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Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia

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Abstract

Maximum acceptable concentrations of metals in food – based on total concentrations – have been established in many countries. To improve risk assessment, it would be better to take into account bioaccessible concentrations. A total of seven species of molluscs from France, UK and Hong Kong was examined in this study including clams, mussels, oysters, scallops and gastropods. The species which have total metal concentrations higher than the most severe food security limits are mainly oysters (all of the three studied species), the gastropod *Buccinum undatum* for cadmium and zinc, and scallops for cadmium. The lowest bioaccessibility (in % extractability with gut juices) was observed for silver (median for all of the species: 14%), it was moderate for lead (median: 33%) and higher for cadmium, zinc and copper (medians were respectively 54%, 65%, and 70%). In most cases, bioaccessible concentrations remained higher than the safety limits, except for cadmium in scallops and zinc in *B. undatum*. The influence of feeding habit (masticated or swallowed, addition of vinegar or lemon) on metal bioaccessibility in oysters is limited. On the contrary, cooking the gastropods decreased the bioaccessibility of metals, except silver.

Keywords: Metal bioaccessibility; Clam; Mussel; Oysters; Scallop; Gastropods

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1. Introduction

The nutritional value of shellfish has been investigated to estimate their contribution to dietary needs, particularly in countries where the socioeconomic level is not very high (Pak et al., 1985; Blanco Metzler and Montero Campos, 1992; Lebiedzińska et al., 2004). Marine mussels, for example, provide a cheap source of protein for human consumption. For *Perna viridis* there is about 60% protein (calculated on a dry weight basis) in mussel soft tissues (Choo and Ng, 1990). From the nutritional point of view, the mussel is an important food source for supplying essential elements (e.g. Ca, Fe) and certain vitamins such as niacin, thiamin and riboflavin (Cheong and Lee, 1984). An epidemiological study in Japan showed that seafood was the largest source of vitamins B6 (16–23% of total intake) and B12 (77–84%) in the diet (Yoshino et al., 2005). The beneficial effect of marine $n-3$ (also called omega-3) polyunsaturated fatty acids (PUFA) on coronary heart disease (CHD) is still a topic of discussion. Schmidt et al. (2006) suggested that the current recommendations (i.e. patients with established CHD should be advised an average intake of marine $n-3$ PUFA of at least 1 g/day) should not be changed. Many species of fish and shellfish are rich sources of omega-3 fatty acids (Ackman, 2000). A positive impact of seafood consumption on bone mineral density has also been reported (Zalloua et al., 2007). Meeting nutritional requirements of antioxidants (particularly zinc) to defend human beings against xenobiotic-induced oxidative stress and associated toxic

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hepatitis (Stehbens, 2003) could be improved through zinc-rich seafood consumption.

On the other hand, seafood products are accumulators of a number of pollutants, the most studied of which are biomagnifying substances such as PCBs or methylmercury. Cadmium which is strongly bioaccumulated in shellfish, particularly oysters, is a metal with high toxic effects, has an elimination half-life of 10–30 y, and accumulates in the human body, particularly the kidney. Exposure to cadmium occurs through intake of contaminated food or water, or by inhalation of tobacco smoke or polluted air. Environmental exposure to cadmium is associated with renal dysfunction, increased calciuria, osteoporosis, and a risk of fractures (Ogawa et al., 2004; Nawrot et al., 2006). In 1993, the International Agency for Research on Cancer (IARC) classified cadmium as a human carcinogen. As reviewed by Verougstraete et al. (2003) and Navarro Silvera and Rohan (2007), there is compelling evidence in support of positive association between cadmium or cadmium compounds and lung cancer. In addition, some findings appear to support the hypothesis that cadmium exposure increases prostate cancer risk (Vinceti et al., 2007).

Advisories against pollutants may therefore conflict with dietary recommendations. Thus balancing the risks and benefits of seafood consumption is clearly a topic of interest. Much has been done in the case of fish (Gochfeld and Burger, 2005) but risk assessment has not been so well-developed in the case of shellfish. In the European Community, the maximum permitted concentration of cadmium in oysters is 1 mg kg^{-1} fresh weight (CE, 2006) and the current deliberation by CODEX to adopt the same value, poses significant threats to oyster farming in several areas in the world (Geffard et al., 2002b; Kruzynski, 2004). In the case of essential metals such as copper and zinc, consumption of seafood can contribute to the attainment of the recommended levels of intake, particularly in developing countries where nutritional deficiency of zinc is widespread, but beyond a certain level even these elements can become toxic (Bragigand et al., 2004). However, the total amount of an ingested contaminant in the diet does not always reflect the amount that is available to the consumer. There is therefore a pressing need to determine the bioaccessibility of both essential and non-essential metals in oysters and other metal-rich seafood products in order to improve health risk assessment.

Approaches to assess the bioaccessibility of metals from materials consumed either accidentally or intentionally in the diet have been recently reviewed (Klein et al., 2003; Intawongse and Dean, 2006). A particular emphasis has been placed on the parameters that influence gastrointestinal extraction, including gastric and intestinal pH, food constituents, residence time and particle size. Thus simple methods based on the well-established influence of pH on metal speciation (Bragigand et al., 2004) need to be improved. Two step procedures simulating gastrointestinal digestion have been proposed (Cabañero et al., 2004; Kulkarni et al., 2007) but the most complete pattern is provided by three step models integrating the role of saliva in the mouth (Versantvoort et al., 2005).

The concepts of bioaccessibility and trophic bioavailability are often used concurrently. Their precise meanings have been discussed by Versantvoort et al. (2005). Trophic bioavailability should be used to describe the proportion of a chemical ingested with food which enters the systemic circulation. Release of a chemical from ingested food is a prerequisite for uptake and assimilation. Thus, the determination of bioaccessibility of a food-bound contaminant, as measured by its extractability from the food, can be used as an indicator of its maximum trophic bioavailability. In this study, we have quantified the bioaccessibility of trace metals in shellfish food products by measuring their extractability in a three step *in vitro* digestion process mimicking human gut digestion (Versantvoort et al., 2005).

The aim of this study, therefore, is to examine bioaccessibility (measured as extractability) of trace metals, both essential and non-essential in commercial shellfish species from Western Europe (France, UK) and Asia (Hong Kong). Depending on feeding habits, seafood products are eaten fresh or cooked and may be ingested concomitantly with additives (vinegar, lemon), the acidic character of which can change the bioaccessibility of metals. Thus the influence of culinary practices will be taken into account. Depending on the level of contamination, metal handling strategies lead to different modes of storage (binding to detoxificatory proteins, biomineralization as granules) which influence subsequent trophic transfer (Ng et al., 2005 and literature cited therein). Thus metal bioaccessibility was investigated in bivalves originating respectively from clean sites and sites historically impacted by mining activities, the Gironde estuary (France) and Restronguet Creek (UK) (Boutier et al., 2000).

2. Materials and methods

2.1. Collection

A total of 7 species of shellfish was examined in this study. Green mussels *Perna viridis* were collected from the rocky shore bordering the sandflat at Hoi Sing Wan, Tolo Harbour, Hong Kong, and scallops *Chlamys nobilis* were obtained from Daya Bay, Shenzhen, China. Clams *Marcia hiantina* were purchased from the local seafood market at Saikung, Hong Kong, where they were on sale for human consumption after collection in the (unspecified) local SE China area. The oysters *Saccostrea cucullata* were collected from the rocky shore at the Hong Kong University of Science and Technology, near Saikung, Hong Kong; *Crassostrea gigas* from clean (Noirmoutier Island) and contaminated (Gironde estuary) sites, along the French Atlantic coast; *Ostrea edulis*, from clean (south coast of Britanny, France) and contaminated (Restronguet Creek, Cornwall, England) sites. Specimens originating from clean sites will be termed controls in the following text. The neogastropod molluscs *Buccinum undatum*, both fresh and boiled were purchased from a supermarket in Nantes, France.

2.2. Metal analysis

Nalgene bottles were used to store all reagents. All labware was soaked in 10% hydrochloric acid, rinsed three times with deionised water and dried in a desiccator sheltered from atmospheric dust. Soft tissues of marine molluscs were digested by heating with suprapure concentrated nitric acid (Carlo Erba), then metal levels in these different acid solutions were determined after dilution with deionised water by flame (FAAS) (Cu, Zn) or electrothermal atomic absorption spectrophotometry (EAAS) (Ag, Cd and Pb) with the Zeeman effect (Varian SpectrAA55, Varian SpectrAA800 spectrophotometers). Standard addition analyses were performed in an iso-medium and added concentrations of each element were + 125, 250, 500, 1000 ng Cu and Zn^{-1} mL for FAAS and + 6.25, 12.5, 25 ng Ag^{-1} mL, + 0.5, 1, 2 ng Cd^{-1} mL, + 25, 50, 100 ng Pb^{-1} mL for EAAS. When the metal concentrations were determined separately in different tissues, the total concentration was recalculated from the mass of tissue and quantity of metal in these tissues. The results were expressed as mg metal kg^{-1} wet weight. The analytical methods have been validated through international intercalibration exercises and internal quality controls (Table 1).

2.3. In vitro digestion

Extractions were carried out separately in digestive glands and remaining soft tissues of clams, mussels and oysters *S. cucullata*. In scallops, adductor muscles only were treated. In neogastropods, oysters *C. gigas* and *O. edulis*, the soft tissues were submitted as a whole to *in vitro* digestion. Mimicking of human digestion was carried out according to the procedure described by Versantvoort et al. (2005). Briefly, it consists of a three step procedure simulating the digestive processes in the mouth, stomach and small intestine. The food matrix was first minced at 4 °C, exposed to artificial saliva at pH 6.8 for 5 min, then artificial gastric juice at pH 1.3 was added for 2 h, and thirdly a mixture of artificial duodenal juice, bile and HCO_3^- at pH 8.1–8.2 was added for a further 2 h. The incubation temperature was 37 °C. After centrifugation at 2800 g for 5 min, the supernatants and pellets were separated. Freeze-dried supernatants and pellets were acid digested and metal content was determined as described in Section 2.2. Extractability (and hence bioaccessibility) was calculated as the percentage of metals recovered in the supernatant. The number of replicates was $n = 3$ for all samples except *C. gigas* from the Gironde and *B. undatum* where $n = 6$.

In some cases, it was impossible to carry out the treatment of samples immediately after collection. Thus they were frozen for a few days at −20 °C. The influence of freezing on extractability (bioaccessibility) was taken into account, comparing extractability in the digestive gland of frozen or fresh bivalves *S. cucullata*, *P. viridis* and *M. hiantina* ($n = 3$ for each category).

Many seafood products are eaten after cooking. Thus, metal bioaccessibility (as extractability) was determined from soft tissues of *B. undatum* submitted or not to cooking (boiled in salted water; $n = 6$ for each treatment).

Depending on consumers, oysters can be "swallowed" or masticated normally, increasing the surface of contact between food and digestive fluids. Certain consumers eat oysters after adding vinegar or lemon juice. The influence of chewing and vinegar were assessed in oysters *C. gigas* and *O. edulis* originating from reference and contaminated sites. The influence of lemon juice, in *C. gigas* from the Gironde estuary (metal-rich site) was also assessed.

2.4. Statistics

Medians, means, standard deviations and linear regressions were calculated using Excel. Non-parametric statistics were used, including comparison by Mann-Whitney tests for two groups and Kruskal-Wallis tests for three groups or more (SPSS).

3. Results

3.1. Metal concentrations in soft tissues

Metal concentrations in soft tissues are shown in Table 2. Oysters *C. gigas* and *O. edulis* originating from contaminated sites generally showed significantly higher metal concentrations than the controls. However, no inter-site difference was observed for lead and zinc concentrations in *C. gigas* from the Gironde. Considering metal concentrations in the whole soft tissues, oysters *S. cucullata* were the strongest metal accumulators among bivalve species originating from SE China. The most important interspecies differences were observed for essential elements (copper: nearly two orders of magnitude; zinc: more than two orders of magnitude). Very high concentrations of cadmium were determined in the adductor muscle of the scallops *C. nobilis*, and very high concentrations of cadmium and silver in the whole soft tissues of the neogastropods *B. undatum*.

Table 1
Results of external and internal quality controls

	Ag	Cd	Cu	Pb	Zn
Fish homogenate IAEA-407 (Wyse et al., 2003)					
Our value (Lab. Code: 57)		0.189 (0.014)	3.19 (0.47)	0.08 (0.02)	68.31 (2.51)
Certified value		0.189 (0.019)	3.28 (0.40)	0.12 (0.06)	67.1 (3.8)
Z-scores[a]		0.00	− 0.22	−2.67	0.14
Mussel tissues SRM 2976 (NIST)					
Our value	0.016 (0.006)	0.79 (0.02)	3.99 (0.44)	1.12 (0.07)	127 (1)
Certified value	0.011 (0.005)	0.82 (0.16)	4.02 (0.33)	1.19 (0.18)	137 (13)
Z-scores[a]	1.00	− 0.19	− 0.09	−0.39	− 0.76
Mussel tissue BCR N° 278R					
Our value	0.19 (0.02)	0.354 (0.011)	9.2 (0.3)	2.04 (0.054)	85.0 (2.0)
Certified value	–	0.348 (0.007)	9.45 (0.13)	2.00 (0.04)	83.1 (1.7)
Z-scores[a]	–	0.86	− 1.92	1.00	1.11
TORT-2, NRC					
Our value	–	25.1 (0.6)	105 (2)	0.35 (0.02)	186 (7)
Certified value	–	26.7 (0.6)	106 (10)	0.35 (0.13)	180 (6)
Z-scores[a]	–	− 2.70	− 0.10	0.00	1.00

Mean concentrations in mg kg^{-1} dry wt and standard deviation for our values, 95% confidence interval for certified values.

[a] $|Z| \leqslant 2$: performance is acceptable; $2 < |Z| \leqslant 3$: questionable; $|Z| > 3$: unacceptable (Thompson and Wood, 1993).

Table 2
Metal concentrations in soft tissues of edible molluscs expressed in mg kg^{-1} wet weight

Species	Organ	*n*	Ag	Cd	Cu	Pb	Zn
Buccinum undatum	*in toto*	6	0.63	**1.7***	5.4	0.37	**61***
			(0.05)	(1)	(1.3)	(0.14)	(25)
Crassostrea gigas Controls	*in toto*	3	0.11	0.04	13	0.28	**92***
			(0.06)	(0.01)	(4)	(0.09)	(37)
Crassostrea gigas Gironde	*in toto*	3	0.42	0.68	**58***	0.45	**217***
			(0.22)	(0.41)	(29)	(0.28)	(112)
Ostrea edulis Controls	*in toto*	3	0.19	0.048	6.4	0.2	**97***
			(0.11)	(0.014)	(3.4)	(0.04)	(20)
Ostrea edulis Restronguet Creek	*in toto*	3	0.08	0.19	**78***	**7.0***	**500***
			(0.01)	(0.06)	(46)	(1.4)	(220)
Saccostrea cucullata	Remaining soft tissue	6	0.28	0.86	134	0.7	**800***
			(0.07)	(0.19)	(76)	(0.5)	(223)
Saccostrea cucullata	Digestive gland	6	0.82	0.41	148	0.8	**244***
			(0.53)	(0.04)	(18)	(0.2)	(73)
Saccostrea cucullata	*in toto*	6	0.43	0.71	**141***	0.7	**618***
			(0.19)	(0.11)	(52)	(0.3)	(133)
Perna viridis	Remaining soft tissue	6	0.011	0.041	1.1	0.3	4.2
			(0.006)	(0.009)	(0.4)	(0.1)	(1.3)
Perna viridis	Digestive gland	6	0.077	0.081	3.2	0.7	10.5
			(0.02)	(0.021)	(1.7)	(0.1)	(4.9)
Perna viridis	*in toto*	6	0.017	0.045	1.3	0.3	4.8
			(0.005)	(0.009)	(0.5)	(0.1)	(1.6)
Marcia hiantina	Remaining soft tissue	6	0.025	0.084	0.9	0.2	4.8
			(0.007)	(0.022)	(0.4)	(0.1)	(1.4)
Marcia hiantina	Digestive gland	6	0.19	0.26	3.4	0.2	12.8
			(0.03)	(0.13)	(1.7)	(0.2)	(9)
Marcia hiantina	*in toto*	6	0.041	0.099	1.1	0.16	5.4
			(0.008)	(0.023)	(0.4)	(0.13)	(2)
Chlamys nobilis	Adductor muscle	12	0.036	**4.2***	1.8	0.39	33
			(0.012)	(2)	(0.9)	(0.19)	(20)

Metal concentrations in bold with an asterisk are higher than at least one MPL listed in Table 3.

Table 3
Maximum permissible levels (MPL) (in mg kg^{-1} wet weight) of metals in food in different countries or regions

	Cd	Cu	Pb	Zn
Shellfish molluscs				
European Community	1^{a}		1.5^{a}	
Hong Kong	2^{b,c}		6^{b}	
Australia	2^{d}	30^{e}	2^{d}	
USA	3–4^{f}		1.5–1.7^{f}	
Food category not specified				
Brazil	1^{g}	30^{g}	2^{g}	50^{g}
Thailand		26.6^{h,i}	1.33^{h,i}	133^{h,i}
Malaysia	1^{j}	30^{j}	2^{j}	100^{j}

[a] CE, 2006.
[b] HKEPD, 1997.
[c] Excluding oysters and scallops.
[d] FSANZ, 1996.
[e] Australian Government, 2006.
[f] USFDA, 1990.
[g] ABIA, 1991.
[h] MPHT, 1986.
[i] Recalculated from concentration in mg kg^{-1} dry wet using a conversion factor of 5.
[j] Malaysian Food Regulation, 1985.

Maximum permissible levels (MPLs) of toxic metals in food have been established in different countries (Table 3). In Table 2, the values higher than at least one MPL are depicted with an asterisk. Cadmium concentrations in the whole soft tissues of *B. undatum* and in the muscle of *C. nobilis* pose a risk as well as lead concentrations in oysters from Restronguet Creek. In oysters from the metal-rich Gironde estuary, the specimens showing the maximum cadmium concentrations were also above the lowest MPL. For copper, oysters from Hong Kong waters as well as European oysters from contaminated sites showed mean concentrations higher than the Australian MPL specifically established for molluscs. For zinc, no MPL has been established specifically for molluscs. Considering general regulations for food safety, mean zinc concentrations were higher in all of the studied oyster species (originating either from contaminated or reference sites) as well as in *B. undatum*. Among scallops, the specimens showing the maximum zinc concentrations were also above the lowest MPL.

In *S. cucullata*, *P. viridis* and *M. hiantina*, metals were analysed separately in the digestive gland and remaining soft tissue (Table 2). Silver concentrations were always lower in remaining soft tissue, the difference being significant in mussels and clams. In the case of cadmium, no significant differences were found except for *P. viridis* in which higher concentrations were detected in the digestive gland. For copper, the concentration was significantly higher in the digestive gland of all of the three species. For lead, the concentration was higher in the digestive gland of mussels only. In the case of zinc, the only significant difference was found in oysters, the remaining soft tissue of which showed higher zinc concentrations than the digestive gland.

3.2. Influence of freezing on metal bioaccessibility

The differences between metal bioaccessibility in fresh or frozen tissues are depicted in Fig. 1. For most of the samples, no significant differences were found, except a depletion of zinc bioaccessibility from the digestive gland of frozen mussels, whereas bioaccessibility of zinc increased in the digestive gland of frozen oysters as well as the bioaccessibility of silver in the digestive gland of frozen mussels.

3.3. Metal bioaccessibility in different species

Bioaccessibility (in % extractability) of metals from soft tissues of different species of interest are shown in Table 4. For different species (*S. cucullata*, *P. viridis*, *M. hiantina*) and different metals, significant differences of bioaccessibility were observed between digestive gland and remaining soft tissue. However, there was no clear trend to indicate a higher bioaccessibility in one tissue or the other. Human consumers generally ingest the whole soft tissues of such bivalves.

Considering the edible tissues of different species, silver was the least bioaccessible metal with a median value of bioaccessibility of 14%, a minimum of 3% in *S. cucullata* and a maximum of 33% in *O. edulis* (control). For lead, these parameters were higher, being respectively of 33% for the median, 19% for the minimum (*S. cucullata*) and 52% for the maximum (*B. undatum*). For cadmium, copper and zinc, the medians were respectively 54%, 70% and 65%. For all of these three metals, the maximum (respectively 84%, 97%, 82%) was always observed in oysters *C. gigas* originating from the metal-rich Gironde estuary. For cadmium and copper the minimum bioaccessibility was determined in the adductor muscle of scallops (respectively 20% and 38%); for zinc the minimum (34%) was observed in the soft tissues of mussels.

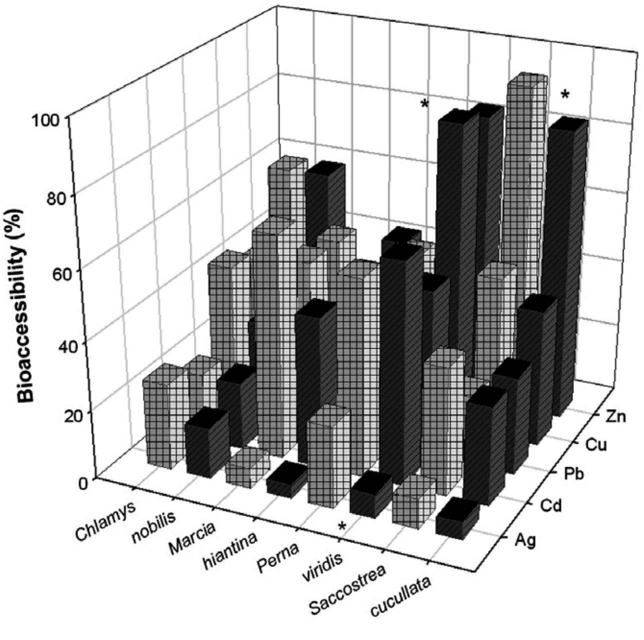

Fig. 1. Bioaccessibility (in %) of different metals in bivalve molluscs determined in fresh or frozen tissues (digestive gland of oysters, mussels and clams, adductor muscle of scallops). Asterisks between two bars indicate a significant difference between treatments.

For the whole soft tissues, the potential influence of the total metal concentration on metal bioaccessibility has been examined taking into account all of the three species of oysters originating from contaminated or reference sites. Silver and zinc bioaccessibility decreased significantly with increasing total concentration (Fig. 2). No significant difference was demonstrated for cadmium, copper and lead.

For the digestive gland of clams and mussels, the relationship between bioaccessibility and total metal concentration is depicted in Fig. 3. These parameters are significantly and negatively correlated in the case of silver and the same trend was observed in the digestive gland of oysters *S. cucullata* and in the adductor muscle of the scallops (not shown). Bioaccessibility decreased with increasing total copper concentration in all of the studied organs, but this relationship was significant only for the digestive gland of clams and mussels (Fig. 3). The reverse was observed for cadmium in the digestive gland of clams and mussels (Fig. 3), whereas no trend was observed in the digestive gland of oysters *S. cucullata* and in the adductor muscle of the scallops (not shown). In the case of zinc, a negative trend was always observed but the relationship was never significant. For lead, the relationship was not consistent between species, a positive trend (not significant) being observed in clams and the reverse in oysters (not shown).

3.4. Influence of cooking on metal bioaccessibility in the gastropods Buccinum undatum

Bioaccessibility (in % extractability) of metals from soft tissues of *B. undatum* subjected or not to cooking (boiled in salted water) is shown in Fig. 4. Cooking had no effect on the bioaccessibility of silver, whereas bioaccessibility was significantly lowered for cadmium, copper, lead and zinc ($p < 0.05$).

3.5. Influence of feeding habits on metal bioaccessibility in oysters

No influence of chewing was observed in *C. gigas* from both sites, nor in *O. edulis* from Restronguet Creek. In *O. edulis* from the reference site, the influence of chewing was not consistent, an increased bioaccessibility being observed for cadmium (66% vs 42% on average) and zinc (71% vs 57%) and the reverse for lead (30% vs 35%), whereas no

Table 4
Bioaccessibility (mean and SD expressed in %) of metals in soft tissues of gastropod and bivalve molluscs

Species	Organ	$N =$	Ag	Cd	Cu	Pb	Zn
Buccinum undatum	*in toto*	6	14	72	90	**52**	73
			(6)	(12)	(9)	(18)	(17)
Crassostrea gigas Controls	*in toto*	3	14	62	82	33	78
			(2)	(3)	(7)	(7)	(4)
Crassostrea gigas Gironde	*in toto*	3	8	**84**	**97.4**	34	**82**
			(3)	(13)	(0.8)	(13)	(3)
Ostrea edulis Controls	*in toto*	3	**33**	66	74	28	71
			(11)	(14)	(8)	(2)	(2)
Ostrea edulis Restronguet Creek	*in toto*	3	22	62	80	28	59
			(2)	(3)	(8)	(2)	(4)
Saccostrea cucullata	Remaining soft tissue	3	2	77	86	16	81
			(1)	(8)	(1)	(9)	(15)
Saccostrea cucullata	Digestive gland	3	5	29	30	28	84
			(4)	(3)	(15)	(6)	(4)
Saccostrea cucullata	*in toto*	3	*3*	68	61	*19*	81
			(1)	(7)	(5)	(7)	(14)
Perna viridis	Remaining soft tissue	3	34	31	58	43	24
			(17)	(8)	(25)	(7)	(5)
Perna viridis	Digestive gland	3	6.8	64	88	48	84
			(0.8)	(2)	(7)	(4)	(4)
Perna viridis	*in toto*	3	19	36	63	44	*34*
			(9)	(7)	(20)	(4)	(5)
Marcia hiantina	Remaining soft tissue	3	23	49	82	37	63
			(2)	(2)	(6)	(3)	(3)
Marcia hiantina	Digestive gland	3	4	44	24	31	44
			(1)	(18)	(10)	(22)	(16)
Marcia hiantina	*in toto*	3	24	48	68	37	60
			(16)	(6)	(3)	(5)	(5)
Chlamys nobilis	Muscle	3	15	*20*	*38*	29	60
			(11)	(5)	(33)	(10)	(39)

The minimum bioaccessibility registered for a given metal in the whole soft tissues of different studied species is underlined and in italics (e.g. *3* in the case of Ag); The equivalent maximum bioaccessibility is in bold (e.g. **33** in the case of Ag).

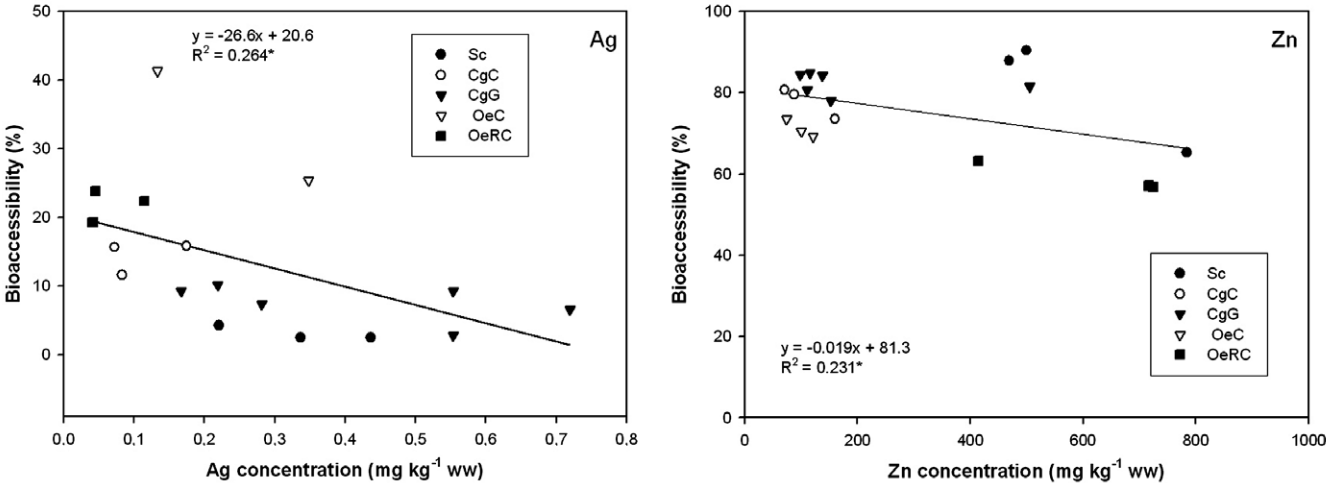

Fig. 2. Relationship between total metal concentrations and bioaccessibility in the whole soft tissues of oysters. Sc: *Saccostrea cucullata*; CgC: *Crassostrea gigas*, control; CgG: *C. gigas* from the metal-rich Gironde estuary; OeC: *Ostrea edulis*, control; OeRC: *O. edulis* from the metal-rich Restronguet Creek. *significant at the 95% level.

significant differences in bioaccessibility were observed for silver and copper.

The influence of co-ingestion of oyster flesh and vinegar was examined in both species originating from two different sites and submitted or not to chewing (8 conditions for each of the five studied metals). In most cases (similarly distributed among species), no significant differences were observed. When differences were significant, bioaccessibility

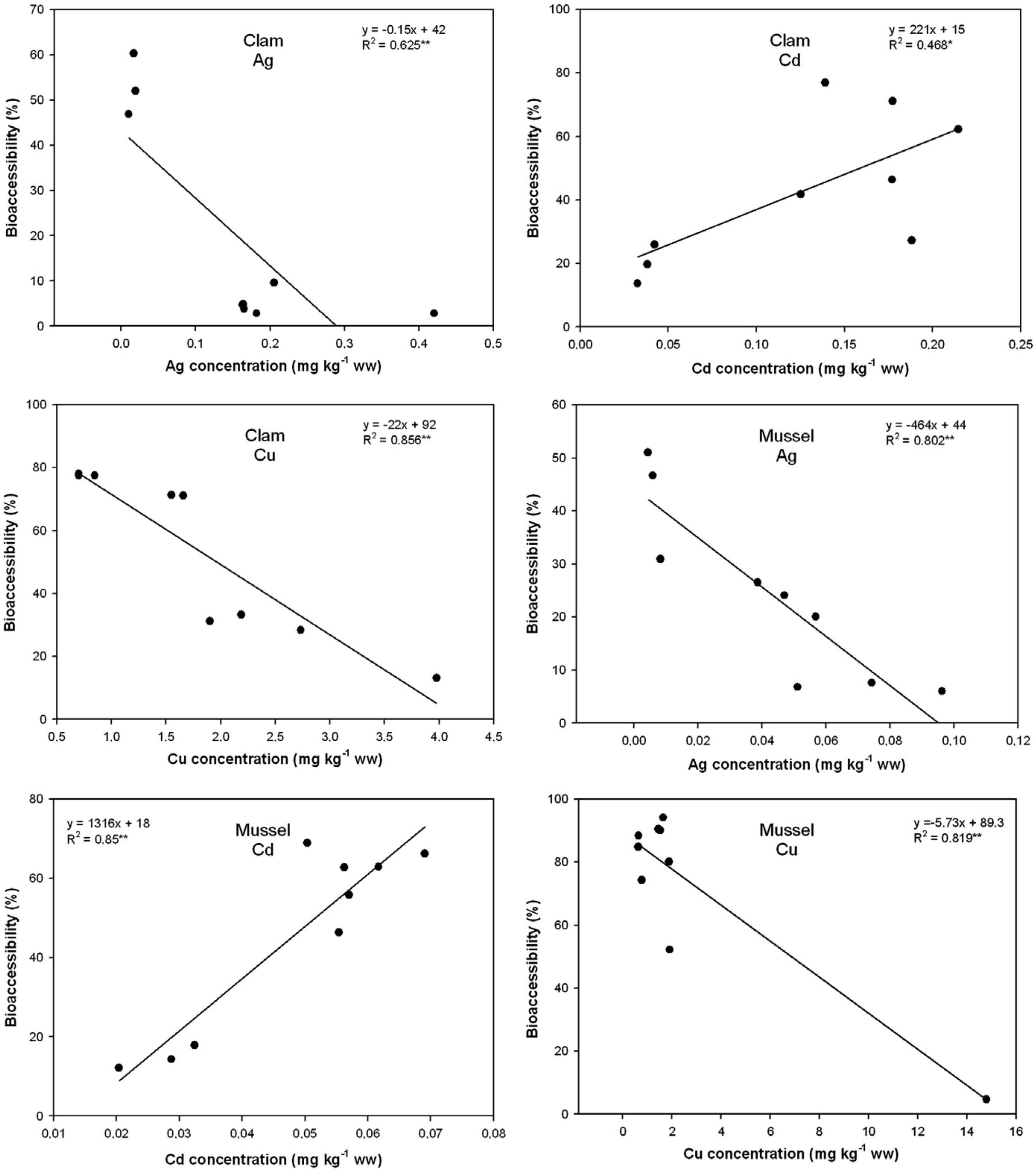

Fig. 3. Relationship between total metal concentrations and bioaccessibility in the digestive gland of clams and mussels. *Significant at the 95% level; **significant at the 99% level.

of silver, copper and lead was generally decreased by the addition of vinegar and conversely increased (3 cases) for cadmium. For zinc, only one significant difference (decrease) was shown (Table 5).

No consistent effect of lemon juice was observed in metal-rich oysters *C. gigas* from the Gironde estuary. For silver and lead, no significant differences were shown whereas bioaccessibility was significantly lowered for

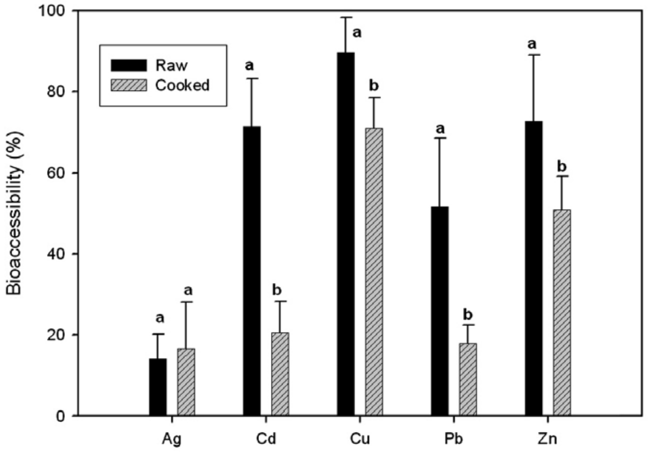

Fig. 4. Influence of cooking on metal bioaccessibility in *Buccinum undatum*. Bars with different letters indicate significant differences (at the 95% level) between bioaccessibilities (%).

copper (only for chewed oysters) and increased for cadmium (only for swallowed oysters) and zinc (Table 5).

4. Discussion

To safeguard public health, maximum acceptable concentrations of toxic contaminants have been established in various countries. Thus in the framework of the numerous legislative measures, many monitoring programmes have been carried out to investigate the health quality of marine species used as human seafood, including molluscs, in Asian and European regions (Franco et al., 2002; Marcotrigiano and Storelli, 2003; Liang et al., 2004; Yap et al., 2004; Leblanc et al., 2006; Marti-Cid et al., 2007).

Interspecies differences in metal concentrations are well-documented and in a number of cases, the biochemical bases responsible for these differences have been demonstrated. Oysters accumulate high concentrations of zinc in detoxified granules while mussels excrete much accumulated zinc in granules from the kidney. Thus oysters are strong accumulators of zinc whereas mussels are weak net accumulators or partial regulators of zinc (Rainbow, 1992 and literature cited therein). This explains why in our study the highest zinc concentrations were determined in oysters. Silver concentrations were also higher in the different species of oysters studied here compared to mussels or clams as reported by others (Cantillo, 1998; Franco et al., 2002). Similarly, oysters are strong accumulators of cadmium, again in agreement with the comparatively high levels of cadmium in oysters from the Gironde estuary and Hong Kong waters. Average tissue cadmium concentrations compiled by Egan et al. (2006) for the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) from European and Far Eastern regions were as follow: 1.38 mg kg^{-1} in oysters ($n = 4478$, SD = 1.24), 0.43 mg kg^{-1} ($n = 2239$, SD = 0.40) in mussels, 0.18 mg kg^{-1} in scallops ($n = 74$, SD = 0.31); 0.19 mg kg^{-1} in other bivalves ($n = 232$, SD = 0.20). Surprisingly, in the scallop *C. nobilis* examined in the present study, very high concentrations of cadmium were documented in the adductor muscle which is the commonly eaten part, particularly in products intended for freezing. The strong capacity of scallops to bioaccumulate numerous trace elements at high concentrations in their tissues has been related to their very efficient storage detoxification systems in the digestive gland and kidneys whereas the concentrations in muscles were not so high (Bustamante and Miramand, 2005 and literature cited therein). However, in scallops, the gut is tightly attached to the adductor muscle and many findings indicate that the scallop gut burden can reach cadmium concentrations up to 800 mg kg^{-1} wet weight (Kruzynski, 2004 and literature cited therein). Thus, it cannot be ruled out that the high cadmium levels determined in scallop adductor muscles from Hong Kong resulted from the presence of pieces of gut, not separated from the adductor muscle. Even in unpolluted waters, neogastropods such as *Nucella (Thais) lapillus*, *Murex brandaris* and *Buccinum undatum* accumulate cadmium at high concentrations (Bouquegneau and Martoja, 1982; Amiard-Triquet et al., 1983 and literature cited therein). *Rapana venosa*, a muricid neogastropod, showed higher cadmium concentrations than *Neverita didyma*, a naticid gastropod, the difference reaching more than one order of magnitude at two contaminated sites (Liang et al., 2004). The neogastropod *Thais clavigera* is able to accumulate Cd very efficiently from the food without any noxious effect (Cheung et al., 2006). Exposure to cadmium is responsible for metallothionein induction in both *N. lapillus* and *T. clavigera*, and metal-rich granules (MRG) have also been shown to be involved

Table 5
Influence of additives on metal bioaccessibility from oysters (mean expressed in %)

			Silver		Cadmium		Copper		Lead		Zinc	
			Chewed	Swallowed	Chewed	Swallowed	Chewed	Swallowed	Chewed	Swallowed	Chewed	Swallowed
C. gigas	Control	Vinegar	14 ↘ 5		62 ↗ 81				33 ↘ 19			
C. gigas	Gironde	Vinegar	8 ↘ 4				97 ↘ 79		34 ↘ 19		82 ↘ 71	
O. edulis	Control	Vinegar				42 ↘ 28	74 ↘ 49	79 ↘ 53		30 ↘ 15		
O. edulis	Restronguet	Vinegar	21 ↘ 11	22 ↘ 4	62 ↗ 84	57 ↗ 71		84 ↘ 65				
C. gigas	Gironde	Lemon				89 ↗ 95	97 ↘ 93				82 ↗ 95	74 ↗ 95

Values shown only when differences between treatments were significant (Mann-Whitney, $p < 0.05$; $n = 3$ for all samples except chewed *C. gigas* from the Gironde where $n = 6$).

in cadmium storage in detoxified form (Leung and Furness, 2001; Cheung et al., 2006).

For a long time, the distribution of metals among organs – or organotropism – in living organisms has been a topic of interest for metal ecophysiologists. Organotropism depends on the role of each organ (such as direct uptake from water in the gills, metabolism in the digestive gland), the physiological handling strategies specific for each element and the degree of exposure. Thus, it is difficult to establish a general pattern, relevant for most of species and metals (see for instance Bebianno et al., 1993; Duquesne and Coll, 1995; Mouneyrac et al., 1998; Geffard et al., 2004). For many marine food species, including oysters, mussels and clams, the soft tissues are typically eaten whole.

Our data show that the species which have metal concentrations higher than the food security limits are mainly oysters (all of the three studied species), *B. undatum* for cadmium and zinc, and scallops (adductor muscle) for cadmium. High levels of a toxic element pose a serious problem for seafood products which support important economic activities both at local (oyster farming) and international (shellfish export trade) levels (Kruzynski, 2004 for British Columbia; Boutier et al., 2000 for Marennes-Oléron, France). In certain countries, the problem has been taken into account in determining safety limits (Table 3). Thus it has been proposed to improve the risk assessment of eating metal-rich seafood by taking into account trophically bioavailable metal concentrations instead of total metal concentrations.

Bioaccessibility is highly variable depending on the metal studied. In the present study, the lowest bioaccessibility was observed for silver. In the stomach, where the low pH is due to the presence of hydrochloric acid, silver is precipitated as silver chloride. In addition, it has been shown in numerous species that silver is detoxified as silver sulphide which is a very stable complex, not easily released into solution in the conditions prevailing in the gut lumen (Berthet et al., 1992). In oysters, the fraction of silver stored in insoluble form, most probably as silver sulphide, increased with Ag contamination (Martoja et al., 1988; Berthet et al., 1992; Mouneyrac et al., 1998). The fact that silver bioaccessibility decreased in oysters, clams and mussels with increasing accumulated silver concentration (Figs. 2 and 3) is in agreement with these literature data. The low bioaccessibility of silver is consistent with its low bioavailability since oral administration of radiolabelled silver to mice, rats, monkeys and dogs has shown that 90% or more of oral doses were not absorbed (WHO, 1977).

The limited bioaccessibility of lead may also be related to its mode of storage. A review by Marigomez et al. (2002) indicates that lead is present in numerous cell types in molluscan tissues, being distributed both in lysosomes and cytosol. More precisely, in oysters *C. gigas*, lysosomes are the major intracellular structures responsible for lead storage in the gills, digestive tract and digestive gland. The abundance of lysosomes and their lead contents vary according to the total lead concentration in the soft tissues (Amiard et al., 1995). Considering all the categories of food used for human consumption, the rate of lead absorption after ingestion can range from 3% to 80%. It is strongly affected by food intake, much higher rates of absorption occurring after fasting than when lead is ingested with a meal. Absorption is also affected by age, typical absorption rates in adults and infants being 10% and 50%, respectively (WHO, 2000).

For cadmium, considering the edible tissues of all the different studied species, the median bioaccessibility corresponded to 54% of total concentrations, with clear interspecific differences. The cadmium-rich adductor muscle of scallops showed the lowest bioaccessibility, but, for other species, the higher the total cadmium concentration the higher its bioaccessibility. The same pattern was apparent when considering individual species (Fig. 3). In molluscs, including neogastropods and oysters studied here, metallothionein induction is important for cadmium detoxification (Amiard et al., 2006). This soluble protein is easily degraded in the digestive tract of a consumer, thus releasing cadmium initially bound in the seafood product. Insoluble ligands are also involved in cadmium storage in oysters (Boisson et al., 2003) and neogastropods (Cheung et al., 2006). Wallace and Luoma (2003), studying the trophic transfer of trace metals between bivalves and shrimps, identified a proposed Trophically Available Metal (TAM) fraction, which included not only trace metals bound to the soluble fraction but also metals bound to cell organelles. More recent studies, dealing again with invertebrates, have shown that different subcellular components of accumulated trace metals, including cellular debris and even metal-detoxification granules, appear to be trophically available to different degrees to different consumers (Rainbow et al., 2007). The absorption or bioavailability of cadmium from the gastrointestinal tract is generally considered to be slightly lower in experimental mammals than in humans. For the majority of species tested, the absorption of cadmium can range from 0.5% to 3.0% of the dose administered, while in humans a range of 3.0–8.0% can be found. The composition of the diet, including fibre, protein and carbohydrates can also affect the bioavailability of cadmium (WHO, 2004). These absorption rates are remarkably low compared to cadmium bioaccessibility in seafood products.

The patterns of storage are more or less similar for copper and zinc since metallothionein and insoluble ligands are concurrently involved in detoxified storage. However, bioaccessibility often decreased with increasing total concentration, suggesting an increasing predominance of storage in the form of less easily degraded complexes. For both copper and zinc, which are essential trace elements, bioaccessibility is clearly an overevaluation of trophic bioavailability. Turnlund (1989) have used stable isotope methodology to study copper absorption in human adults. In individuals fed a low copper diet (0.78 mg Cu day^{-1}), absorption was 55.6%, whereas it was only 36.3% for a recommended dietary allowance (1.68 mg Cu day^{-1}) and 12.4% for a high copper diet (7.53 mg Cu day^{-1}). Persons with adequate nutritional levels of zinc absorb approximately 20–30% of all ingested zinc, while greater proportions of dietary zinc are absorbed in zinc-deficient subjects if presented in bioaccessible form (quoted in WHO, 2001).

Using the total metal concentrations (Table 2) and the percentages of bioaccessibility estimated here (Table 4), bioaccessible concentrations were recalculated and compared to the most severe safety limits (Table 3). In most cases, bioaccessible concentrations remained higher than the safety limits except in the case of cadmium in scallop muscle and zinc in the whole soft tissues of *B. undatum*. To assess the risk due to the consumption of these seafood products, the number of individuals of each category of shellfish which has to be eaten to reach the maximum tolerable intake recommended by the World Health Organisation has been calculated (Table 6). Of course, these numbers are overestimates since other food sources contributing to metal intake have been ignored. The contribution of seafood to the diet is highly variable, but may be very high in certain groups. An inquiry into seafood consumption in France has been published recently (Leblanc et al., 2006). Even the highest consumers do not eat sufficient quantities of gastropods *B. undatum* (>94 g per week in 5% women living in four harbours) and oysters *C. gigas* (>144 g per week in 5% men from the same areas) to reach safety limits. In Taiwan, 9% of people consume more than 18.6 g oyster flesh per day, with extreme values (>139 g per day) in a subsistence fishermen group, thus leading to toxic metal overdoses (Han et al., 2000).

The influence of feeding habits (use of additives such as vinegar or lemon juice) on metal bioaccessibility in oysters is limited. Even when oysters were not masticated, the decreased contact between food and digestive juice had only a marginal effect. On the contrary, cooking the gastropods *B. undatum* (boiled in salted water) decreased the bioaccessibility of all the studied metals, except silver. In different organs of fish, Atta et al. (1997) had observed a consequent decrease of cadmium, copper, lead and zinc concentrations after steaming or baking. During thermal processing, the application of heat hastens protein degradation and loss of weight and water, and so chemical contaminants may also be affected by the heat applied (Lind et al., 1995; Burger et al., 2004; Cabañero et al., 2004). On the one hand, protein degradation can improve protein digestibility, facilitating metal release and therefore increasing bioaccessibility as shown for instance in the case of selenium in herring (Shen et al., 1997, quoted by Cabañero et al., 2004). On the other hand, the loss of water and weight may be accompanied by a loss of the more labile fractions, and metals remaining in the cooked tissues are then less accessible as shown in *B. undatum*.

The mitigation of seafood risk and elevation of consumer confidence are very important in the context of the development of international trade in seafood. The crucial role of environmental quality on seafood quality is well-recognized and filter-feeders are even used in “Mussel Watch” programmes as biomonitors of chemical contamination in coastal areas all over the world. This underlines the importance of traceability, associated with the monitoring of environmental quality in areas where high production of shellfish can take place. For instance, the decrease of Cd levels in oysters from the Gironde estuary (the most cadmium-polluted estuary in France) over recent decades has been well-documented in the framework of the French Mussel Watch (Claisse et al., 2006). The last published values were medians obtained from oysters analysed twice a year from 2000 to 2004. They still reached concentrations higher than 10 mg kg^{-1} dry weight, equivalent to 2 mg kg^{-1} wet weight. Samples collected in 2006 for the present study showed a supplementary decrease, the mean concentration being even lower than the safety limit of 1 mg kg^{-1} wet weight applicable in EU countries.

Table 6
Maximum consumption of seafood products originating from Chinese and Western Europe regions, and provisional tolerable weekly intake (PTWI) or provisional maximum tolerable daily intake (PMTDI) as assessed by the World Health Organisation

	PMTDI mg kg^{-1} bw	PTWI mg kg^{-1} bw	PTWI per individual[e]	Food product	Maximum consumption kg per week	Maximum number of shellfish[f]
Cadmium		0.007[a]	0.49	Gastropod *B. undatum*	0.41	41
Copper	0.05–0.5[b]		24.5–245	Oyster *C. gigas* Gironde	0.44–4.4	73–730
				Oyster *O. edulis* Restronguet	0.39–3.9	65–650
				Oyster *S. cucullata*	0.28–2.8	47–470
Lead		0.025[c]	1.75	Oyster *O. edulis* Restronguet	0.88	147
Zinc	0.3–1[d]		147–490	Oyster *C. gigas* Gironde	0.82–2.73	137–455
				Oyster *O. edulis* Restronguet	0.50–1.66	83–277
				Oyster *S. cucullata*	0.29–0.98	48–163

[a] WHO (2005).

[b] WHO (1982a).

[c] WHO (1999).

[d] WHO (1982b).

[e] Standard weight of a man of 70 kg.

[f] Calculated considering a standard weight of 10 g wet weight of soft tissues for *B. undatum*, 6 g for oysters.

Depuration is a strategic approach to improve the food quality of shellfish. Elimination kinetics has given rise to a number of studies dealing with metals or radioactive tracers in filter-feeders. Generally, elimination curves are biphasic with the first phase corresponding to the elimination of the digestive tract content. This practice may be important to improve the sanitary quality, for instance when the gut content is more contaminated than the flesh as evoked for scallops (Kruzynski, 2004). Similarly, McKay and Halliwell (1994) have shown that the reduction of sediment content in mussels, cockles, limpets and whelks reduced concomitantly their radionuclide content, a fact which must be considered for dose assessment. The second phase of kinetic curves corresponds to the elimination of truly absorbed metals and its length varies for different metals and oyster species (Han et al., 1993; Lim et al., 1998; Geffard et al., 2002a). When the half-life is only a few days or a few weeks, depuration represents an opportunity to lower metal concentrations below recommended safety limits.

In conclusion, this study confirms that depending on the metal, the use of global concentrations or bioaccessible concentrations can lead to great differences in the quality assessment of seafood. In the case of essential metals (copper, zinc), bioaccessibility was generally high and then, the bias of using total concentrations to decide of food security is not important. On the other hand for elements which are considered among the most toxic such as silver, lead and cadmium, bioaccessible concentrations are generally consistently lower than total concentrations. In this case, it may be relevant to take into account the former to carry out a more accurate assessment of seafood quality, meeting the needs of both human health security and the economic interests of shellfish farmers. Models have been tested to estimate the probability for the exposure to exceed a fixed safe level for seafood consumers (Tressou et al., 2004). Such models could be improved by taking bioaccessibility into account. However, for certain species accumulating metals to a very high degree, neither total concentrations nor bioaccessible concentrations will meet security thresholds. The way of cooking or preparing seafood also influences metal bioaccessibility. Thus, as is too rarely done (Muñoz et al., 2005), it is recommended that bioaccessibility is considered when estimating the dietary intake of metals by human consumers.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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