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Abstract**—** Oyster samples (*crassostrea gigas*) were collected from seven mariculture site in Quiberon bay and gulf of Morbihan (South Brittany coastal area) for the investigation of metallic trace elements (MTE) assessment. Cu, Zn, Cd and Pb were analyzed by using inductively coupled plasma–mass spectrometry after acid digestion process. Oyster tissue, SRM1556a was used to validate the methods and the results show a good agreement with the certified values. The mean levels of Cu, Zn, Cd and Pb in oysters flesh were 3.57, 32.8, 0.04 and 0.24 mg/kg *wet wt*., respectively. MTE 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 heavy metals, both oysters' species had the potential of being used as biomonitors to control the aquatic contaminations of heavy metals.

Keywords**—***Crassostrea Gigas*, Metallic Trace Element, South Brittany Coastal Area, Human Consumption.

I. Introduction

THE pacific or Japanese oyster, C. *gigas* is the most cultivated oyster species worldwide. It has been introduced for cultivation in mariculture area in many countries including France. For the purpose of assessing coastal environmental quality, C. *gigas* species have been proposed as sentinel organisms for marine ecotoxicological tests, because they are very sensitive to pollutant and provide a rapid response sensitive to pollutants [1]. Additionally, C. gigas is widely cultivated for human consumption and has important commercial value [2].

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Aquatic animals accumulate large quantities of xenobiotics and the accumulation depends upon the intake and elimination from the body [3]. Living organisms require trace amounts of some elements, including Co, Cu, Fe, Mn, Va, Sr and Zn. Many of these metallic trace elements (MTE) exist naturally, at background levels in the environment. Zn, Fe, Cu and Mn are biologically essential and play an important role as cofactors in enzymatic processes [4]. Among different aquatic organisms, oyster, mussel and clams accumulate large quantities of MTE in marine environment.

Oyster species are often used as indicator of estuary and coastal health. Since oysters are suspension feeders that filter water, they can retain small particles within large volumes of seawater and concentrated metals and other pollutants. Therefore, they are ideal pollution indicators and are frequently used in environmental assessment and monitoring [5]. The ability of oyster to accumulate high concentrations of MTE in its soft tissue is well documented [6]–[8].

II. Materials & Methods

A. Sampling & Sample Treatment

Oyster sample were collected at the oyster mariculture site, located in the Quiberon bay and gulf of Morbihan (Figure 1). 322 specimens covering a wide size–range (59 to 181 mm length) 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 ice–chest and preserved at 4°C while shipped back to the laboratory.

Prior to analysis, each individual sample was shucked and washed with running deionized water (Mili–Q). 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.

B. Analytical Method

Content of MTE was measured in pure white muscle and in all soft tissue excluding shell (in–toto). Data were analyzed as total metal concentrations on a wet weight basis and are expressed in mg/kg *wet wt*.

The soft tissues were weighted to approximately of 3g (weight weight) in a PTFE digestion container. Nitric acid, hydrochloric acid and hydrogen peroxide were added to each sample and left to predigest.

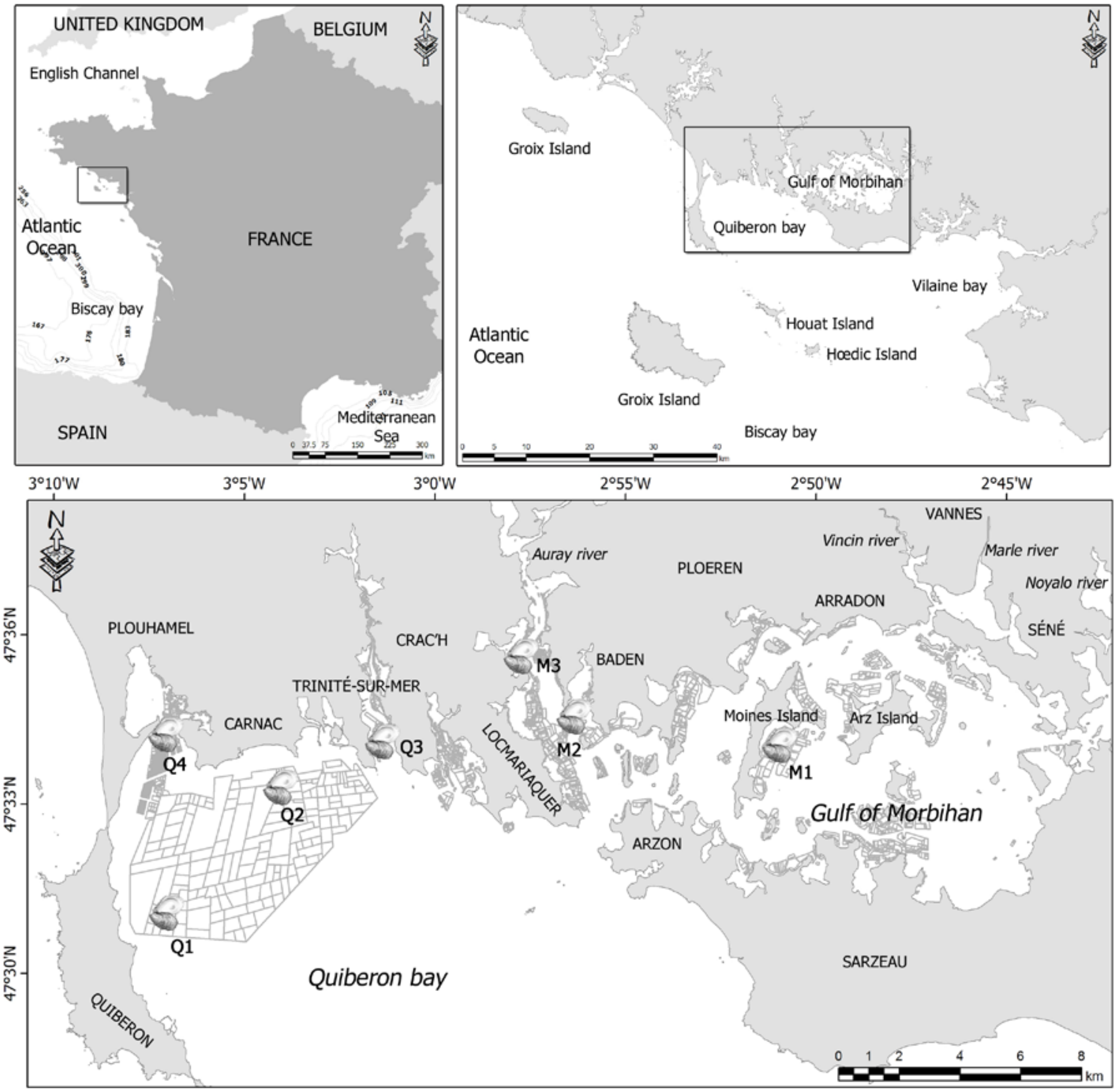

Fig. 1 Map showing sampling location of oyster samples in South Brittany coastal area

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 hours at 150°C. After cooling, the solution was transferred into a polypropylene tube and diluted with 5% nitric acid.

The values of MTE (Cu, Zn, Cd, Pb) were measured in oyster sample by using Inductively Coupled Plasma Mass Spectrometry [9], [10]. Blanks were provided through all determinations. All analyses were carried out in thrice using the external calibration method. Analytical quality was assessed using standard reference material, SRM1556a (Oyster tissue). Recoveries of Cu, Zn, Cd and Pb were 99%, 97%, 102% and 97%, respectively. 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 MTE in biological samples.

III. Results & 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 [11] and accumulate trace metals in proportion to the integrated ambient availabilities [12]. Under normal conditions, as much as 387 L of water is pumped through the gills in a single day [13] and therefore oysters accumulate a large amount of MTE by the ingestion of phytoplankton and organic particles as well as direct uptake from solution. So they could be used as biomonitors provide integrated measures of the supply of MTE available to them in an environment, accumulating the MTE taken up from all sources such as from water and from food [14], [15].

The results of the analyzed MTE (Cu, Zn, Cd, Pb) in C.

gigas flesh collected from Quiberon bay and gulf of Morbihan were presented in Table 1. According to the data, there is a wide range of values for the MTE contents in all the samples. Generally, the concentration of the MTEs analyzed exhibited the following order: Zn>Cu>Pb>Cd.

TABLE 1
TOTAL SHELL LENGTH AND MTE LEVELS IN SOFT TISSUE

Location		Length (mm)	MTE (mg/kg *dry wt.*) Cu	Zn	Cd	Pb
Q1 (n=40)	Mean	93	3.51	31.5	0.03	0.20
	Min	79	0.26	4.61	0.001	0.05
	Max	119	6.35	68.9	0.11	0.37
Q2 (n=37)	Mean	94	3.58	32.8	0.02	0.13
	Min	67	0.98	9.82	0.001	0.02
	Max	112	5.66	67.4	0.05	0.29
Q3 (n=45)	Mean	142	5.33	50.1	0.05	0.22
	Min	114	0.75	14.1	0.001	0.06
	Max	181	9.57	82.1	0.08	0.41
Q4 (n=49)	Mean	101	2.57	34.1	0.04	0.15
	Min	73	0.61	11.6	0.001	0.05
	Max	130	14.5	83.9	0.09	0.29
M1 (n=53)	Mean	100	2.61	26.9	0.04	0.31
	Min	59	0.56	4.52	0.01	0.09
	Max	145	7.58	62.8	0.09	0.78
M2 (n=47)	Mean	104	3.70	28.2	0.04	0.28
	Min	59	1.25	8.57	0.01	0.07
	Max	126	13.4	90.6	0.11	0.77
M3 (n=51)	Mean	93	3.91	27.6	0.04	0.33
	Min	70	0.96	8.63	0.01	0.03
	Max	119	12.1	52.0	0.08	0.89
All samples (n=322)	Mean	104	3.57	32.8	0.04	0.24
	Min	59	0.26	4.52	0.001	0.02
	Max	181	14.5	90.6	0.11	0.89

We observed that higher concentration of Cu and Zn occurred in C. gigas collected from Quiberon bay mariculture farm compared to gulf of Morbihan mariculture farm. Due to a lack of information for the determination in different food commodities, no maximum levels have been settled by Commission of European Communities (EC) for Cu and Zn. Therefore, we compared with Food and Agricultural Organization [16] limits for Cu and Zn (30 mg/kg *wet wt.*). None of our samples were exceed the maximum limit for Cu while some samples were above the maximum limit for Zn for all mariculture farms.

On the other hand, higher concentration of Cd and Pb occurred at gulf of Morbihan compared to mariculture farms located at Quiberon bay. Cd levels did not exceed the maximum residual level of 1.0 mg/kg *wet wt.* set by Commission of the European Communities [17]. However, some samples especially from mariculture farm located at gulf of Morbihan were higher than European legislation, 0.5 mg/kg *wet wt.* [17].

Shellfish including C. *gigas* are important source of dietary protein in south Brittany and provide a livelihood for many 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 soft part of the oyster, therefore in this study, the MTE analysis is examine in–toto or oyster flesh.

To safeguard public health, maximum acceptable concentrations of toxic contaminants have been established in various countries. Using the total MTE concentration in our study (Table 1), bio–accessible concentrations were compared to the most severe safety limits (Table 2). In most cases, bio–accessible concentrations remained lower than the safety limits except in the case of Zn concentration. 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 accumulation in bivalve above the portion which is normally required in the catalytic activity of enzymes [18].

TABLE 2
MAX PERMISSIBLE LEVELS (MPL) FROM DIFFERENT REGIONS

	MTE (mg/kg *wet wt.*) Cu	Zn	Cd	Pb
Shellfish mollusk				
European Communities [17]	–	–	1	1.5
Hong Kong [19]	–	–	2	6
Australia [20]	30	150	2	2
Spain [21]	20	–	1	5
Food category not specific				
Malaysia [22]	30	50	1	2
Thailand [23]	20	133	–	1
Brazil [24]	30	50	1	2

International scientific committees such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) generally use the safety factor approach for the establishing acceptable of tolerable intakes of substances that exhibit thresholds of toxicity. JECFA derives tolerable intakes for contaminants, expressed on either a daily or a weekly basis (Table 3). Many contaminants are not removed rapidly from human body and for them, provisional tolerable weekly intakes (PTWIs) are allocated. The term 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. For contaminants that are not known to accumulate in the body, JECFA establishes provisional maximum tolerable daily intakes (PMTDIs). The term of maximum is used because this designation is often applied to trace elements that are both essential nutrients. In such situations, a range is designated, with the lower value representing the level of essentially and the upper value the PMTDI [16].

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 WHO has been calculated. Table 4 calculates the number of individuals of each category of oyster which can be eaten for one week continuously before reach the maximum tolerable intake based on an adult standard body weight of 65kg.

TABLE 3
MAXIMUM CONSUMPTION OF SEAFOOD PRODUCTS AND PTWI/PMTDI AS ASSESS BY WHO [16]

	PTWI (mg/kg body weight)	PMTDI (mg/kg body weight)
Cu		0.5
Zn		1.0
Cd	0.007	
Pb	0.025	

TABLE 4
MAXIMUM CONSUMPTION OF OYSTERS AND PTWI BASED ON STANDARD WEIGHT OF 65KG

	PTWI/individual (mg/kg body weight)	Max consumption (kg per week)	Max number of oysters
Cu	228	15.6	575
Zn	455	5.02	184
Cd	0.45	4.13	152
Pb	1.62	1.83	67

An inquiry into seafood consumption in France has been reviewed by Leblanc [25]. The average consumption of shellfish in south Brittany was 276 g/week for an adult (Leblanc et al., 2006). For cultivated C. gigas, JECFA established a PTWI for Cd of 0.007 mg/week for a 65kg adult. By consider the mean of weekly shellfish consumption in south Brittany of 276g per person, and the maximum Cd levels in examined samples, weekly intake calculated was 0.25 mg (276g x 0.89mg/1000g) per person. The estimated PTWI of Cd was below the established PTWI value. In order to exceed the PTWI level, a 65kg adult should consume 4.13kg (1.63mg x 1kg/0.89mg) of C. gigas in–toto or 152 individual (in–toto weight, 27.2g).

We concluded that, from the PTWI estimation for all MTE studied of a 65kg adult, the maximum numbers of C. gigas from mariculture farm located at South Brittany coastal that can be consumed before reach the PTWI value was 1.83kg C. gigas flesh or 67 individuals weekly.

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