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Levels of Trace Elements in Tissue of *Ostrea edulis* and *Crassostrea gigas* from Quiberon Bay, Brittany, France

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ABSTRACT

The concentration of trace elements, copper, zinc, cadmium and lead in the two oyster species (*Ostrea edulis*) and (*Crassostrea gigas*) collected from mariculture sites in the Quiberon bay were studied to investigate metal contamination in the surrounding area and its significant risk assessment to human who consumed the both species. Cu, Zn, Cd and Pb were analyzed by using inductively coupled plasma-mass spectrometry after acid digestion process. Certified reference materials, Oyster tissue, SRM1556a was used to validate the methods and the results shown a good agreement with the certified values. The levels of Cu, Zn, Cd and Pb in oyster flesh were 0.26-14.5, 4.61-83.9, 0.01-0.11 and 0.02-0.41 mg kg^{-1} wet weight. Element levels in *C. gigas* were generally higher than *O. edulis*. Element concentrations in the flesh were assessed for human consumption according to Provisional Tolerable Weekly Intake (PTWI) and Provisional Tolerable Daily Intake (PTDI). Due to their bioaccumulation capacity of trace elements, both oysters' species had the potential of being used as biomonitors to control the aquatic contaminations by trace elements.

Key words: Bioaccumulators, *Ostrea edulis*, *Crassostrea gigas*, mariculture, Quiberon bay

INTRODUCTION

Quiberon bay may receive natural and anthropogenic contaminants by human activities from various sources located surrounding the bay through river and surface runoff. These contaminants including trace elements are toxic to aquatic organisms and cause their lethal or sub-lethal deterioration (Silva *et al.*, 2001; Wang *et al.*, 2005) when the contaminants reach a certain levels. Trace elements contamination status of the aquatic environment can be assessed by studying the water, sediments and marine organisms (Hamed and Emara, 2006).

To study the trace elements contamination in the marine environment, several types of marine organisms has been used, such as seaweed (Giusti, 2001; Rainbow, 2002), mollusks (Shulkin *et al.*, 2003; Maanan, 2008), bivalve (De Mora *et al.*, 2004; Mostafa *et al.*, 2009) and marine fish (Canli and Atli, 2003; Agusa *et al.*, 2005). The levels of trace elements in marine organism such as mollusks and bivalves are often significantly higher than in other constituents of the marine environment due to their habitat and feeding habits (Sankar *et al*., 2006). Hence, both species are the reliable tool for identifying the trace elements contamination as they exhibit greater spatial sensitivity (Goldberg and Bertine, 2000; Perez *et al*., 2004). The bioaccumulation of sediment-bound elements by both benthic species is extremely important to the food webs and their eventual transfer back to human by consuming these organisms (Lim *et al*., 1995).

The Quiberon bay is successfully used for commercial shellfish cultivation such as *O. edulis* and *C. gigas* with a significant market in this region for both local consumption and export revenue. However, little data on pollutants in both shellfish are available in this region, necessitating the generation of baseline data in this region. Therefore, the work reported in this study is a first attempt to use the oysters, *O. edulis* and *C. gigas* in a biomonitoring study investigating trace elements pollution in the Quiberon bay and to evaluate both oysters' species as a public health food hazard. Thus, the aim of present study was to evaluate the concentration of copper, zinc, cadmium and lead in *O. edulis* and *C. gigas* collected from mariculture farm in Quiberon bay.

MATERIALS AND METHODS

Apparatus and reagents: ELAN 9000 inductively coupled plasma mass spectrometry (Perkin Elmer) was used for the determination of elements. The working parameters are listed below: RF power 1000 W; carrier gas flow rate 0.92 L min^{-1}; sample uptake rate 1.0-1.5 mL min^{-1}. The orifice of platinum sampling cone and sample cone is Nickel (±30), respectively. Nitric, hydrochloric and hydrogen peroxide acids are guarantee reagent in analytical grade. The stock calibration standards are from Merck chemicals. Standards for calibration curves are prepared by diluting the stock solution with Mili-Q water. The mixture of 1.0 μg mL^{-1} Sc (45), Ge (72), In (115) and Bi (209) in 1% v/v nitric acid is used as calibration internal standard.

All glass and plastic wares had been washed in detergent, rinsed in distilled water and then soaked in 5% nitric acid and then rinsed with de-ionized water for several times. All the lab wares were dried in oven prior to analysis.

Sampling and sample treatment: Oyster sample were collected at the oyster mariculture site, located in the Quiberon Bay (Fig. 1) during December 2010. Two hundred and twelve specimens covering a wide size-range were chosen for analysis. Sediment adhering to the oyster shell was washed thoroughly in the field with in-situ seawater. All the samples were stored in an ice-chest at 4°C while shipped back to the laboratory. In the laboratory, all samples were washed with running distilled water. Prior to analysis, samples were classified, weighed and measured and the whole soft tissue were removed from their shells by stainless steel scalpel blades and then thoroughly rinsed with distilled water to remove extraneous impurities and their wet weights recorded.

Oyster species: The kind of oyster species, the specific way of food intake and the shell lengths of oyster are as follows according to reference book in conjunction with a catalogue of marine molluscs (Liang *et al*., 2004):

- ***Ostrea edulis*:** Common name: European flat oyster, Mollusca (Bivalvia), Family: Ostreidae, tropical type: herbivorous (grazing), with the typical shell length of 53.2-71.5 mm
- ***Crassostrea gigas*:** Common name: Pacific oyster, Mollusca (Bivalvia), Family: Ostreidae, tropical type: herbivorous (grazing), with the typical shell length of 67.3-180.8 mm

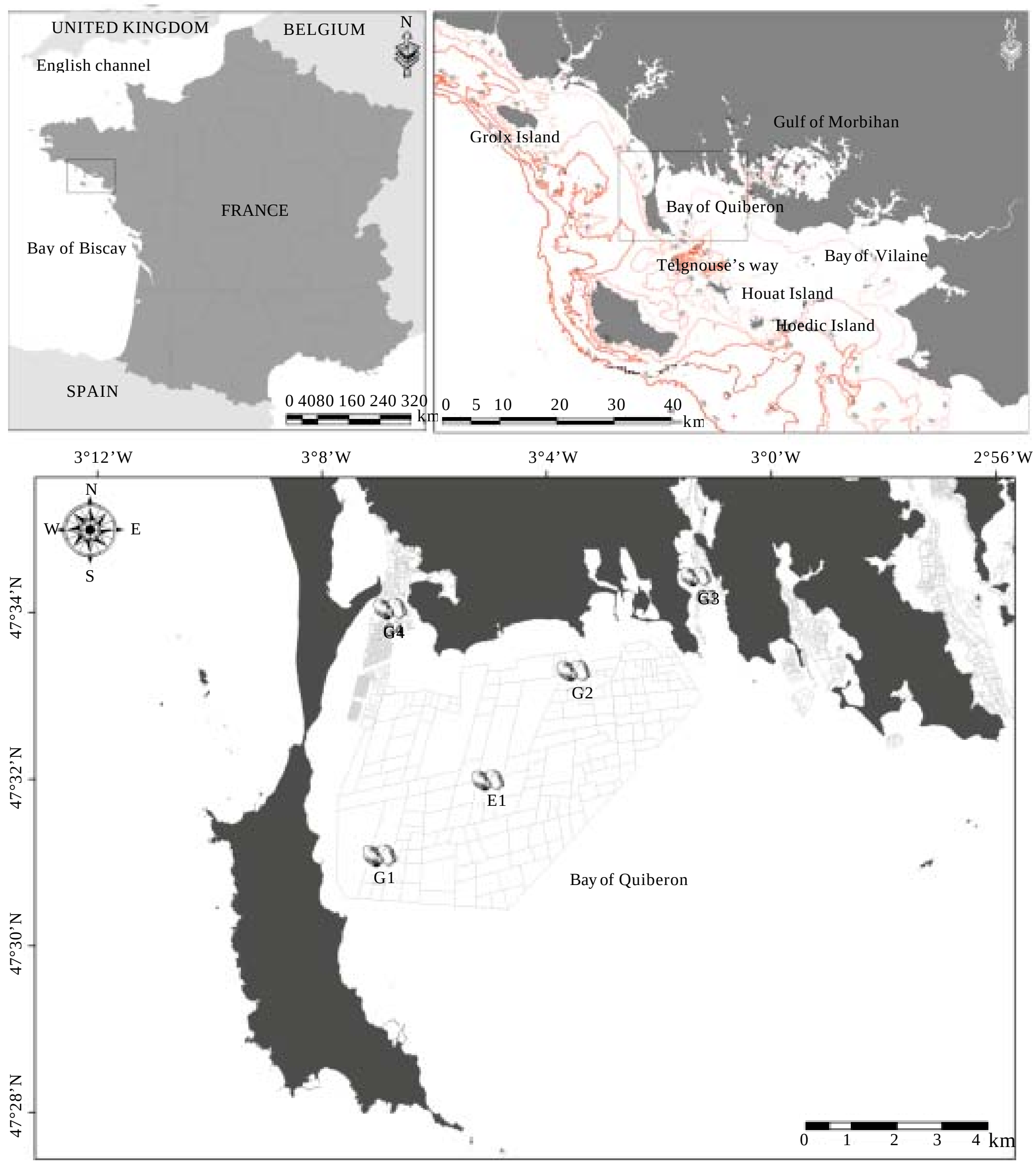

Fig. 1: Map showing sampling locations of *Ostrea edulis* (E) and *Crassostrea gigas* (G)

Analytical methods: The soft tissues were weighted to approximately of 3 g (wet weight) in a PTFE digestion container. Nitric acid, hydrochloric acid and hydrogen peroxide were added to each sample and left to predigest. Reagent blanks were processed simultaneously. Thereafter, the container was covered and placed in a stainless steel bomb, which was the sealed with a screw closure to avoid any acid leakage and placed in an oven. The oven temperature was kept for 6 h at 150°C. After cooling, the solution was transferred into a polypropylene tube and diluted with 5% nitric acid.

Table 1: Recovery of trace elements from certified reference materials

	Standard reference materials 1566a (Oyster tissue)		
Elements	Certified	Found	Recovery (%)
Copper	66±400	66.9±0.8	101.3±0.7
Zinc	830±57	835.8±1.3	100.7±1.4
Cadmium	4.2±0.4	4.09±0.07	97.4±0.7
Lead	0.46±0.04	0.46±0.02	99.6±1.2

The values of heavy metals (Cu, Zn, Cd and Pb) were measured in oyster sample by using Inductively coupled plasma mass spectrometry (Kamaruzzaman *et al.*, 2008a, b). Blanks were provided through all determinations. All analyses were carried out in thrice replicate using the external calibration method. Oyster tissue, SRM1556a certified reference materials were used to prove the precision and accuracy of the method and the results were reported in Table 1. The determined values of all elements were in good agreement with the certified values, suggesting that the proposed method was feasible for the determination of selected heavy metals in biological samples.

Statistical analysis: Statistical analysis, t-test by SPSS software was used to investigate the variations in trace elements concentration in the organisms studied and the sampling site. The levels of significance for the statistical analyses were set at 0.05.

RESULTS AND DISCUSSION

Oyster is a non-migrant species of long life, has a world-wide distribution, a reasonable size and easy to sample and an ability to concentrate numerous pollutants. Oysters accumulate metals such as copper and zinc and can tolerate very high metal concentrations, without apparent detrimental effects (Lin and Hsieh, 1999) and accumulate trace metals in proportion to the integrated ambient availabilities. Under normal conditions, as much as 387 L of water is pumped through the gills in a single day (Ingle and Whitfield, 1968) and therefore oysters accumulate a large amount of heavy metals by the ingestion of phytoplankton and organic particles as well as direct uptake from solutions. Therefore, they could be used as a biomonitors to provide integrated measures of the supply of trace metals available to them in an environment and accumulating the metal taken up from all sources such as from water and from food (Rainbow, 1995).

The estimation of some trace elements (Cu, Zn, Cd and Pb) within soft tissues of *O. edulis* and *C. gigas* was determined as mg kg^{-1} wet weight. Table 2 show the mean concentration and range of individual concentrations of each metal measured by the above-mentioned method. Overall, Zn concentrations are the most abundant element among all the interest elements (with an average of 34.9 mg kg^{-1} wet b.wt., ranged from 4.61-83.9 mg kg^{-1} wet weight) followed by Cu (an average of 3.56 mg kg^{-1} dry b.wt., ranged between 0.26 and 14.5 mg kg^{-1} wet b.wt.). Oysters collected from G1 farm had the lowest content of both Zn and Cu concentrations and also, G4 farm located at the innermost part of the bay recorded the highest content of both elements. Statistically analysis of t-test showed that concentration for both Zn and Cu were significantly different in the studied oyster samples.

The average concentration of Pb recorded in oyster samples from the five locations were 0.18 mg kg^{-1} wet weight. Oysters collected from G3 farm located at Trinite-sur-Mer site showed the highest concentrations of Pb, 0.41 mg kg^{-1} wet weight. While oysters from G2 showed the lowest

Table 2: The total shell length and trace elements levels in soft tissue from Quiberon bay

Station	Total length (mm)	Trace elements (mg kg^{-1} wet weight) Cu	Zn	Cd	Pb
E1 (n = 41)					
Mean	63.3	2.80	26.00	0.04	0.21
Min.	53.2	0.58	6.76	0.01	0.10
Max.	71.5	7.22	51.10	0.09	0.34
G1 (n = 40)					
Mean	93.3	3.51	31.50	0.03	0.20
Min.	79.0	0.26	4.61	0.01	0.05
Max.	119.0	6.35	68.90	0.11	0.37
G2 (n = 37)					
Mean	94.1	3.58	32.80	0.02	0.13
Min.	67.3	0.98	9.82	0.01	0.02
Max.	112.0	5.66	67.40	0.05	0.29
G3 (n = 45)					
Mean	142.0	5.33	50.10	0.05	0.22
Min.	114.0	0.75	14.10	0.01	0.06
Max.	181.0	9.57	82.10	0.08	0.41
G4 (n = 49)					
Mean	101.0	2.57	34.10	0.04	0.15
Min.	73.7	0.61	11.60	0.01	0.05
Max.	130.0	14.50	83.90	0.09	0.29
Average (n = 212)	98.7	3.55	34.9	0.04	0.18

concentrations, 0.02 mg kg^{-1} wet weight. From the t-test analysis, it can be conclude that there was no significant variation in concentrations of Pb between all the farms.

Cd recorded the lowest concentration range in oyster samples among all trace elements studied. The average Cd concentration was 0.04 mg kg^{-1} wet weight, ranged from 0.01 to 0.11 mg kg^{-1} wet weight. The highest concentration of Cd was observed at G1 farm located at the southern part of the bay. The t-test analysis showed that there is no significant different between all farms for Cd concentrations.

Generally, the mean concentration of trace elements studied can be arranged from high to low content as Zn>Cu>Pb>Cd. The high concentration of trace elements in oysters may be due the winter season where the oyster samples were collected in December (Regoli, 1998) presumably due to a loss of metals stored in gonad tissues during spawning (O'Leary and Breen, 1998). The dilution or concentration effects of changing body weight on soft tissue metal concentration provide an alternative explanation for temporal shifts (Sokolowski *et al*., 2004). Overall, higher trace element concentrations were recorded at G3, near the shipping activities area at Trinite-sur-Mer and G4, the innermost part of the bay.

Figure 2 shows the relationship between trace elements content with the size of the oysters. It has been suggested that accumulated trace elements concentration in mollusk vary significantly with body size (Mubiana *et al*., 2005), which occasionally requires correcting these effects before spatial comparisons are made on large scale. However, the influence of an animal's size (age) on soft tissue, element concentration appears to be ambiguous and can depend on local environmental conditions (Przytarska *et al*., 2010). Riget *et al*. (1996) suggested that the relationship between size and element concentrations should be taken into account particularly in areas not affected by human impact or anthropogenic input.

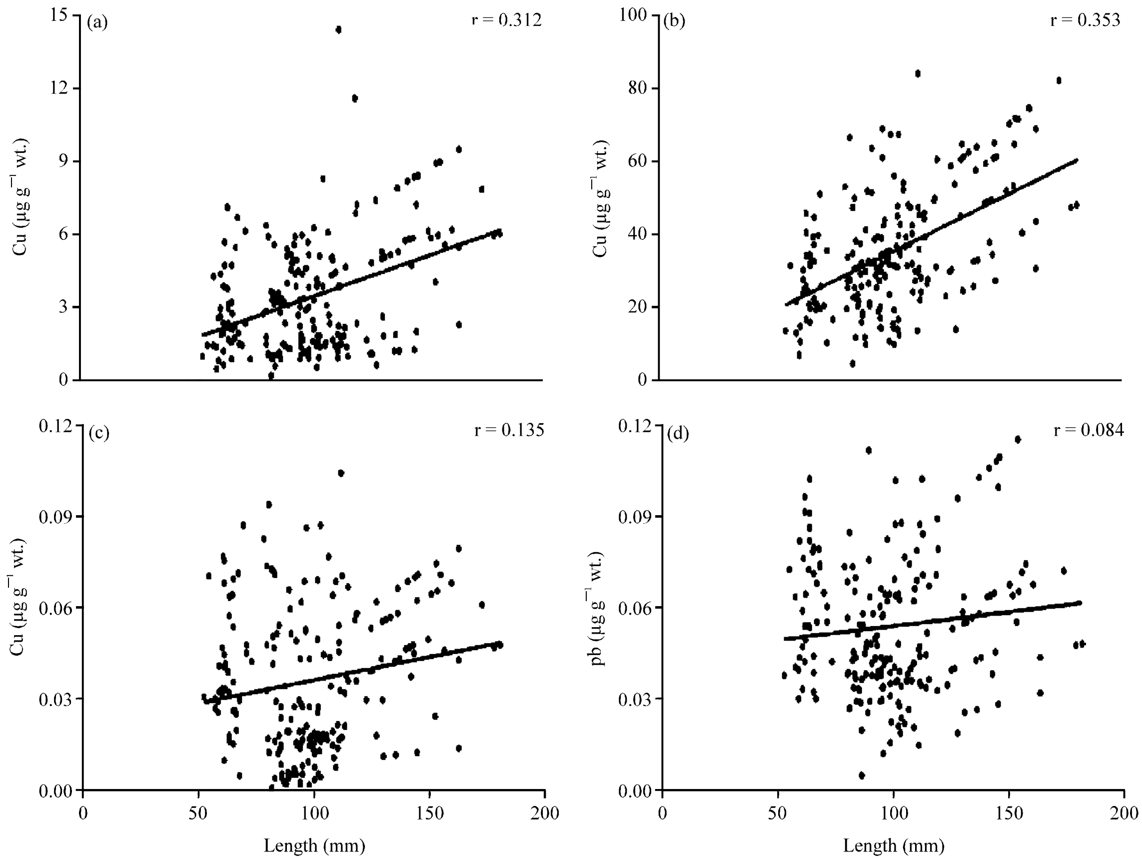

Fig. 2(a-d): Relationship between trace elements (a) Copper, (b) Zinc, (c) Cadmium and (d) Lead concentration and oyster size (in the term of length)

Oysters from Quiberon bay are used as a local food source for the coastal communities. Depending on consumer, those oysters can be "swallowed" or masticated normally, increasing the surface of contact between food and digestive fluids. Consumer will consume whole oyster soft part and therefore public health limits on the concentrations of toxic elements are usually quoted in terms of fresh (wet) weight. Using the total element concentration in Table 2, bio-accessible concentrations were calculated and compared to the most severe safety limits (Table 3). In most cases, bio-accessible concentrations remained lower than the safety limits except in the case of Zn concentration was comparable with the safety value. The ability of bivalves to eliminate Zn is well known. Yet the question remains as to the reason for the particularly high participation of zinc in bivalve metal accumulation above the portion which is normally required in the catalytic activity of enzymes (Martincie *et al.*, 1984).

International scientific committees such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), regional scientific committees such as European Union and national regulatory agencies generally use the safety factor approach for the establishing acceptable of tolerable intakes of substances that exhibit thresholds of toxicity. Table 4 shows the PTWI or PMTDI as assessed by the World Health Organization. JECFA derives tolerable intakes, expressed on either a daily or a weekly basis, for contaminants (WHO, 1983). Many contaminants are not removed rapidly from human body and for them, Provisional Tolerable Weekly Intakes (PTWIs) are allocated. The term

Table 3: Maximum permissible levels (MPL) of trace elements in food in different countries or regions

	MPL (mg kg^{-1} b.wt.)				
	Cu	Zn	Cd	Pb	References
Shellfish mollusk					
European Community			1	1.5	EC (2006)
Hong Kong			2	6	HKEPD (1997)
Australia	30		2	2	FSANZ (2005)
USA			3.5	1.6	USFDA (1990)
Food category not specific					
Malaysia	30	50	1	2	MFR (1986)
Thailand	26.6	133		1.33	MPHT (1986)

Amiard *et al*. (2008)

Table 4: Maximum consumption of seafood products and provisional tolerable weekly intake (PTWI) or provisional maximum tolerable daily intake (PMTDI)

	PTWI (mg kg^{-1} b.wt.)	PMTDI (mg kg^{-1} b.wt.)
Copper	--	0.5
Zinc	--	1.0
Cadmium	0.007	--
Lead	0.025	--

WHO (1983)

Table 5: Maximum consumption of oysters from the Quiberon bay and provisional tolerable weekly intake (PTWI) based on standard weight of an adult of 65 kg

	PTWI per individual (mg kg^{-1} b.wt.)	Species	Maximum consumption (kg week^{-1})	Maximum No. of shellfish
Cu	228	*O. edulis*	31.70	4953
		C. gigas	23.80	1460
Zn	455	*O. edulis*	8.90	1390
		C. gigas	5.54	340
Cd	0.46	*O. edulis*	5.11	798
		C. gigas	5.75	353
Pb	1.63	*O. edulis*	4.79	748
		C. gigas	3.97	244

tolerable is used because it signifies permissibility rather than acceptability for the intake of contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods.

JECFA has established regulatory guidelines regarding the dietary intake of trace elements from shellfish. To assess the risk due to the consumption of these oyster products, the number of individuals of each species from mariculture area which has to be eaten to reach the maximum tolerable intake recommended by the World Health Organization has been calculated (Table 5). For example, JECFA established a PTWI for Pb of 0.025 mg kg^{-1} b.wt. week^{-1} which was equivalent to 1.63 mg week^{-1} for a 65 kg adult. By consider the means of weekly shellfish consumption in south Brittany of 276 g per person (Leblanc *et al*., 2006) and the minimum and maximum Pb levels in examined samples, weekly intake calculated ranged from 0.005 mg (276 g×0.10 mg/1000 g) to 0.11 mg (276 g×0.34 mg/1000 g) per person for Pb in cultivated *C. gigas*. The estimated PTWI of Pb for *C. gigas* is far below the established PTWI, 0.025 mg kg^{-1} body weight. In order to exceed the PTWI level, a 65 kg adult should consumed 3.97 kg (1.63 mg×1 kg/0.41 mg) of *C. gigas* flesh or 244 individual (in-toto weight, 8.7 g).

CONCLUSION

A long term monitoring program for measuring the contents of various metals oysters is essential as marine organisms consumed by human may accumulate these harmful substances at a high level. Knowledge concerning the levels of toxic metals within these valuable food resources is necessary to carry out meaningful toxicological evaluation experiments, since metals bound to tissues of marine organisms may be toxic due to metal-metal interaction and altered absorption, distribution and secretion characteristics. Generally, Cu, Zn, Cd and Pb concentrations in the flesh or muscle of the studied oyster, *C. gigas* and *O. edulis* were in the recommended permissible level for human consumption based on several standards from WHO (1983) and from the European Communities (EC, 2006) regulation.

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