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Subcellular and body distributions of 17 trace elements in the variegated scallop *Chlamys varia* from the French coast of the Bay of Biscay

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Abstract

Seventeen elements were analysed in the organs and tissues of the variegated scallop *Chlamys varia*, from the Atlantic coast of France. Concentration levels were determined in scallops of different sizes sampled in contaminated (La Rochelle Bay) and clean (Ré Island) sites. Greater concentrations of Ag, Al, Ce, Cr, La, Mo, Nd, Ti, and V were found in the digestive gland while As, Cd, Co, Cu, Mn, Ni, Pb, and Zn were the highest in the kidneys. In the digestive gland, most of the metals were found in the insoluble fraction while As, Co, Cd, Mo, Ni, and V appeared to be mostly bound to soluble compounds. Among tissues, the adductor muscle always displayed the lowest trace element concentrations. According to size, Ag and Cd showed significantly higher concentrations in larger individuals, while Co and Zn were higher in the smallest ones. According to the sampling area, most of the metals, Ag, Al, Ce, Co, Cu, La, Mn, Nd, Pb, and Zn, showed significantly higher concentrations in La Rochelle Bay compared to the Ré Island, reflecting differing inputs from industrial, domestic and harbour activities. However, Cr, Mo, Ni, Ti, and V concentrations did not display significant differences between sites and As and Cd were significantly higher at the Ré Island. This study highlighted the ability of the variegated scallop *C. varia* to concentrate numerous trace elements to high levels, even those reported as poorly bioavailable for marine biota, such as rare earth elements.

Keywords: Heavy metals; Trace elements; Rare earth elements; Bioaccumulation; Subcellular distribution

1. Introduction

Among filter feeders, scallops have been reported to be useful biomonitors to survey marine pollution. Indeed, numerous studies have demonstrated their ability to concentrate trace elements, even in areas far from anthropogenic sources such as the Antarctic Ocean (e.g., Mauri et al., 1990; Berkman and Nigro, 1992; Viarengo et al., 1993), with seasonal variations in the concentrations at various stages of their lives (Bryan, 1973). However, the distribution of trace

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elements between body tissues has been rarely considered. Furthermore, earlier studies of elemental analysis in scallops have focused only on a few heavy metals. Among them, Cd has received particular attention since it was first detected by a spectrographic method in *Pecten maximus* by Fox and Ramage (1931). The strong capacity of scallops to bioaccumulate numerous trace elements at high concentrations in their tissues is related to their very efficient detoxification systems involving lysosomal compartmentalisation, coprecipitation on phosphate granules and binding to soluble proteins such as metallothioneins (George et al., 1980; Ballan-Dufrançais et al., 1985; Stone et al., 1986; Fowler and Gould, 1988; Mauri et al., 1990; Viarengo et al., 1993). These detoxification processes seem to be particularly efficient in the digestive gland and the kidneys of Pectinidae. Therefore, such tissues have been proposed as valuable monitors of the availability of stable trace metals in the sea (Bryan, 1973).

The aim of this study was to provide information on the concentrations, and tissue and subcellular distributions of 17 trace elements in the tissues and organs of the variegated scallop *Chlamys varia* from the Bay of Biscay. *C. varia* is a common seashell on the rocky shore of the French Atlantic coasts, although rare in the English Channel. It is encountered in important shoals in Brittany and in Charente-Maritime where this species is targeted by a commercial fishery as well as for leisure activities (Letaconnoux and Audouin, 1956; Quéro and Vayne, 1998). Comparisons have been made between scallops from an area subjected to anthropogenic inputs (industrial, domestic, and harbour), i.e., La Rochelle Bay, and from a clean site close to a commercially exploited scallop bed, i.e., Ré Island. At each site, elemental concentrations, distribution among tissues and organs, and size-related variations in concentrations were examined. Moreover, the partitioning of trace elements between soluble and insoluble subcellular fractions of the digestive gland was investigated as this organ has been reported to concentrate numerous trace elements to high levels of in Pectinidae.

2. Material and methods

2.1. Sampling and sample preparation

For this study, 130 specimens were collected by hand in La Rochelle Bay ($n=90$) and in Saint-

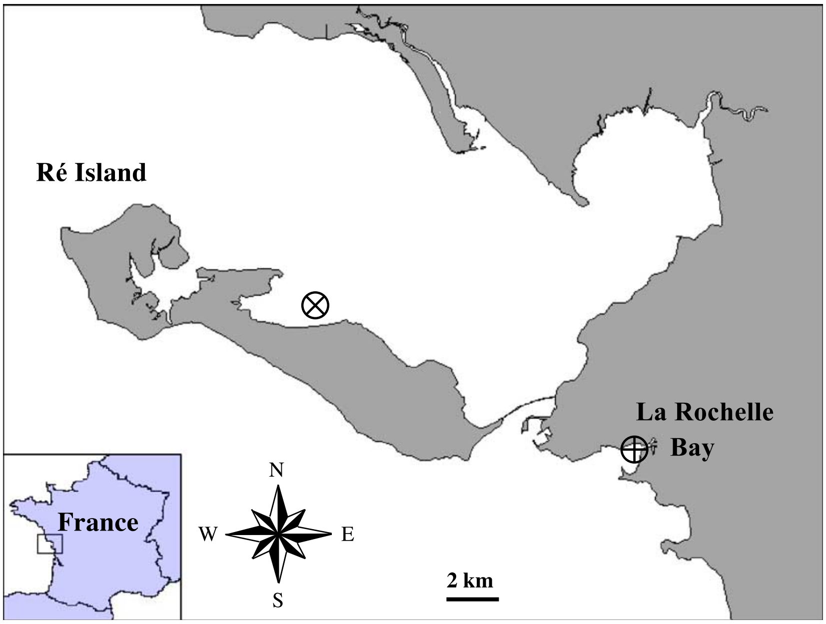

Fig. 1. Sampling location sites for the variegated scallops *C. varia*. Saint Martin en Ré (⊗), La Rochelle Bay (⊕).

Martin en Ré ($n=40$) in Ré Island in February 1996 (Fig. 1). Scallops were pooled into size classes of 10 individuals according to the height of the shell and were depurated for 48 h in clean seawater to eliminate faecal and pseudofaecal material. The size of scallops from Ré Island ranges from 32±1 mm (three pools of 10 individuals) to 44±2 mm (10 pooled individuals), and those from La Rochelle Bay from 33±1 to 45±2 mm (nine pools of 10 individuals). The low number of large scallops in Ré Island was due to the fishing pressure in this area where the biggest individuals (>40 mm) are targeted for harvesting, while any kind of shellfishing is forbidden in the La Rochelle Bay sampling site because of possible bacterial contamination.

For each scallop, adductor muscle, gonad, gills, kidneys, and digestive gland were separated from the rest of the soft parts, and pooled for each defined size class. The remaining tissues including the mantle, the foot, the intestine and the heart, were also analysed in order to calculate the whole trace element content of the soft parts.

2.2. Subcellular fractionation

To investigate the partitioning of trace elements between soluble and insoluble subcellular fractions of the digestive gland, 40 scallops (size=45±2 mm) were collected in winter 1996 in La Rochelle Bay and were depurated as described above.

Four pools of 10 individuals were made with the digestive glands removed from the soft parts of the 40 scallops. They were subsequently homogenised with a mortar and pestle on ice with 10 vol. of 0.02 M Tris–HCl buffer 0.25 M sucrose with 1 mM phenylmethylsulfonylfluoride (PMSF, as protease inhibitor) and 5 mM dithiothreitol (DTT, as reducing agent), at pH 8.6. The homogenates were centrifuged at $100\,000\times g$ for 1 h at 4 °C in a Beckman LE-70 ultracentrifuge. Particle-free supernatants (cytosols) were separated from the pellet. Aliquots of the homogenates, cytosols, and pellets were analysed for trace elements.

2.3. Trace element analyses

Tissue samples were dried for several days at 80 °C to constant weight. Whenever it was possible, two aliquots of approx. 300 mg of each homogenised dry sample were digested with 4 ml of 65% ultrapure HNO_3 and 0.3 ml of ultrapure 70% $HClO_4$ at 80 °C until the solution was clear. Then acids were evaporated and residues were dissolved in 0.3 N ultrapure nitric acid.

Cadmium, copper, and zinc were determined by atomic absorption spectrophotometry. Other elements (Ag, Al, As, Ce, Co, Cr, La, Mn, Mo, Nd, Ni, Pb, Ti, and V) were analysed by ICP-MS. Appliances used for trace element determination were a Varian spectrophotometer Vectra 250 Plus with deuterium background correction and a Varian ICP-MS Ultra Mass 700.

Reference tissues, dogfish liver DOLT-2 (NRCC), dogfish muscle DORM-2 (NRCC), lobster hepatopancreas TORT-2 (NRCC), and Bush Branches and Leaves (GBW 07602) were treated and analysed in the same way as the samples. The results for standard reference materials displayed recoveries of the elements ranging from 86% to 112%. The detection limits were (μg g^{-1} dry wt) 0.002 (Ag), 0.005 (Al), 0.15 (As), 0.005 (Cd), 0.017 (Ce), 0.005 (Co), 0.005 (Cr), 0.027 (Cu), 0.017 (La), 0.017 (Mn), 0.017 (Mo), 0.017 (Nd), 0.002 (Pb), 0.002 (Ni), 0.033 (Ti), 0.005 (V) and 0.15 (Zn).

2.4. Data analyses

Trace element concentrations in tissues are given on a dry weight basis (μg g^{-1} dry wt) while the distribution percentages were calculated based on wet weight. Comparison of metal concentrations between sites and between sizes was tested by the non-parametric Kruskall–Wallis test using MINITAB 13.1 Software. Changes in trace element distribution among scallop tissues were tested for significance by the G procedure for multiple comparisons (Zar, 1996). The significance level for statistical analyses was always set at $\alpha=0.05$.

3. Results

3.1. Elemental concentrations and tissular distribution

Trace element concentrations in the tissues and organs of variegated scallops *C. varia* from the French coast of the Bay of Biscay are reported in Table 1. The digestive gland contained the highest

Table 1
Trace element concentrations (μg g^{-1} dry wt) in the tissues and organs of the variegated scallop *Chlamys varia* from the coast of Charente-Maritime, Bay of Biscay, France

Metal	Sampling area	*n*	Size (mm)	Digestive gland	Kidney	Gills	Gonads	Muscle	Whole soft parts
Ag	La Rochelle	3	33±1	12.1±2.9	5.63±0.58	0.44±0.24	6.48±0.99	0.57±0.26	3.08±0.38
	La Rochelle	3	39±1	27.8±10.2	8.30±2.43	0.44±0.11	8.66±2.82	0.44±0.11	6.47±1.39
	La Rochelle	3	45±2	61.2±29.1	5.01±3.77	0.36±0.10	11.62±2.23	0.45±0.14	11.02±4.09
	Ré Island	3	32±1	3.23±0.97	0.94±0.44	0.28±0.19	1.99±0.48	0.02±0.01	1.05±0.15
	Ré Island	1	44±2	5.36	0.86	0.28	5.50	0.03	1.85
Al	La Rochelle	3	33±1	2511±690	222±112	1353±580	1072±370	71±24	901±299
	La Rochelle	3	39±1	1763±54	157±73	407±175	981±231	42±7	727±97
	La Rochelle	3	45±2	1951±239	199±113	487±145	1084±114	38±15	920±190
	Ré Island	3	32±1	1418±360	169±78	467±60	161±40	18±4	493±51
	Ré Island	1	44±2	478	88	264	516	19	195
As	La Rochelle	3	33±1	23.6±6.4	36.0±15.0	23.1±6.3	26.5±19.4	14.3±4.7	18.3±2.0
	La Rochelle	3	39±1	17.9±4.9	41.2±25.7	15.1±2.5	18.9±4.9	8.6±0.9	14.4±2.1
	La Rochelle	3	45±2	12.5±7.5	19.2±8.6	22.2±9.1	20.4±4.5	10.5±3.3	14.9±2.6
	Ré Island	3	32±1	21.5±2.0	43.5±12.0	39.6±5.6	30.5±6.1	15.8±3.8	25.0±4.8
	Ré Island	1	44±2	10.8	25.4	29.2	25.0	12.7	16.0
Cd	La Rochelle	3	33±1	22.3±0.6	46.1±1.7	2.91±0.06	1.89±0.08	0.55±0.09	4.9±0.2
	La Rochelle	3	39±1	32.7±5.0	47.0±14.2	3.65±1.37	2.29±0.22	0.59±0.10	7.1±1.4
	La Rochelle	3	45±2	39.6±2.5	52.4±10.0	4.43±0.84	2.38±0.20	0.59±0.13	8.0±1.3
	Ré Island	3	32±1	24.2±1.2	39.6±0.8	2.57±0.33	2.80±0.13	0.36±0.06	5.6±0.3
	Ré Island	1	44±2	35.3	42.5	3.18	1.97	0.71	8.5
Ce	La Rochelle	3	33±1	10.47±3.88	2.45±2.16	6.02±3.93	6.38±2.72	0.25±0.05	3.98±0.49
	La Rochelle	3	39±1	10.61±1.09	2.03±2.27	4.01±1.90	5.06±0.73	0.18±0.04	3.59±0.11
	La Rochelle	3	45±2	10.85±1.73	1.11±0.44	6.02±3.93	5.80±0.86	0.32±0.22	4.50±0.49
	Ré Island	3	32±1	3.17±0.82	0.30±0.21	0.07±0.01	1.00±0.31	0.04±0.02	0.92±0.13
	Ré Island	1	44±2	1.17	0.15	0.05	1.97	0.03	0.36
Co	La Rochelle	3	33±1	2.60±0.31	27.50±4.06	1.40±0.38	1.55±0.82	0.24±0.08	1.52±0.26
	La Rochelle	3	39±1	2.13±0.29	24.29±6.32	0.59±0.48	1.22±0.23	0.13±0.01	1.33±0.11
	La Rochelle	3	45±2	1.79±0.64	9.71±2.14	1.17±0.41	1.25±0.20	0.15±0.09	1.04±0.19
	Ré Island	3	32±1	2.28±0.68	16.48±5.20	1.14±1.11	1.55±0.23	0.14±0.03	1.19±0.16
	Ré Island	1	44±2	0.84	5.75	0.91	0.92	0.11	0.52
Cr	La Rochelle	3	33±1	7.82±1.71	7.58±2.51	4.80±1.88	2.49±1.26	1.08±0.50	3.69±0.80
	La Rochelle	3	39±1	8.13±1.05	4.34±1.60	2.79±1.50	2.61±3.75	0.84±0.18	3.18±0.04
	La Rochelle	3	45±2	5.98±2.53	4.37±0.95	5.61±3.17	6.20±1.50	0.73±0.21	3.31±0.60
	Ré Island	3	32±1	8.14±3.05	7.77±3.24	4.75±2.98	4.86±3.02	0.93±0.19	3.55±0.96
	Ré Island	1	44±2	3.07	8.77	6.88	5.66	1.17	2.59
Cu	La Rochelle	3	33±1	68±4	288±13	14±2	33±4	4±0	30±3
	La Rochelle	3	39±1	77±10	246±39	10±1	33±7	4±1	23±3
	La Rochelle	3	45±2	135±9	271±14	12±2	40±4	4±1	21±1
	Ré Island	3	32±1	24±3	73±34	9±2	17±2	2±1	9±1
	Ré Island	1	44±2	22	33	14	19	2	10
La	La Rochelle	3	33±1	7.90±3.15	2.11±1.49	5.06±3.24	5.73±3.31	0.22±0.03	2.96±0.58
	La Rochelle	3	39±1	8.12±0.84	1.96±0.79	2.79±1.73	4.35±0.94	0.21±0.03	2.79±0.14
	La Rochelle	3	45±2	7.53±2.52	1.28±0.23	4.26±2.15	5.09±0.68	0.32±0.32	2.74±0.44
	Ré Island	3	32±1	0.28±0.16	0.22±0.10	0.46±0.03	0.53±0.12	0.03±0.03	0.32±0.05
	Ré Island	1	44±2	0.08	0.11	0.23	0.79	0.02	0.12
Mn	La Rochelle	3	33±1	174±75	10282±3163	155±34	169±80	30±17	265±36
	La Rochelle	3	39±1	132±32	11446±5144	117±11	151±47	36±10	339±143
	La Rochelle	3	45±2	113±47	11855±4517	150±53	134±65	42±13	276±93
	Ré Island	3	32±1	58±19	1286±173	93±43	68±23	8±5	64±5
	Ré Island	1	44±2	49	3594	49	66	7	90

Table 1 (*continued*)

Metal	Sampling area	*n*	Size (mm)	Digestive gland	Kidney	Gills	Gonads	Muscle	Whole soft parts
Mo	La Rochelle	3	33±1	7.40±1.31	4.10±1.72	2.97±1.08	4.31±1.38	0.72±0.33	2.34±0.56
	La Rochelle	3	39±1	6.37±1.53	2.74±1.06	1.74±0.40	3.54±0.76	0.39±0.07	1.94±0.29
	La Rochelle	3	45±2	4.26±2.75	2.65±1.47	2.29±0.66	4.10±0.54	0.47±0.13	1.68±0.29
	Ré Island	3	32±1	7.62±0.79	4.40±1.62	3.29±0.72	4.08±2.61	0.40±0.08	2.45±0.44
	Ré Island	1	44±2	4.25	4.23	2.96	9.87	0.54	2.01
Nd	La Rochelle	3	33±1	5.22±2.06	0.81±0.18	2.23±0.84	4.31±1.35	0.13±0.03	1.65±0.25
	La Rochelle	3	39±1	5.21±0.46	0.47±0.06	1.57±1.11	2.78±0.83	0.08±0.02	1.64±0.07
	La Rochelle	3	45±2	5.66±0.83	0.58±0.21	2.05±1.55	3.44±0.86	0.14±0.11	1.97±0.58
	Ré Island	3	32±1	1.23±0.49	0.12±0.07	0.34±0.04	0.91±0.45	0.02±0.01	0.39±0.09
	Ré Island	1	44±2	0.52	0.05	0.18	1.32	0.01	0.18
Ni	La Rochelle	3	33±1	6.74±1.21	20.59±5.40	4.23±1.68	3.19±0.60	1.60±0.42	2.67±0.37
	La Rochelle	3	39±1	6.08±0.75	14.51±0.07	2.24±0.78	3.38±0.45	0.58±0.35	2.72±0.18
	La Rochelle	3	45±2	5.73±1.06	10.79±0.66	3.10±1.05	3.83±1.50	0.67±0.22	3.73±1.09
	Ré Island	3	32±1	6.94±2.23	20.94±5.28	5.04±2.74	8.40±4.26	0.78±0.21	3.60±0.67
	Ré Island	1	44±2	3.30	10.66	6.20	5.26	0.91	2.44
Pb	La Rochelle	3	33±1	3.43±0.87	58.80±3.29	3.47±0.51	8.71±3.13	0.89±0.05	3.14±0.40
	La Rochelle	3	39±1	3.81±0.81	45.38±7.88	2.03±0.70	6.54±2.05	0.78±0.22	2.89±0.27
	La Rochelle	3	45±2	4.17±0.56	41.57±3.74	2.31±0.38	5.65±0.80	1.01±0.52	2.66±0.21
	Ré Island	3	32±1	2.63±0.45	28.44±6.97	2.10±0.56	2.33±0.75	1.67±0.17	1.91±0.07
	Ré Island	1	44±2	1.05	20.28	1.94	3.16	0.85	1.29
Ti	La Rochelle	3	33±1	38.2±13.2	12.1±5.4	25.8±8.8	46.7±22.4	4.84±2.28	25.8±9.1
	La Rochelle	3	39±1	54.5±25.2	2.8±1.5	22.3±12.5	31.7±8.0	2.04±0.27	24.2±4.1
	La Rochelle	3	45±2	64.5±27.7	10.6±6.1	21.4±11.9	37.2±7.9	3.25±2.35	32.2±12.3
	Ré Island	3	32±1	42.6±30.3	7.2±3.6	18.6±2.4	10.8±2.7	1.73±0.67	17.8±6.4
	Ré Island	1	44±2	12.8	3.6	9.1	19.7	1.70	7.5
V	La Rochelle	3	33±1	11.36±2.16	3.45±1.47	4.31±2.07	3.59±0.56	0.22±0.08	3.22±0.77
	La Rochelle	3	39±1	8.35±1.17	2.32±0.82	3.09±1.13	4.30±0.66	0.21±0.03	2.97±0.32
	La Rochelle	3	45±2	8.22±1.62	3.31±0.43	4.87±1.16	4.55±0.14	0.29±0.18	2.87±0.42
	Ré Island	3	32±1	8.10±1.57	2.00±0.67	2.60±0.23	1.69±0.02	0.08±0.06	2.53±0.27
	Ré Island	1	44±2	3.35	1.30	1.42	3.03	0.02	1.15
Zn	La Rochelle	3	33±1	518±68	38168±4603	569±105	885±171	175±25	1062±132
	La Rochelle	3	39±1	346±24	34831±2451	313±20	542±62	175±73	1003±97
	La Rochelle	3	45±2	344±44	34212±1885	383±27	477±103	174±24	799±48
	Ré Island	3	32±1	204±30	14868±5742	312±134	672±153	83±14	420±39
	Ré Island	1	44±2	110	12566	109	242	70	313

n: number of pool of 10 individuals.

concentrations of Ag, Al, Ce, Cr, La, Mo, Nd, Ti, and V. Second, kidneys exhibited the highest concentrations of As, Cd, Co, Cu, Mn, Ni, Pb, and Zn. The adductor muscle of *C. varia* always displayed the lowest trace element concentrations. In this tissue, concentrations were remarkably low for nonessential elements such as Ag, Cd, Pb, or rare earth elements and also for most of the essential ones, especially Co, Cu, and Mo (Table 1). Compared to these low muscle concentrations, the gills and gonads of *C. varia* displayed generally relatively high concentrations of trace elements.

The distribution and relative body burdens of elements in *C. varia* have been calculated on the basis of the fresh tissue weights (Table 2). Among tissues, the digestive gland of *C. varia* accounted for only about 12% of the soft part weight but the percentage body burdens of Ag, Al, Cd, Ce, Co, Cr, Cu, Mo, Nd, Ni, and V were high in this tissue (Table 2). It is also remarkable that despite their very low percentage of the soft part weight (less than 2%), kidneys displayed the highest proportions of Mn and Zn (up to 80%) and also high burdens of Pb (up to 40%). The proportions of trace elements accumulated in these organs clearly indicate their detoxification functions. Body burdens of all elements in gills and gonads were generally low (i.e., <10%), and these organs represented a small fraction of the soft part weight.

Table 2
Distribution (% of fresh weight) of body burdens of trace elements in the tissues and organs of the variegated scallop *Chlamys varia* from the coast of Charente-Maritime, Bay of Biscay, France

Metal	Sampling area	*n*	Size (mm)	Digestive gland	Kidney	Gonads	Gills	Muscle	Remaining tissues
Ag	La Rochelle	30	33±1	54±5	4±2	4±1	1±1	10±5	26±4
	La Rochelle	30	39±1	69±14	3±1	2±2	1±0	4±0	21±11
	La Rochelle	30	45±2	85±6	1±0	1±1	0±0	3±1	10±4
	Ré Island	30	32±1	53±10	1±0	3±1	1±1	1±0	41±10
	Ré Island	8	44±2	64	1	3	0	1	31
Al	La Rochelle	30	33±1	44±6	1±0	2±1	10±3	5±2	38±6
	La Rochelle	30	39±1	45±2	1±0	3±1	3±2	3±1	45±4
	La Rochelle	30	45±2	42±8	0±0	2±0	2±0	3±1	50±10
	Ré Island	30	32±1	50±4	0±0	0±0	5±2	2±0	42±2
	Ré Island	8	44±2	58	1	2	5	5	29
As	La Rochelle	30	33±1	18±2	5±4	2±1	7±2	43±11	25±5
	La Rochelle	30	39±1	21±5	7±5	2±1	6±1	36±10	27±9
	La Rochelle	30	45±2	15±9	2±1	2±1	7±3	44±13	30±14
	Ré Island	30	32±1	17±3	2±1	2±0	8±1	33±2	38±2
	Ré Island	8	44±2	18	2	2	7	45	27
Cd	La Rochelle	30	33±1	62±2	19±6	1±0	3±1	6±1	9±3
	La Rochelle	30	39±1	72±2	15±3	1±0	2±0	4±1	6±1
	La Rochelle	30	45±2	79±3	10±2	0±0	2±1	4±1	5±1
	Ré Island	30	32±1	74±5	9±4	1±0	2±1	3±1	11±1
	Ré Island	8	44±2	84	6	0	1	4	6
Ce	La Rochelle	30	33±1	42±16	1±1	3±1	10 ±7	4±1	40±8
	La Rochelle	30	39±1	54±5	1±2	3±1	6±3	3±1	33±5
	La Rochelle	30	45±2	47±8	0±0	2±0	5±3	4±1	42±9
	Ré Island	30	32±1	58±2	0±0	1±0	0±0	2±1	38±0
	Ré Island	8	44±2	70	1	5	0	4	21
Co	La Rochelle	30	33±1	24±3	38±11	2±0	6±2	9±4	21±7
	La Rochelle	30	39±1	27±3	44±9	1±0	2±2	5±1	20±8
	La Rochelle	30	45±2	33±12	17±5	2±1	5±2	10±5	33±12
	Ré Island	30	32±1	38±5	19±3	2±0	5±4	7±1	30±2
	Ré Island	8	44±2	41	16	2	7	12	23
Cr	La Rochelle	30	33±1	32±2	4±1	2±1	8±3	18 ±9	36±10
	La Rochelle	30	39±1	45±4	4±2	1±2	5±3	15±4	30±6
	La Rochelle	30	45±2	35±12	3±0	3±1	8±5	15±6	36±14
	Ré Island	30	32±1	41±9	3±1	2±0	6±1	14±3	34±6
	Ré Island	8	44±2	32	5	2	11	26	24
Cu	La Rochelle	30	33±1	44±2	28±7	3±1	4±1	10±1	13±6
	La Rochelle	30	39±1	55±1	25±3	2±1	2±1	8±1	8±1
	La Rochelle	30	45±2	71±3	14±3	2±0	1±0	7±1	5±0
	Ré Island	30	32±1	48±7	10±4	3±1	5±0	15±4	19±8
	Ré Island	8	44±2	56	5	1	6	11	21
La	La Rochelle	30	33±1	43±18	2±1	4±1	12±8	5±1	36±9
	La Rochelle	30	39±1	53±4	2±1	3±1	6±4	4±1	33±4
	La Rochelle	30	45±2	52±15	1±0	3±1	7±3	7±3	30±12
	Ré Island	30	32±1	16±7	1±0	2±0	7±1	5±4	68±11
	Ré Island	8	44±2	19	2	7	8	10	54
Mn	La Rochelle	30	33±1	9±4	74±10	1±0	3±1	6±3	7±5
	La Rochelle	30	39±1	7±1	78±7	1±0	2±1	6±2	7±4
	La Rochelle	30	45±2	8±4	71±10	1±1	3±2	9±1	9±5
	Ré Island	30	32±1	19±2	32±10	2±1	9±4	8±4	30±10
	Ré Island	8	44±2	16	65	1	2	5	12

Table 2 (*continued*)

Metal	Sampling area	*n*	Size (mm)	Digestive gland	Kidney	Gonads	Gills	Muscle	Remaining tissues
Mo	La Rochelle	30	33±1	47±6	4±1	3±1	8±2	17±5	21±3
	La Rochelle	30	39±1	56±8	3±1	3±1	5±2	12±5	21±7
	La Rochelle	30	45±2	42±17	3±2	4±2	7±4	18±7	26±12
	Ré Island	30	32±1	56±2	2±1	2±1	7±2	8±1	25±2
	Ré Island	8	44±2	51	3	5	5	14	22
Nd	La Rochelle	30	33±1	48±14	1±0	5±1	9±5	5±1	32±9
	La Rochelle	30	39±1	57±4	1±0	3±0	6±4	3±1	31±6
	La Rochelle	30	45±2	56±18	1±0	3±1	4±2	4±2	32 ±19
	Ré Island	30	32±1	53±5	0±0	3±0	5±2	2±2	37±1
	Ré Island	8	44±2	66	0	7	3	3	20
Ni	La Rochelle	30	33±1	26±4	12±6	2±1	7±2	25±7	27±9
	La Rochelle	30	39±1	39±7	14±3	2±1	5±2	12±7	28±6
	La Rochelle	30	45±2	40±9	7±1	2±0	5±2	16±7	30±14
	Ré Island	30	32±1	36±9	8±2	3±1	7±1	12±4	34±6
	Ré Island	8	44±2	36	7	2	10	21	24
Pb	La Rochelle	30	33±1	16±3	39±9	5±1	7±1	16±2	17 ±5
	La Rochelle	30	39±1	22±3	38±7	4±1	4±2	15±7	17±4
	La Rochelle	30	45±2	28±3	26±4	3±1	4±1	22±11	17±4
	Ré Island	30	32±1	28±2	21±5	2±1	6±2	18±7	26±6
	Ré Island	8	44±2	21	23	3	6	33	14
Ti	La Rochelle	30	33±1	24±10	1±0	4±0	7±4	13±9	51±11
	La Rochelle	30	39±1	41±14	0±0	3±1	6±3	5±2	45±11
	La Rochelle	30	45±2	41±21	1±0	2±1	3±2	7±5	47±20
	Ré Island	30	32±1	38±14	1±0	1±1	5±1	5±3	50±11
	Ré Island	8	44±2	43	1	3	4	14	35
V	La Rochelle	30	33±1	54±5	2±1	2±1	8±3	4±2	29±4
	La Rochelle	30	39±1	51±5	2±1	3±1	6±3	4±1	34±5
	La Rochelle	30	45±2	54±9	2±0	2±0	8±2	6±3	27±9
	Ré Island	30	32±1	56±4	1±0	1±0	5±1	2±1	35±3
	Ré Island	8	44±2	67	1	2	4	1	24
Zn	La Rochelle	30	33±1	7±2	70±10	1±0	3±1	9±1	10±6
	La Rochelle	30	39±1	6±0	79±0	1±0	2±1	9±1	5±0
	La Rochelle	30	45±2	7±2	70±6	1±0	2±1	13±1	7±2
	Ré Island	30	32±1	12±3	57±10	3±1	5±2	13±3	11±3
	Ré Island	8	44±2	10	64	1	1	14	10

The remaining tissues (mantle, foot, intestine and heart) contained large amounts of Ag, Al, As, Ce, Co, Cr, La, Mo, Nd, Ni, Ti, and V since this tissue accounted for around 31% of the soft part weight. Although adductor muscle represented about 34% of the soft part weight, As was the only element contained in large proportion in this tissue (up to 45%). This difference is attributable to the very low concentrations found for the other trace elements in the adductor muscle of *C. varia* (Table 1).

3.2. Size trends in element concentrations

Table 1 presents the elemental concentrations in the tissues of *C. varia* of different sizes (from 32±1 to 45±2 mm) and possible size effects were investigated on the scallops from La Rochelle Bay representing three sizes from three pools of 10 individuals. Generally, few trace element concentrations (Ag, Cd, Co, Cu, Ni, and Zn) varied significantly with size of whole soft parts or of the tissue under consideration (Fig. 2).

Considering the whole soft parts, concentrations of Ag and Cd were higher in large scallops ($H=7.20$, $p=0.027$ and $H=6.01$, $p=0.050$, respectively), while Co and Zn exhibited significantly higher concentrations in small individuals ($H=6.01$, $p=0.050$ for both metals). For Cd and Ag, these variations are clearly due to the bioaccumulation of Ag and Cd in the digestive gland where concentrations of both metals

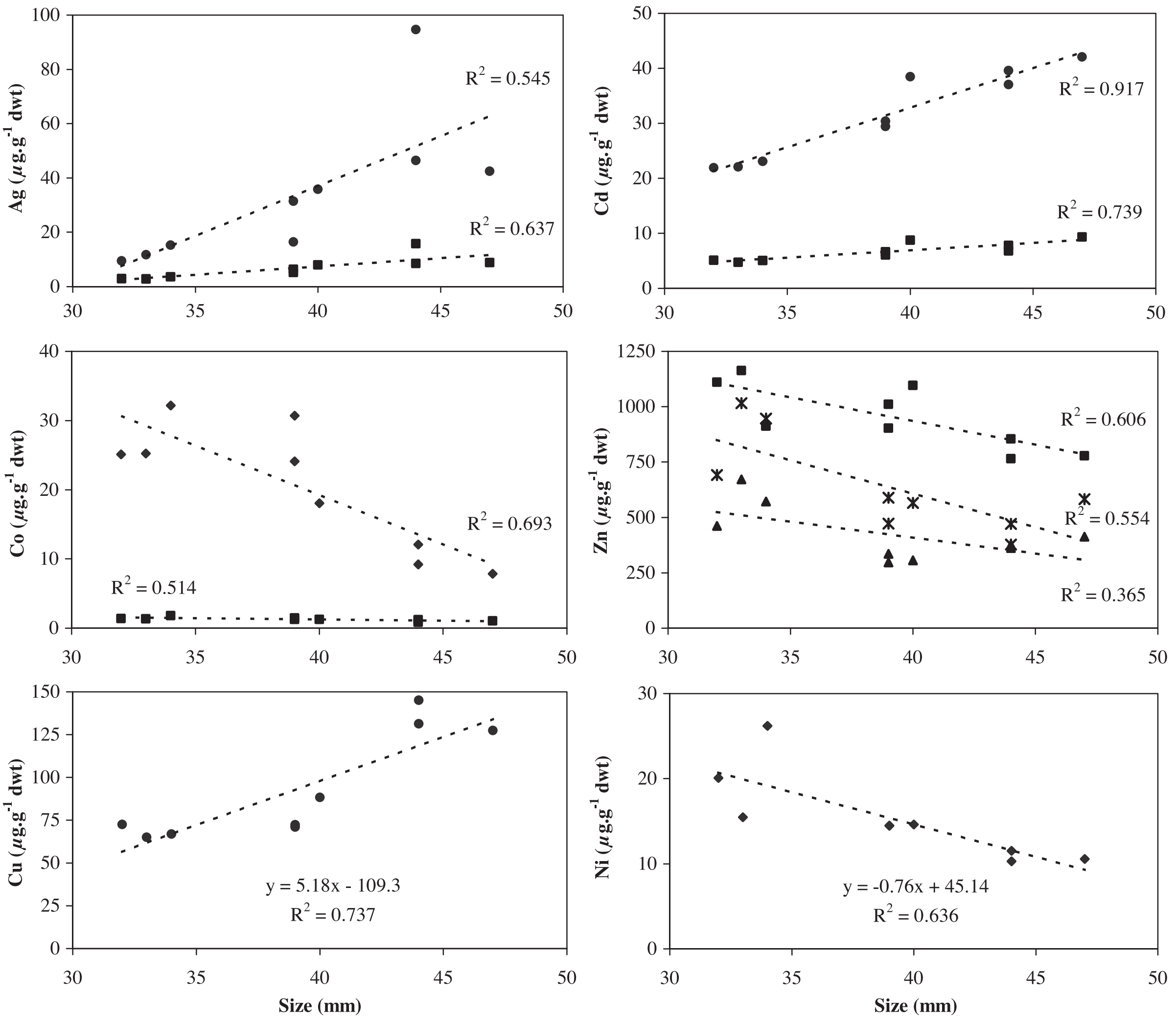

Fig. 2. Significant variations of trace element concentrations with size in the tissues of the variegated scallop *C. varia* from La Rochelle Bay, Bay of Biscay, France. Digestive gland (●), kidneys (◆), gonad (✱), gills (▲), whole soft parts (■).

were also significantly higher in large scallops than in small ones ($H=7.20$, $p=0.027$ and $H=6.49$, $p=0.039$, respectively). For Co, concentrations in kidneys appear to be responsible for the decrease of concentrations with size in the whole soft parts ($H=6.01$, $p=0.050$), while for Zn, it seems to be due to significant concentration decrease in gills and gonad ($H=7.20$, $p=0.027$ and $H=6.01$, $p=0.050$, respectively) but not in whole soft parts.

As for Co, Ni concentrations decreased significantly with size in kidneys ($H=7.20$, $p=0.027$). Finally, Cu concentrations increased with size in digestive gland ($H=6.01$, $p=0.050$), but without any significant effect on allometric relationship in whole soft parts.

A G-test indicated that tissue distribution (% wet wt) was not significantly different ($p<0.05$) between size for all elements except for Ag, which is mainly found in the remaining tissues and muscles in small scallops, and progressively increased in the digestive gland in large individuals (Table 2).

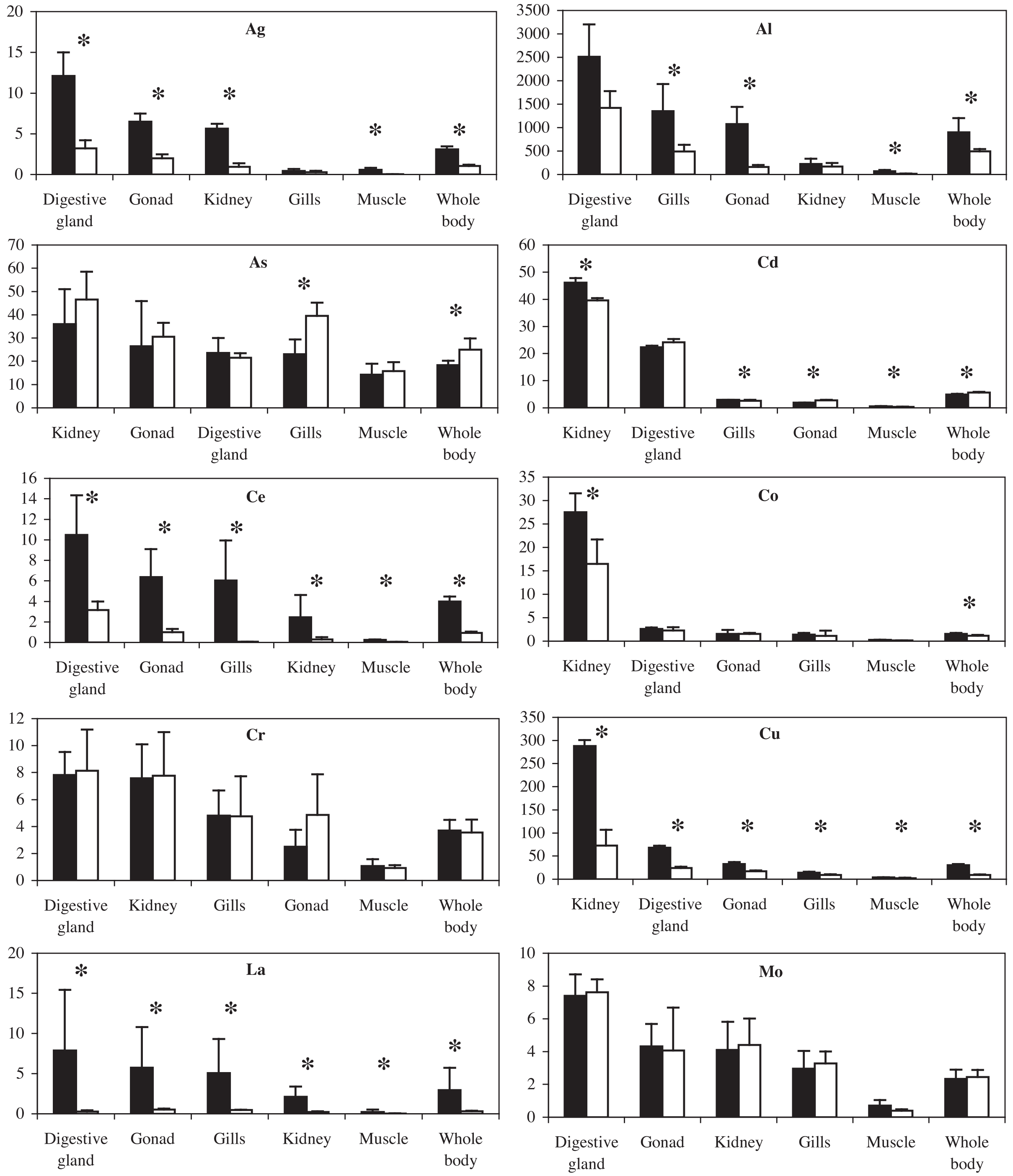

Fig. 3. Comparison of the trace element concentrations in the tissues of the variegated scallop *C. varia* of similar sizes from La Rochelle Bay (size = 33 ± 1 mm, black bars) and Ré Island (size = 32 ± 1 mm, white bars), Bay of Biscay, France.

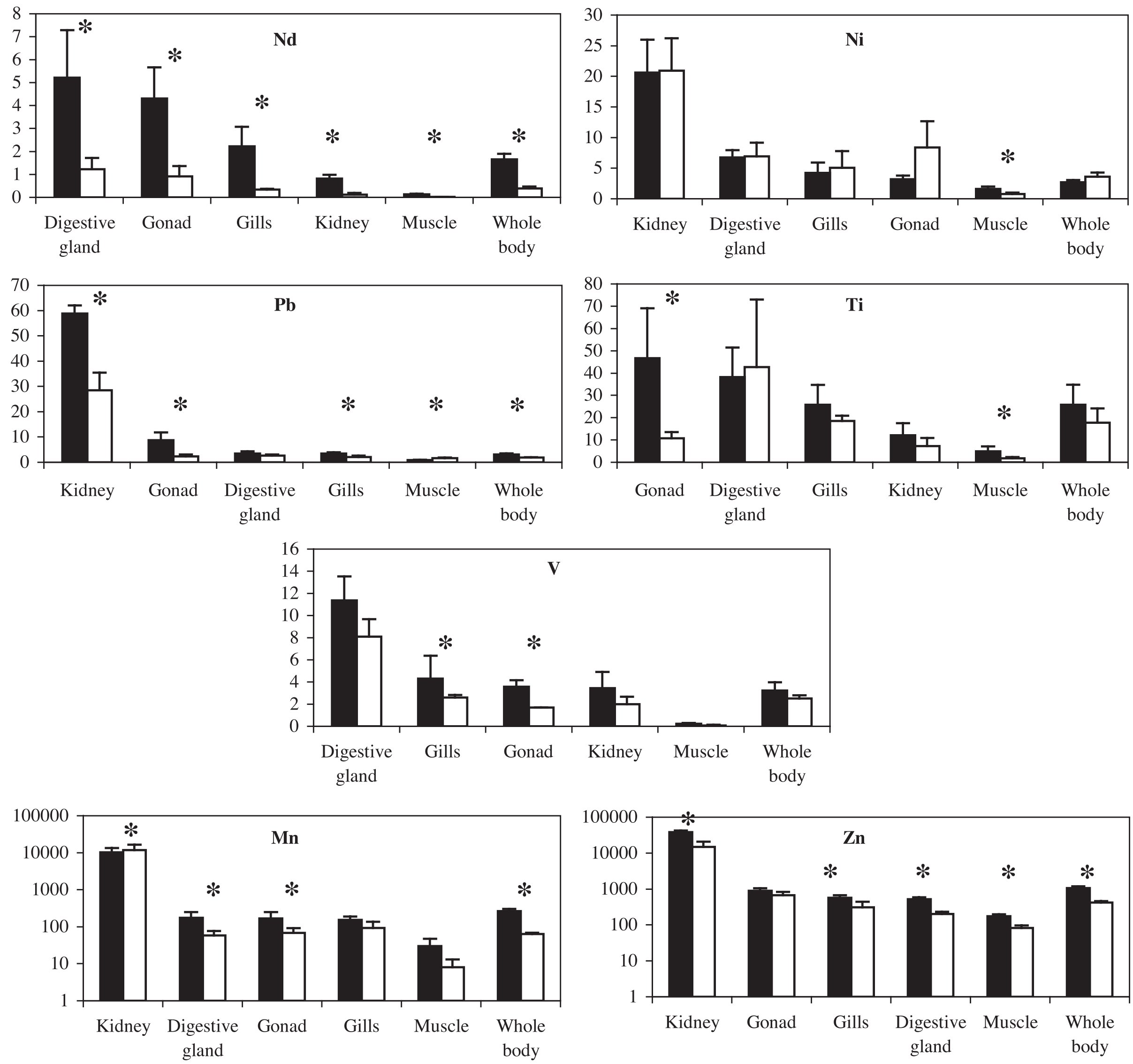

Fig. 3 (*continued*).

3.3. Accumulation differences between sites

Concentrations in the tissues and the whole soft parts of *C. varia* were compared between individuals of similar size from La Rochelle Bay and Ré Island (Fig. 3). Considering the whole soft parts, only a few elements, i.e., Cr, Mo, Ni, Ti, and V, were in the same range at both sampled sites. In contrast, Ag, Al, Ce, Co, Cu, La, Mn, Nd, Pb, and Zn concentrations were significantly higher ($P<0.05$) at La Rochelle while As and Cd concentrations were higher at Ré Island.

3.4. Subcellular distribution within the digestive gland

Fig. 4 presents the proportion of elements contained in the insoluble fraction of the digestive gland homogenates. Elements could be separated following

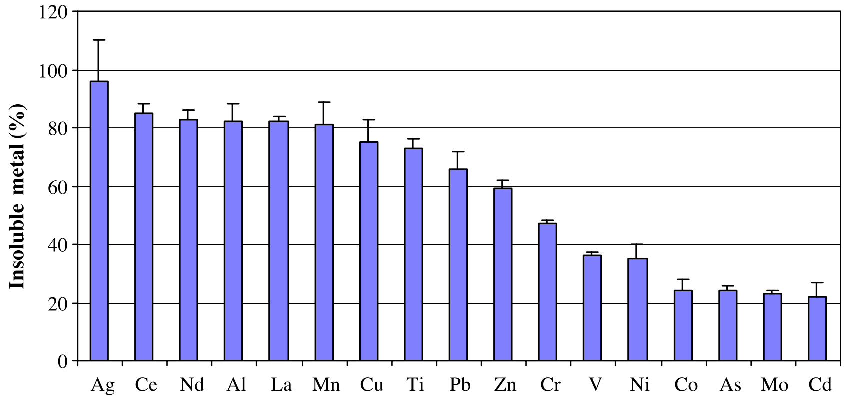

Fig. 4. Trace elements (%) contained in the insoluble fraction of the digestive gland homogenates of the variegated scallop *C. varia* from La Rochelle Bay, Bay of Biscay, France.

their affinity to the insoluble fraction: 96±4% of Ag, 85±3% of Ce, 83±3% of Nd, 82±6% of Al, 82±2% of La, 81±8% of Mn, 75±8% of Cu, 73±3% of Ti, 66±6% of Pb and 59±3% of Zn were found associated to the organelles and membranes of the digestive cells. In contrast, 53±1% of Cr, 64±6% of V, 65±5% of Ni, 76±4% of Co, 76±2% of As, 77±1% of Mo and 78±5% of Cd were found in the cytosolic fraction.

4. Discussion

4.1. Concentration and distribution of trace elements

Among the different tissues analysed, the digestive glands and kidneys of *C. varia* from the French coasts of the Bay of Biscay displayed the highest elemental concentrations. Reported concentrations in these tissues for different scallop species from various locations are given in Table 3 for comparison with the larger scallops from La Rochelle Bay. Observed concentrations of Ag, Cd, Cr, Ni, and Pb in *C. varia* for both tissues were similar to those previously reported. However, concentrations of Al and Cu in digestive gland and kidney and of Mn and Zn in the digestive gland were much higher in *C. varia* than in other species. The reasons for high concentrations of trace elements in the tissues of Pectinidae are typically attributed to their binding to cytosolic proteins in the digestive gland and to their precipitation on granules in kidneys (George et al., 1980; Stone et al., 1986; Fowler and Gould, 1988; Mauri et al., 1990; Viarengo et al., 1993). With the exception of As, Cd, Co, Cr, Mo, Ni, and V, cytosolic proteins seem to have a minor importance in binding of most of the elements, i.e., Ag, Al, Ce, Cu, Nd, La, Mn, Ti, Pb, and Zn. Indeed, large proportions of these elements (59–96%) were found in the insoluble fraction which represents the metals trapped within the organelles, associated to membranes or to granules (Fig. 4). This is particularly obvious in the case of rare earth elements. In fact, the lanthanides have chemistry and sorption/complexation characteristics analogous to transuranic elements such as plutonium or americium (Choppin Torres and Choppin, 1984; Kim et al., 1989; Buckau et al., 1992). In the grant scallop *P. maximus*, most of the ^{241}Am was found in the digestive gland associated with the stomach wall cells and the duct cells, and in lysosomal residual bodies within the digestive cells (Miramand et al., 1991). Residual bodies represent the ultimate form of the digestive processes before excretion in faeces, and are found in the insoluble fraction of digestive gland homogenates (Bustamante, 1998). Therefore, it is likely that rare earth elements would be found in the same structures as ^{241}Am.

The insoluble fraction of the digestive gland cells also contained a remarkably high proportion of Ag, i.e., 96±2% of the total metal. Silver is known to precipitate with sulphur within cells in bivalves (Ballan-Dufrançais et al., 1985; Martoja et al., 1989 Berthet et al., 1990, 1992). Overall, Ag, Al, Br, Ca, Cd, Cu, Fe, Mo, S, Si, and Zn have been detected by both qualitative and quantitative analysis in the

Table 3

Reported mean concentrations of trace elements (μg g^{-1} dry wt) in the digestive gland and kidney of different scallops species from various locations

Species	Location	*n*	Ag	Al	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn	Reference
Digestive gland													
Aequipecten opercularis	English Channel	11	77	43	27	–	4.7	–	–	–	–	228	Bryan (1973)
Adamussium colbecki	Antarctic	30	–	–	142±57	–	1.7 ± 0.4	12.6 ± 3.3	3.4 ± 1.2	–	–	74.9 ± 25.4	Mauri et al. (1990)
Chlamys varia	Bay of Biscay	30	61.2 ± 29.1	1951 ± 239	39.6 ± 2.5	1.79 ± 0.64	5.98 ± 2.53	135 ± 9	113 ± 47	5.73 ± 1.06	4.17 ± 0.56	344 ± 44	Present study
Pecten jacobeus	Mediterranean	30	–	–	41.0 ± 18.2	–	4.8 ± 2.8	16.6 ± 6.6	49.0 ± 23.0	–	–	124 ± 55	Mauri et al. (1990)
Pecten maximus	English Channel	11	13.6	173	321	1.28 ± 0.46	8.1	57.9 ± 27.4	15.6 ± 7.0	3.55 ± 0.67	3.90 ± 0.44	407 ± 123	Bryan (1973)
Pecten maximus	Irish Sea	1	8.9	340	96	0.68	1.8	25	410	0.96	1.7	1100	Segar et al. (1971)
Kidney													
Aequipecten opercularis	English Channel	11	35	58	41	–	6.6	–	–	–	–	54700	Bryan (1973)
Adamussium colbecki	Antarctic	30	–	–	11.6 ± 3.3	–	1.5 ± 0.5	4.0 ± 1.7	16.3 ± 8.9	–	–	199 ± 89	Mauri et al. (1990)
Chlamys varia	Bay of Biscay	30	5.01 ± 3.77	199 ± 113	52.4 ± 10.0	9.71 ± 2.14	4.37 ± 0.95	271 ± 14	11855 ± 4517	10.79 ± 0.66	41.57 ± 3.74	34212 ± 1885	Present study
Pecten jacobeus	Mediterranean	30	–	–	10.7 ± 4.0	–	0.33 ± 0.15	17.5 ± 10.9	6390 ± 3030	–	–	2790 ± 1340	Mauri et al. (1990)
Pecten maximus	English Channel	11	4.3	53.5	79	9.05 ± 3.07	3.9	20.8 ± 10.3	15300 ± 4100	22.9 ± 6.3	159 ± 38	19300 ± 4800	Bryan (1973)

lysosomal system of the digestive gland of *P. maximus*, (Ballan-Dufrançais et al., 1985). With the exception of Cd and to a lesser extend Cu and Zn, information on the subcellular distribution of trace elements in Pectinidae is particularly scarce in the current literature. However, 60–99% of the Cd is usually found in the soluble fraction of the digestive gland of Pectinidae from the field or after experimental exposure to this metal (Evtushenko et al., 1986, 1990; Stone et al., 1986; Lukyanova and Evtushenko, 1989), even if the Antarctic scallop *Adamussium colbecki* appears not to follow this trend with only 30% of soluble Cd (Viarengo et al., 1993). These general observations are consistent with the results obtained for *C. varia* in which the cytosolic fraction of the digestive gland contained 78±5% of the total Cd (Fig. 4). This strongly suggests the presence of mechanisms of detoxification of Cd involving proteins such as metallothioneins (Evtushenko et al., 1986; Stone et al., 1986; Evtushenko et al., 1990; Viarengo et al., 1993; Bustamante, 1998).

As mentioned above, Ag and Cd appeared to be mainly bound to insoluble material and cytosolic proteins, respectively (Fig. 4). Thus, according to the present results both detoxification processes involved in the storage of these metals seem to lead to a long-term bioaccumulation of these metals in the digestive gland of *C. varia*. It is therefore noteworthy that two different detoxification strategies, binding to soluble and insoluble compounds, lead to large concentrations of both toxic metals in this species.

For elements other than Cd found in the cytosolic fraction of the digestive gland, i.e., As, Co, Cr, Mo, Ni, and V, other systems or proteins other than metallothioneins might be responsible of the binding. Co, Cr, Mo, and Ni are essential micronutrients required for function of a large number of proteins. However, both their accumulation and regulation mechanisms are poorly understood because of their generally low concentrations in biota. Recently, investigation of V accumulation in ascidians has shown that specific vanadium-binding proteins (vanabins) were responsible for vanadium accumulation in these organisms (Ueki et al., 2003). In contrast to metallothioneins, vanabins do not use cysteine residues to bind vanadium ions and they might function as metal chaperone proteins rather than proteins for metal storage or detoxification. Vanabins appear to be able to chelate V under its +5 oxidation state and help NADPH to reduce the ion to vanadium(IV), which is easier to carry within the cell. The implication of such proteins in the accumulation of V in Pectinidae should be considered in the future.

Metal concentrations in bivalves may vary with biological factors such as age (size) and sex. However, only Ag, Cd, Co, Cu, Ni, and Zn varied with size in at least one tissue or organ. Similarly to *C. varia* from the Bay of Biscay, Cd also displayed positive accumulation with animal size in *P. maximus* and *Placopecten magellanicus* (Boyden, 1977; Uthe and Chou, 1987). However, no significant relationship was found for the Antarctic scallop *A. colbecki* (Mauri et al., 1990) which was attributed to the influence of various factors such as the contamination of the sampling area and the physiological state of the individuals. In scallops, excretory functions of kidneys might reduce the concentrations of weakly retained elements. This appears to be the case of Ni and Co, which concentrations tend to decrease with size.

4.2. Accumulation differences between sites

Among the different trace elements analysed, Ag, Al, Ce, Co, Cu, La, Mn, Nd, Pb, and Zn concentrations were significantly higher in the scallops from La Rochelle Bay. This could be due to the fact that this area is directly subject to industrial and domestic contamination via several discharge points, but also from more diffusive sources related to harbour activities (e.g., antifouling paints) or installations (e.g., release of Zn by cathodic protection). Therefore, it is not surprising that *C. varia* displayed elevated tissue concentrations of several elements in this area. Contribution of the different local sources might be difficult to determine for most of the elements, but higher rare earth element concentrations in the tissues of *C. varia* are probably related to industrial releases into La Rochelle Bay from the rare earth extracting plant, Rhodia. Very little is known about long-term effects of rare earth elements on marine invertebrates in the field, but experiments have shown that these elements are poorly bioavailable from seawater and also weakly toxic to marine biota (Hirano and Suzuki, 1996). However, the gills of *C. varia* contained high concentrations of Ce, La and Nd relative to muscle. Gills represent a natural pathway for metals dissolved in seawater (Bustamante et al., 2002a). Therefore, if we assume that rare earth elements have low rates of redistribution to other tissues as shown for ^{241}Am (Miramand and Germain, 1986), Ce, La, and Nd concentrations found in the gills would mainly indicate the exposure via seawater. Rare earth elements are also most probably accumulated from food as suggested by the high concentrations found in the digestive gland. High concentrations in gonads might be due to translocation from the intestine into this tissue, which serves both for the expulsion of faeces and gonadal products. Therefore, adsorption of rare earth elements on the intestine walls could occur and be responsible for the relatively elevated concentrations in the gonad of these scallops. The gonad also contained relatively high concentrations of Ag and Pb, which also could be subject to adsorption processes on the intestine walls in the gonad (Table 1, Fig. 3). The ability of *C. varia* to concentrate rare earth elements would be useful to study and to monitor the behaviour of these elements under field conditions. Moreover, lanthanides could also provide particularly interesting information on toxic elements with similar chemical properties, i.e., transuranic elements in the marine environment.

Higher As and Cd concentrations in the scallops from the Ré Island compared to the contaminated site are surprising since there is no identified source of these metals in this area. Similarly, investigation of ^{210}Po in the tissues of *C. varia* and *Mytilus edulis* reported the same unexpected difference between sites (Bustamante et al., 2002b). Uthe and Chou (1987) also reported unexpected high Cd concentrations in the tissues of the scallop *P. magellanicus* from a noncontaminated area. These were attributed to feeding and nutritional inadequacy of the diets. Scallops are filter feeders using different types of food such as phytoplankton, detritus and other suspended matter which are major components of the variation in food quality in the field through variation in their carbon and nitrogen contents (Grant and Cranford, 1991; Alber and Valiela, 1996). Therefore, it would be of particular interest to characterise the food composition and metal concentrations in the food of *C. varia* in both sampling areas.

5. Conclusion

Because of very high trace element concentrations, the digestive gland and kidneys of *C. varia* can be used to monitor trace element availabilities in seawater but only when individuals of similar size are used. Thus, significant differences in concentrations between clean and impacted areas from Charente-Maritime suggested contamination of Ag, Al, Ce, Co, Cu, La, Nd, Pb, Mn, and Zn due to combined domestic, industrial, and harbour releases. The subcellular distribution of trace elements in the digestive gland showed that most of the elements were associated with insoluble material and would have therefore a low bioavailability for the upper trophic levels (Wallace and Lopez, 1996, 1997). Finally, the ability of *C. varia* to concentrate lanthanides would be useful to study the behaviour of trivalent ions in the marine environment with particular attention to actinides.

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