correlation between Cr to factor F2.

3.4. Bioconcentration Factors (BCFs) and Metal Pollution Index (MPI)

The estimated BCFs are depicted in Table 11 and in Table 12. For the soft tissues, the BCFs ranged from 8.7 for Pb observed in January at Zalivé to 23074.8 for Zn in April as well. Regarding the shells, the minimum value was found for

Table 6. Analysis of variances of the trace elements concentrations in the shell of *C. gasar*.

	F value and significance						
Metal	Hg	As	Pb	Cd	Zn	Cu	Cr
Zalivé	314.56*	199.67*	62.27*	218.68*	74.18*	682.68*	257.48*
Zowla	280.67*	112.32*	143.67*	335.08*	181.95*	828.41*	315.02*

Note: *p < 0.05.

Table 7. Student's t test of the mean trace elements concentrations in the shell of *C. gasar* from Lake Zowla-Aného lagoon.

	T value and significance						
	Hg	As	Pb	Cd	Zn	Cu	Cr
January	18.01***	1.48 NS	1.97 NS	3.48***	7.55***	8.06***	4.08***
April	5.14***	9.82***	2.5*	3.15**	2.95**	6.98***	5.73***
July	6.23***	4.89***	2.21*	2.37*	4.37***	6.64***	8.59***

Note: *p < 0.05; **p < 0.01; ***p < 0.001; NS = Not significant.

Table 8. Correlation between the mean trace element concentrations in the soft tissues of *C. gasar* from Zalivé and Zowla.

		Zowla oyster soft tissues						
		Hg	As	Pb	Cd	Zn	Cu	Cr
Zalivé oyster soft tissues	Hg	1	0.86**	0.87**	0.78**	0.70**	0.76**	0.81**
	As	0.52**	1	0.89**	0.77**	0.87**	0.80**	0.79**
	Pb	0.74**	0.59**	1	0.78**	0.81**	0.78**	0.77**
	Cd	0.54**	0.57**	0.61**	1	0.68**	0.68**	0.64**
	Zn	0.34**	0.69**	0.51**	0.49**	1	0.78**	0.69**
	Cu	0.38**	0.56**	0.49**	0.38**	0.74**	1	0.69**
	Cr	0.69**	0.67**	0.65**	0.55**	0.58**	0.71**	1

Note: **p < 0.05.

Table 9. Correlation between the mean trace element concentrations in the shell of *C. gasar* of Zalivé and Zowla.

		Zowla Oyster Shell						
		Hg	As	Pb	Cd	Zn	Cu	Cr
Zalivé Oyster Shell	Hg	1	0.76**	0.86**	0.87**	0.79**	0.84**	0.72**
	As	0.85**	1	0.78**	0.79**	0.68**	0.81**	0.77**
	Pb	0.81**	0.68**	1	0.82**	0.78**	0.84**	0.73**
	Cd	0.86**	0.85**	0.73**	1	0.82**	0.87**	0.79**
	Zn	0.73**	0.70**	0.64**	0.76**	1	0.88**	0.78**
	Cu	0.83**	0.82**	0.71**	0.85**	0.82**	1	0.93**
	Cr	0.72**	0.73**	0.65**	0.77**	0.76**	0.93**	1

Note: **p < 0.05.

Table 10. Student's t-test between the mean trace element concentrations in soft tissues and shells of *C. gasar* from lake Zowla-Aného lagoon.

	T value and signifiance						
Location	Hg	As	Pb	Cd	Zn	Cu	Cr
Zalivé	13.07***	13.94***	13.78***	23.41***	21.67***	19.61***	−9.45***
Zowla	7.97***	9.16***	9.87***	17.78***	18.3***	14.2***	−10.59***

Note: ***$p < 0.0001$.

Table 11. Bioconcentration factors of the soft tissue and shells of Zalivé oysters.

	Tissues	Hg	As	Pb	Cd	Zn	Cu	Cr
January	ST	112.5	135.9	8.7	470.3	20469.5	761.04	39.4
	OS	13.75	24.4	0.6	9.6	670.7	23.75	63.9
April	ST	550	225.6	29.6	746.6	23074.8	968.14	71.9
	OS	112.5	48.7	2.3	25.6	1109.52	59.75	86.2
July	ST	422.2	93.05	17.7	1117.5	13188.9	554.9	41.7
	OS	122.22	30.55	2.6	69.4	915.44	61.2	99.9

Note: ST = Soft tissue; OS = Oyster' shell.

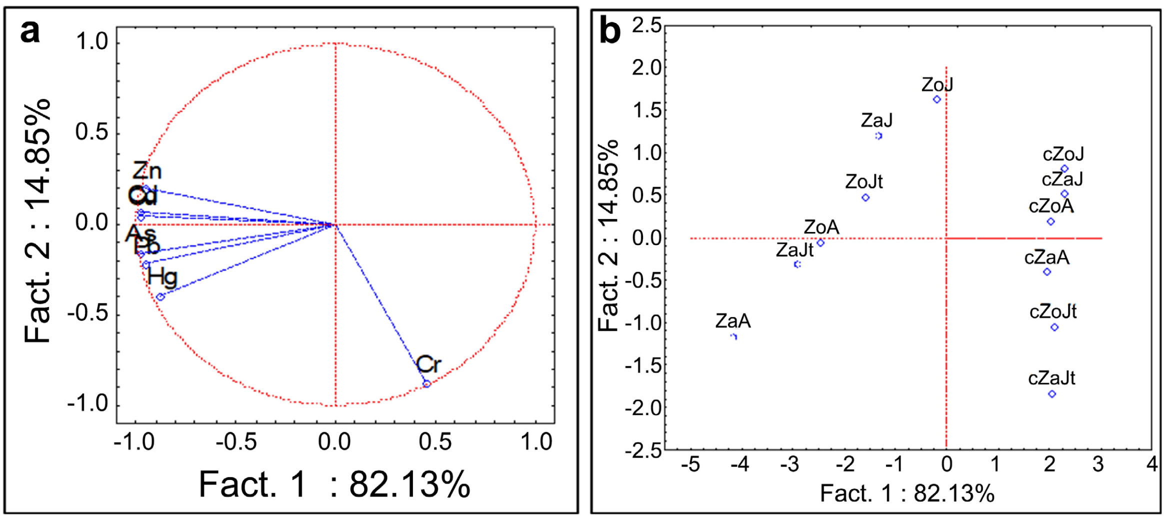

Figure 3. Projection of variables (a) and individuals (b) in factorial designs F1 × F2 (ZaJ = Zalivé/January/Soft tissue; ZaA = Zalivé/April/ Soft tissues; ZaJt = Zalivé/July/Soft tissue; ZoJ = Zowla/January/Soft tissues; ZoA = Zowla/Avril/Soft tissue; ZoJt = Zowla/July/Soft tissues; cZaJ = Zalivé/January/Shell; cZaA = Zalivé/Avril/Shell; cZaJt = Zalivé/July/Shell; cZoJ = Zowla/January/Shell; cZoA = Zowla/April/Shell; cZoJt = Zowla/July/Shell).

Pb in Zalivé (0.6) and the maximum bioaccumulation result was observed for Zn (966.75) in Zowla. Moreover, at both sites, the mean BCFs of the metals were observed in the bivalve soft tissues as follows: Zn > Cu > Hg > As > Cr > Cd and >Pb. The trend was slightly different (Zn > Cr > Hg > Cu > As > Cd and >Pb) with regard to bioaccumulation of elements in the shells.

The Metal Pollution Index (MPI) of tissues calculated for the lake Zowla-Aného lagoon ecosystem (Table 13). The highest MPI was obtained for soft tissue

Table 12. Bioconcentration factors of the soft tissues and shells of Zowla oysters.

		Hg	As	Pb	Cd	Zn	Cu	Cr
January	ST	139.4	90.62	9.9	731.39	18031.8	500.56	86.05
	OS	12.12	27.19	0.94	14.72	386.5	15.68	150.82
April	ST	969.7	193.75	28.1	1269.72	20283	779.28	151.5
	OS	212.12	40.62	3	43.05	966.75	51.12	191.98
July	ST	470.59	123.81	27.5	1024.33	10947.1	515.68	107.7
	OS	156.86	48.86	4.9	67.7	724.1	69.70	263.2

Note: ST = Soft tissue; OS = Oyster' shell.

Table 13. Metal Pollution Index (MPI) of oyster tissues.

Tissue	Location	MPI	Index value	Degree of pollution
Soft tissue	Zalivé	27.3	$20 < MPI \leq 50$	High contamination
	Zowla	11.58	$10 < MPI \leq 20$	Medium contamination
Shell	Zalivé	5.2	$5 < MPI \leq 10$	Low contamination
	Zowla	3.14	$20 < MPI \leq 5$	Very low contamination

collected from Zalivé (27.3) indicating a high contamination and the lowest for shell in Zowla (3.14).

4. Discussion

Anthropogenic activities have been known to be the primary causes of trace metal contamination in waters. Recently, Ouro-Sama *et al.* [20] assess the levels of trace element contaminations of the waters from the western part of the hydrosystem (Lake Togo-Lagoon of Aného) and its spatio-seasonal variations. Their results proved that this aquatic ecosystem is contaminated by Cd, Pb, Cr and Ni with average concentrations that are significantly above WHO standards. Data obtained in this study corroborate these works indicating that the eastern part of the hydrosystem (*i.e.*, Lake Zowla-Aného lagoon) is also contaminated by trace elements, notably Pb, Cd and Cr. Moreover, average levels of elements are higher than those obtained by Rezaie-Boroon *et al.* [37] in Lake Togo for Pb, Cu, As and Zn. In this study, trace element concentrations in water samples decreased in the order: Pb > Cu > Cr > Cd > Zn > As > Hg in the dry season and Pb > Cu > Cr > Zn > Cd > As > Hg in the rainy season. Higher average values were found in wet season in Zalivé for most of the studied elements except Hg. These spatial and seasonal variations highlight marine and floodwater influences in the contamination process of the hydrosystem and suggest that the trace elements mainly originate from the ocean via the intrusion of marine waters loaded with effluents discharged by the phosphate processing plant [20] [21]. In addition, some fertilizers and fungicides based on zinc ethylene-bis-dithiocarbamate and copper oxychloride although non-authorized are commonly used in the lagoon watershed [38], reflecting their source, since such compounds contain Cu and Zn. Finally, an important source of pollutant, but not the least, is sewage because Cu and Zn take part in the formulation of commercial detergents, shampoos, and other personal care products.

The phenomenon of metal bioaccumulation of trace elements was earlier observed in some fish and bivalve species collected from coastal and marine ecosystems in Togo [23] [39] [40] [41]. In this study, a wide variation of element concentration was observed in the investigated oysters where the highest metal contents were for Zn with an average concentration of 969.14 mg/kg and the lowest metal concentrations were for Hg with an average concentration of 0.44 mg/kg. Overall, the ranking of element concentrations in soft tissue reflects the typical metal accumulation in *Crassostrea* oysters and some other bivalve species investigated elsewhere [42] [43] [44]. At both sampling locations, the order of magnitude of element levels is Hg < As < Pb < Cr < Cd < Cu < Zn.

The concentrations of Zn, Cr, Cd, and Pb have shown significant temporal variability at both sampling sites. Metal contents increase between January and April, and decrease from April to July. The effect of seasons on trace metal accumulation was also detected in many other studies [45] [46] [47]. For example, Otchere [48] studying heavy metal burden in the bivalves from lagoons in Ghana, observed that element concentrations may vary in *Crassostrea tulipa* by a factor of two or more during the annual cycle. In general, one expects to find the highest element concentrations at the end of the dry season attributable to varying seasonal amount of organic matter in the environment, water salinity, temperature and physiological changes. In bivalves, these changes are a sign of alterations in the rates of filtration, feeding, growth, respiration and reproduction and changes in their metabolic and biochemical parameters [49] [50].

In the dry season, the arrival of marine waters in Lake Zowla-Aného lagoon increases salinity which is an important factor in the growth of *C. gasar* in the hydrosystem [51]. Thus, increase in tissue mass leads to an increase in the concentrations of trace elements in the soft tissues. Metongo [43] found that the accumulation of trace elements is proportional to the weight of the individuals. This indicates a difference in filtration capacity between small and large individuals, and accumulation of the trace elements during food ingestion [49].

From April to July, the decrease in trace element concentrations in tissues can have two explanations: one linked to reproduction and other linked to food competition with other organisms such as barnacles and gastropods. In fact, *C. gasar* is a filter feeder and planktophagous mollusc. Diet and filtration capacity of a mollusc depend on its physiological developmental stages (larvae, juvenile, adult) [52]. The study carried out by Solitoke *et al.* [51] revealed that the oyster's period of reproduction and eggs laying occurs during April to June in the Lake Zowla-Aného Lagoon hydrosystem. It should be noted that the reproduction phase corresponds to the maturation of the gonads which accumulate reserves and consequently increase the levels of trace elements in nutrients. However, the recruitment phase which corresponds to the emission of gametes requires a high consumption of energy and oxygen, this emission causes a general weakening of individuals which is indicated by a significant loss of weight, a decrease in tissue mass and a release of trace elements [45] [53]. Other authors have explained the decrease in trace element contents in organisms by the availability of food. Food intervenes by its nature and availability by making a large quantity of metal-polluted nutrient available to the molluscs [54] [55] [56]. This variation is also attributable to the phenomenon of desorption and the phenomenon of tide. Indeed, according to Cheggour *et al.* [57], the phenomenon of desorption could also contribute to the bioavailability of trace elements and their seasonal content fluctuation. Kraemerl *et al.* [58] point out that the tidal phenomenon also controls the action of pollutant release and therefore is a determining factor in the bioavailability of trace elements in the environment.

As mentioned previously, concentrations of all the seven elements in oysters from Zalivé (sampling site 1) were considerably higher than those from Zowla (sampling site 2). This inter-site variation in metal concentrations is reported in the literature by different researchers. Indeed, Huanxin *et al.* [59] conducted a study on the distribution of Cd, Cr, Cu, Fe, Mn, Pb, and Zn in oysters in the Gulf of Mexico. The study found that concentrations of metals varied considerably geographically with relatively constant metals ratios. Also, Pourang *et al.* [60] observed significant differences between sampling sites for accumulation of some trace elements in soft tissues (gills) and shells (especially the prismatic layer). These results are consistent with the assumption that relative proximity to the point of introduction of the pollutant, even though the stations are not very far apart, constitute an important aspect that mainly influences the bioavailability and bioaccumulation of metals in oysters. In fact, along the coast of Togo, the littoral drift is stronger eastwards and metal contamination has been found to decrease from the Aného pass to upsteam of the hydrosystem [19] [20]. This direction of circulation of water masses on the coast would explain why oysters collected in Zalivé, a fishing ground closer to the location where the mine tailings are poured into the sea (near Kpémé) are more contaminated than those from Zowla.

The comparisons of trace elements in the present study with other studies are shown in **Table 14**. Metal concentrations at the two studied locations were within the same range (As, Hg and Zn) or well above (Cu, Cd and Pb) the concentrations in same or similar species from previous studies in Togolese waters or elsewhere. Differences might have been caused by discrepancies in the contexts of the studies as well as differences in sources of contamination and individual differences in the bioaccumulation of heavy metal in the aquatic organisms. The concentrations of trace elements recorded in this study are considerably higher than those reported for Cu and Pb in the literature. Regarding Cu, the average contents (255.25 mg/kg in Zalivé and 175.33 mg/kg in Zowla) were far higher than the maximum value of 17 mg/kg measured by Otchere [48] in the

Table 14. Comparisons of trace elements in the present study with those of the literature.

Species	Geographical Area	Element						Reference
		As	Cu	Zn	Cd	Hg	Pb	
C. gasar	Aného Lagoon	0.095 - 0.22	246.47	339.35 - 2488.1	40.31	0.31	10.13	This work
C. gasar	Lake Zowla, Togo	0.087 - 0.18	165.1	291.1 - 1487.3	34.26	0.2	6.43	This work
C. gasar	Aneho lagoon, Togo	30.67 - 94.63			0.36 - 0.51		0.79 - 4.12	[23]
C. gasar	Benya Lagoons, Ghana		17	2350 - 2780	0.21 - 0.74			[48]
C. gasar	Niger Delta/Nigeria		0.001 - 0.05		0.001 - 0.05	<0.001	0.11 - 0.14	[62]
C. gasar	Ivory Coast			2172.65	1.98		2.00	[47]
C. gasar	Senegal		7.2	121.6	2.37			[63]
C. corteziensis	NW Mexico		17.5 - 166.3	245 - 2304	1.5 - 7.4		4.1 - 9.4	[64]
C. corteziensis	Mexico		33.6 - 44.9	263 - 382	0.2 - 0.6	0.03 - 0.08	0.3 - 1.9	[44]
C. gigas	Golf of Californie	0.25 - 0.48	26.2 - 85.0	138.0 - 418.6	9.0 - 21.4	0.003 - 0.04	0.8 - 4.6	[42]
C. gigas	Southeast England		391.36	1972.17	2.19		1.14	[61]
C. palmula	Mexico		23.1 - 112.6	226 - 1745	1.0 - 9	0.17 - 0.57	0.3 - 2.1	[65]
C. rhizophorae	Venezuela		27.5 - 83.0	130 - 877	1.5 - 4.2		2.5 - 3.0	[66]

same species from the Benya Lagoons, Ghana. Nevertheless, these concentrations were lower than that found by Bray *et al.* [61] in non-native Pacific oysters (*Crassostrea gigas*) on the shores of southeast England. These high concentrations of Cu as well as Zn in bivalves' soft tissues have been explained by the role played by these elements in the organism [49] [56]. In the same way, the mean concentrations of Pb in oysters collected in different tropical lagoons and estuaries were also significantly lower than those found in present study (10.45 and 7.15 mg/kg respectively).

Hg exhibits the lowest concentrations in soft tissues at both sites. Similar observations were also made by Páez-Osuna and Osuna-Martínez [65] for *Crassostrea palmula* in Mexico. This low Hg content can be attributed to its bioavailability and mobility. Indeed, the stability, the formation and the successive destruction of Hg complexes in relation to the "turnover" of proteins are factors which influence the mobility of Hg in biological systems and, therefore, govern its location and distribution in different organs [67].

A number of studies have reported on metal accumulations in the shell or in shell layers of oyster and other mollusc species from various sites [68] [69] [70] [71] [72]. From these works, it appears that mollusc shells tend to select some of the metals found around them in the environment and store them in their shells, and that animal behavior differs according to the environmental conditions in each region. In the present study, lower concentrations of all metals analyzed occurred in the shell than in the soft tissues, excepting chromium which was considerably higher in the shells. The low element concentration found in the shells rather than soft tissues is in agreement with the results reported in previous studies on different bivalve species [68] [72]. Usually, the soft tissue of oysters showed on average several times the element concentration in shells. However, since the soft parts accounted for only 19.5% ± 4.1% of the total live weight of the oysters, Wolfe [72] considered that shells contained nearly 45% of the total metal in *Crassostrea gasar* oysters.

A mollusc's ability to accumulate metals from a medium in its tissue can be estimated using the Bioconcentration Factor (BCF). According to Swaley *et al.* [73], a BCF > 1000 in bivalves indicates either a slow and significant accumulation of the environmental pollutant, or an accumulation of the pollutant through the food chain. In this study, Zn has high BCF ranging from 1109 to 23,074. Such values would indicate that the mollusk has accumulated large quantities of essential metals in its tissue. On the other hand, based on our results, *C. gasar* was apparently tolerant to the intake of Cu, Cr, Hg and As (all recorded moderate cumulative factor with BCFs ranging between 100 and 1000). Pb exhibited low potential factor (BCF = 8.7 - 28.1) at both sampling sites. Though Pb (0.32 - 0.59 mg/l) had the highest concentration in the lagoon waters compared to the other heavy metals, yet it presented a low BCF value. Such a low value indicates that the mollusc was unwilling to absorb the trace elements from water possibly because of Pb toxicity [74].

The results obtained revealed that the average values of Cd (40.31 mg/kg), Pb (10.13 mg/kg), Zn (896.85 mg/kg) and Cu (255.25 mg/kg) in oyster samples were well above the respective reference values. Meanwhile the mean values of Hg (0.31 mg/kg) As and Cr (13.34 mg/kg) were lower than reference standards. However, despite these apparently low levels of mercury and arsenic in oyster (*C. gasar*) tissue, they are still a potential public health concern, since mercury is a toxic metal that bio amplifies through food webs. These results indicate that oyster meat consumers are probably exposed to some potential health risks.

5. Conclusion

This work quantified trace element concentrations in a mangrove oyster and water collected in a tropical lagoon ecosystem to characterize oyster trace metal bioaccumulation in this Togolese coastal region. It is concluded that *C. gasar* from Lake Zowla and Aného lagoon presented concentrations of all the metals studied, which demonstrates its accumulating and bioindicator characteristics. Trace metal concentrations in soft tissue and shell were statistically significantly higher in April (at the end of the dry season). Significantly higher metal concentrations were determined in oysters from Aného lagoon (Zalivé sampling site), confirming the level of contamination of the water and sediments of this part of the hydrosphere. The levels of Cd, Pb, Zn and Cu were found to exceed the permissible limits for human consumption. Moreover, the average value of MPI has shown that the trace element concentration in tissues is at a high contamination level at Zalivé and medium contamination level at Zowla. It is recommended to reduce the consumption of these bivalves in order to minimize the serious effects of metals on human health.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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