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Heavy metals in the Antarctic scallop *Adamussium colbecki*

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ABSTRACT: Cu, Fe, Cr, Cd, Mn and Zn concentrations were determined in different organs of the Antarctic scallop *Adamussium colbecki* (Smith) and compared with those found in *Pecten jacobaeus* L., a scallop of temperate waters, and with literature values for other Pectinidae. The digestive gland of *A. colbecki* was the target organ for Cu, Fe, Cr and Cd, whereas Mn and Zn were found mainly in the kidney. Cd concentration in the digestive gland of *A. colbecki* was higher than that in the same organ of *P. jacobaeus*, indicating a marked ability of the Antarctic scallop to concentrate this metal. However, in *A. colbecki* renal concentrations of both Mn and Zn were considerably lower than those measured in *P. jacobaeus* and other Pectinidae, and may be related to the scarcity of concretions observed in its kidney.

INTRODUCTION

Better insight into the ecology of the Antarctic is today of great importance considering the increasing interest shown in the resources of this continent. Collecting new environmental data will serve as the baseline for evaluating future environmental impact of pollutants in this remote area.

This study is a first determination of heavy metal levels (Cu, Fe, Cr, Cd, Mn, Zn) in a bivalve mollusc of the Antarctic Sea bottoms, *Adamussium colbecki*.

Bivalves are the classic organisms of choice for studies on heavy metal accumulation (Bryan 1976), because of their poor capability of regulating ion concentrations in internal fluids and of their high tolerance, metal ions being sequestered in excess of metabolic requirements (George 1980). Consequently, high accumulation values are observed without apparent toxic effects. *Adamussium colbecki* was chosen for 2 main reasons: (1) It is one of the largest bivalves in the Antarctic marine environment and makes up most of the benthic biomass (Nicol 1966a, b). Hence, this mollusc can be considered one of the most significant species in the area. (2) It is found throughout the Antarctic coasts and over a wide bathymetric range (Dell 1972). This is of interest for future comparisons with specimens from other research stations in the Antarctic. *A. colbecki* is a gonochoristic species and is closely related systematically to Pectinidae from temperate seas, such as *Lissopecten*, *Pecten* and *Chlamys*.

Pecten jacobaeus L., a hermaphroditic species of temperate waters, was used for comparison between *Adamussium colbecki* and other non-Antarctic scallops. Data on heavy metal levels in other species of Pectinidae were also used for comparison with *A. colbecki*.

MATERIALS AND METHODS

Adamussium colbecki (Smith), (superfam. Pectinoidea, fam. Amussidae), was sampled in Terranova Bay, Ross Sea, in austral summer 1987/88 during an Italian expedition to the Antarctic, supported by the Progetto Nazionale Antartico. Samples of *A. colbecki* collected by dredging from depth of 40 to 70 m were immediately frozen at −30 °C and maintained at this temperature until processed for heavy metal analysis.

In the laboratory, specimens were subdivided into 2 groups of 30 each. One group was used for organ metal analysis and the other to measure the relation between body weight and metal levels in the body. In the latter case, all soft parts of scallops with shell length between 4.2 and 7.9 cm were analysed. For Group 1, metals were determined in mantle, gills, gonad plus foot, kidney, digestive gland and muscle. After partial thawing the adductor muscle was dissected to enable opening of the valves; sex was determined for all individuals.

The bean-shaped gonads, mature at sampling time, were bright orange in females and yellowish in males.

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After determination of the total fresh weight of the soft parts, organs of scallops of the first group were dissected. For evaluating individual variations every sample processed for analysis consisted of a single whole organ, except for kidneys, which consisted of pooled samples from 3 individuals. Dry weight was determined after drying at 105 °C for 24 h.

Mineralization of the samples was carried out by wet digestion with concentrated (BDH Aristar) nitric acid first for 24 h at room temperature, then on a hot plate; the mineralized dry residue was then diluted in 2 % nitric acid. The same digestion procedures were repeated for lobster standards (National Research Council Canada) to check the accuracy of the analytical methods.

Samples of *Pecten jacobaeus* (superfam. Pectinoidaea, fam. Pectinidae) with ripe gonads, collected in the northern Adriatic Sea in June 1988, were processed for metal analysis using the same methodologies described for organs of *Adamussium colbecki*.

Analysis was by atomic absorption spectrophotometry (AAS) (IL mod. S11 equipped with a deuterium background corrector and an IL mod. 755 graphite furnace). Cu, Cr and Cd were determined by carbon rod, Fe, Mn and Zn by flame atomization.

The mean percentage contribution of each organ to the total metal body burden was computed in order to compare metal content in the organs. Univariate analysis of variance (ANOVA) was applied to evaluate metal differences between males and females in gonads and in total body. A regression analysis was developed to assess the influence of body size on metal concentrations.

Samples of digestive gland fixed in situ (Antarctic) with buffered 10 % formalin were cut into pieces of 1 mm and washed in 0.1 M cacodylate buffer (pH 7.3) with 8 % sucrose added. Half of this material was postfixed in 1 % osmium tetroxide. All samples were then dehydrated in ethanol and embedded in Epon-Araldite mixture and viewed in a Philips 400 electron microscope fitted with an EDAX energy dispersive X-ray analyzer.

Unstained sections of 300 nm were mounted on aluminium grids and used for X-ray microanalysis; sections of 70 to 100 nm, stained with aqueous uranyl acetate and lead citrate, were used for conventional electron microscopy.

RESULTS

Concentrations of Cu, Fe, Cr, Cd, Mn, Zn in organs and mean percentage contributions of each organ to the total metal body burden of the soft parts of both *Adamussium colbecki* and *Pecten jacobaeus* suggest that Cu, Fe, Cr and especially Cd in both species are mainly concentrated in the digestive gland, and that this organ contributes the highest percentage to the total body burden (Table 1). Although the organs of *P. jacobaeus* usually showed higher concentrations of metals, *A. colbecki* showed a strong accumulation of Cd in the digestive gland: the value is more than 3 times that measured in *P. jacobaeus* (Fig. 1). Mn and Zn were found mainly in the kidney (Table 1); in *A. colbecki*, the concentrations of Mn and Zn are respectively 400 and 14 times lower than in *P. jacobaeus* (Figs. 2 and 3). This explains the different percentage contribution of kidney to the total metal body content (12 and 5.3 % in *A. colbecki* for Mn and Zn respectively; 60.9 and 23.3 % in *P. jacobaeus*).

Males and females of *Adamussium colbecki* showed similar body concentrations of metals. However, female gonads contained higher Cd concentrations than males (Table 2). The female portion of the hermaphrodite

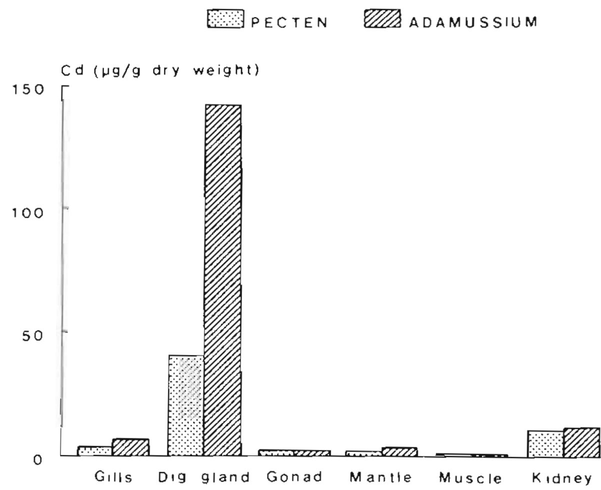

Fig. 1. *Adamussium colbecki* and *Pecten jacobaeus*. Cadmium concentrations in the organs

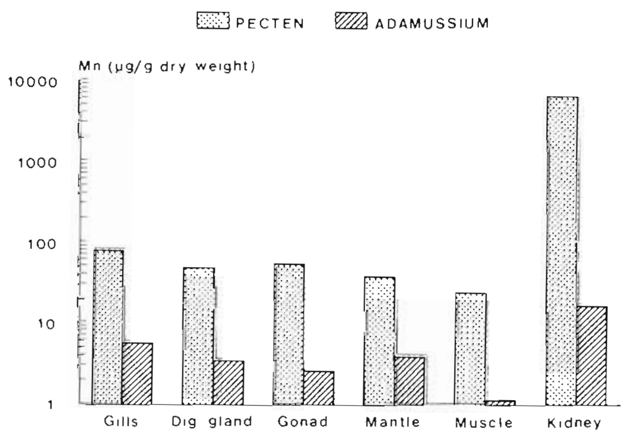

Fig. 2. *Adamussium colbecki* and *Pecten jacobaeus*. Manganese concentrations in the organs

Table 1 *Adamussium colbecki* and *Pecten jacobaeus*. Heavy metals in the organs (1987/88). $\bar{x}$: mean concentration ($\mu g\ g^{-1}$ dry wt); SD: standard deviation; %: mean percentage contribution of the organs to the total metal body burden (soft parts)

Metal	Organ	*Adamussium colbecki*			*Pecten jacobaeus*		
		$\bar{x}$	SD	%	$\bar{x}$	SD	%
Cu	Gills	6.5	2.2	12.3	6.3	2.1	10.8
	Dig. gland	12.6	3.3	50.3	16.6	6.1	38.0
	Gonad	4.7	2.2	8.0	10.3	3.8	24.6
	Mantle	3.5	1.8	10.1	3.3	0.9	11.7
	Muscle	1.6	0.9	17.5	1.1	0.3	12.2
	Kidney	4.0	1.7	1.7	17.5	10.9	2.7
Fe	Gills	119	45	10.9	593	184	17.0
	Dig. gland	292	63	56.6	1350	457	52.0
	Gonad	87.9	36.1	7.3	181	62	7.3
	Mantle	62.5	27.1	8.9	256	89	15.1
	Muscle	28.4	8.1	14.9	41.0	16.0	7.7
	Kidney	69.2	31.7	1.4	336	152	0.9
Cr	Gills	0.88	0.05	10.0	1.7	0.7	10.2
	Dig. gland	1.7	0.4	40.4	4.8	2.8	38.4
	Gonad	0.82	0.28	8.5	2.3	1.0	19.5
	Mantle	0.95	0.05	17.0	1.5	0.4	18.5
	Muscle	0.31	0.09	20.2	0.33	0.11	13.2
	Kidney	1.5	0.5	3.9	0.33	0.15	0.2
Cd	Gills	6.8	1.1	2.1	3.7	1.9	5.0
	Dig. gland	142	57	93.1	41.0	18.2	73.6
	Gonad	2.4	1.7	0.7	2.4	1.2	4.5
	Mantle	3.5	0.9	1.7	2.0	0.6	5.4
	Muscle	0.94	0.34	1.7	1.2	0.4	10.3
	Kidney	11.6	3.3	0.8	10.7	4.0	1.3
Mn	Gills	5.7	1.5	18.1	53.1	24.5	5.6
	Dig. gland	3.4	1.2	22.9	49.0	23.0	7.0
	Gonad	2.5	1.0	7.3	54.0	29.2	8.0
	Mantle	3.8	1.1	19.1	8.1	3.4	1.8
	Muscle	1.1	0.6	20.6	23.9	14.3	16.7
	Kidney	16.3	8.9	12.0	6390	3030	60.9
Zn	Gills	114	33	13.2	60.7	20.8	5.6
	Dig. gland	74.9	25.4	18.3	124	55	15.4
	Gonad	98.7	20.7	10.3	97.1	25.0	12.6
	Mantle	123	46	22.3	63.7	37.4	12.1
	Muscle	46.2	13.0	30.6	50.6	11.6	30.9
	Kidney	199	89	5.3	2790	1340	23.3

Fig. 3. *Adamussium colbecki* and *Pecten jacobaeus*. Zinc concentrations in the organs

gonad of *Pecten jacobaeus* showed significantly higher concentrations of Cu and Zn than that of the male (Table 2).

Body size expressed as total dry weight of soft parts correlated with whole body soft part concentrations of Cd, Mn and Zn in *Adamussium colbecki* (Table 3), whereas it did not affect Cu, Fe and Cr. Cd, Mn and Zn decreased as body weight increased, as shown by double logarithmic coordinates (Fig. 4).

A characteristic feature of the digestive cell in *Adamussium colbecki* was the presence of a highly developed lysosomal system, composed of primary lysosomes, heterophagosomes and residual bodies. X-ray microanalysis showed Fe and Cu, which were constantly associated with heterophagosomes, whereas Zn was present mainly in the residual bodies. Cr, Cd and

Table 2. *Adamussium colbecki* and *Pecten jacobaeus*. Differences in metal concentrations between male and female gonads: results of ANOVA. Only those metals with significant differences at 0.05 level are reported; there was no significant difference in concentrations of Fe, Cr, and Mn. $\bar{x}$: mean concentration ($\mu g\ g^{-1}$ dry wt); df: degrees of freedom

A. colbecki		**Cd**						
	$\bar{x}$	F	df	p				
Female gonad Male gonad	4.2 1.6	7.6	11	< 0.01				
P. jacobaeus		**Cu**				**Zn**		
	$\bar{x}$	F	df	p	$\bar{x}$	F	df	p
Female gonad Male gonad	11.2 3.9	9.7	7	< 0.05	139 52.1	8.7	7	< 0.05

Mn were not detected in the cells of the digestive gland.

X-ray microprobes also showed Na, Mg, P, Si, S, K, and Ca in heterophagosomes and residual bodies. Si appeared to be confined to small needle-like particles contained within lysosomal vesicles (Fig. 5).

DISCUSSION

Metal concentrations in bivalves usually vary with biological factors such as body size (age) and sex. According to Boyden (1974) the influence of body size can be expressed by a power function:

$$[\text{metal}] = aw^{b} \quad (1)$$

or:

$$[\text{metal}]w^{-1} = aw^{b-1} \quad (2)$$

where [metal] = soft body metal content; $[\text{metal}]w^{-1}$ = metal concentration in $\mu g\ g^{-1}$ dry wt; w = body weight (in g); and a and b = constants.

On a double logarithmic scale Eq. (2) gives the following straight line relation:

$$\log\,[\text{metal}]w^{-1} = \log a + (b-1)\log w \quad (3)$$

In *Adamussium colbecki*, the regressions are significant only for Cd, Mn and Zn, the concentrations of these 3 metals decreasing steadily with body weight throughout the whole size range of samples. Similar relations with regression coefficients $(b-1) < 0$ were found by Boyden (1977) for Mn and Zn in *Pecten maximus*. On the other hand, Cd displayed a positive regression coefficient in older individuals, probably due to 'accumulation of the metal within specific tissue'. *Adamussium colbecki* does not show a similar trend for Cd with respect to body size. In this scallop the relation between metal level and body size might be influenced by factors such as contamination of sampling area and physiological state of the individuals (Latouche & Mix 1982, Fischer 1983).

When considering the total body, no differences in metal concentrations were found between males and females of *Adamussium colbecki*. However, gonadal concentrations of Cd were higher in females than in males. The female portion of the hermaphroditic gonad of *Pecten jacobaeus* showed higher concentrations of both Mn and Zn than the male. Differences in metal concentrations between males and females have previously been reported for the bivalve *Choromytilus meridionalis* (Watling & Watling 1976, Orren et al. 1980). According to Latouche & Mix (1982), the higher gonadal level of Mn and Zn in females of *Mytilus edulis* is related to gametogenesis. Females of the wedge shell *Donax trunculus* show higher Mn and Zn concentrations than males when the gonads are ripe (Mauri & Orlando 1983). This is due to higher gonadal concentrations of these 2 metals in females compared to males.

Table 3. *Adamussium colbecki*. Statistical data on relation (Eq. 3) between metal body concentrations ($\mu g\ g^{-1}$ dry wt) and body weight (g dry wt). Only regressions with $b-1 \neq 0$ are reported. n: number of individuals

Metal	a	Intercept (log a)	Regression coefficient (b−1)	Correlation coefficient (r)	n	p
Cd	39.26	1.594	−0.34	0.653	30	< 0.001
Mn	6.09	0.785	−0.55	0.804	30	< 0.001
Zn	113.77	2.056	−0.55	0.795	30	< 0.001

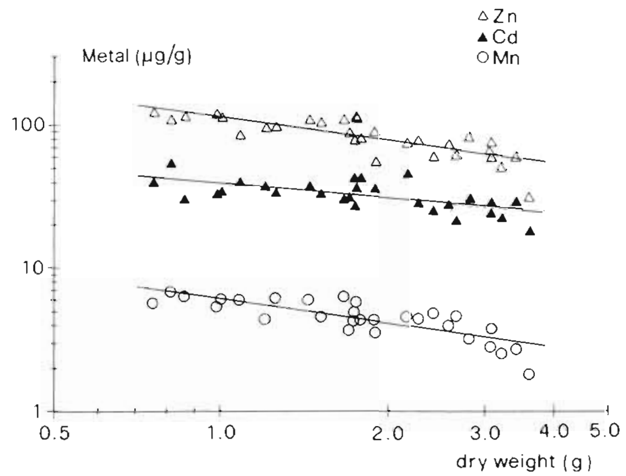

Fig. 4. *Adamussium colbecki*. Relation between metal concentrations (µg g^{-1} dry wt) and body weight. Only those regressions with $b-1 \neq 0$ are shown

The higher concentration of Cd in the female gonad of *Adamussium colbecki* might be related to the abundance of lysosomes in oocytes. The digestive gland of *A. colbecki* seems the target organ for Cu, Fe, Cr and Cd, whereas Mn and Zn tend to accumulate in kidneys.

X-ray microanalysis of the digestive gland showed that Cu, Fe and Zn are compartmentalized within the lysosomal system, particularly in heterophagosomes and residual bodies. This finding confirms data from previous studies on the intracellular localization of these metals in Pectinidae (Ballan-Dufrancais et al. 1985) as well as in other molluscs (George 1983). Although a high level of Cd was detected by AAS in *Adamussium colbecki* digestive gland, this metal was not revealed in unstained sections by X-ray microanalysis. This failure could be explained by the fact that Cd is mainly present in the cytosol, bound to thioneine-like proteins (Viarengo et al. 1987). However a selective loss of Cd during processing for electron microscopy cannot be excluded.

The abundant needle-like particles, which appear to be mainly composed of Si, were also observed in *Pecten maximus* (Ballan-Dufrancais et al. 1985), and might be derived from sand or plankton organisms suspended in seawater. This finding also suggests that endocytosis probably occurs in *Adamussium colbecki* as in other filter-feeding bivalves: *Pecten maximus* (Ballan-Dufrancais et al. 1985), *Cardium edule* (Owen 1970) and *Mytilus edulis* (Coombs 1980).

Metals were distributed between organs in a similar pattern in *Adamussium colbecki* and in *Pecten jacobaeus*. Concentrations in *A. colbecki* were lower, with the exception of Cd which is strongly accumulated in the Antarctic bivalve. It is surprising to find such high Cd concentrations in a species endemic to the Antarctic, where metal contamination appears unlikely. Therefore this high Cd level might represent a strong capacity in this species for concentrating this element. Variations in the ability to concentrate Cd have been described among members of the Pectinidae family. For example, Bryan (1973) suggested that differences between cadmium concentrations in *P. maximus* and *Chlamys opercularis*, collected from the same area of the English Channel, may be due to interspecific diversity.

Pectinidae strongly concentrate Mn and Zn in their kidneys (Table 4). While *Pecten jacobaeus* seemed to follow this pattern, *Adamussium colbecki* showed comparatively lower renal concentrations of Mn and Zn. In fact, this organ made up a low percentage of the total body metal content (12 % Mn, 5.3 % Zn) in *A. colbecki*, whereas the contribution was much higher (60.9 % Mn, 23.3 % Zn) in *P. jacobaeus*. In this respect, *A. colbecki* is similar to *Patinopecten yessoensis*, which concentrates Mn and Zn to a low degree (Table 4). These low renal concentrations might be due to a low bioavailability of these 2 metals. For Mn, this hypothesis could be supported by the low levels in all other organs (Fig. 2), whereas in the case of Zn, organ concentrations, with the exception of the kidney, appear similar to those in *P. jacobaeus* (Fig. 3). However we cannot exclude, as already seen for Cd, interspecific diversity between the 2 scallops. It should be noted that Mn and Zn in the kidneys can be sequestered in intra- and extracellular concretions described in Pectinidae as well as in many other bivalves (Carmichael et al. 1979,

Table 4. Renal concentrations (µg g^{-1} dry wt) of Mn and Zn in Pectinidae

	Mn	Zn	Sampling area	Source
Chlamys opercularis	17300	40800	English Channel	Bryan (1973)
Pecten maximus	15300	19300	English Channel	Bryan(1973)
Pecten maximus	-	32000	Scotland	George et al. (1980)
Pecten novae-zelandiae	-	2630	New Zealand	Brooks & Rumsby (1965)
Patinopecten yessoensis	10	174	Japan	Ishii et al. (1985)
Adamussium colbecki	16	199	Antarctic	Present study
Pecten jacobaeus	6390	2790	North Adriatic	Present study

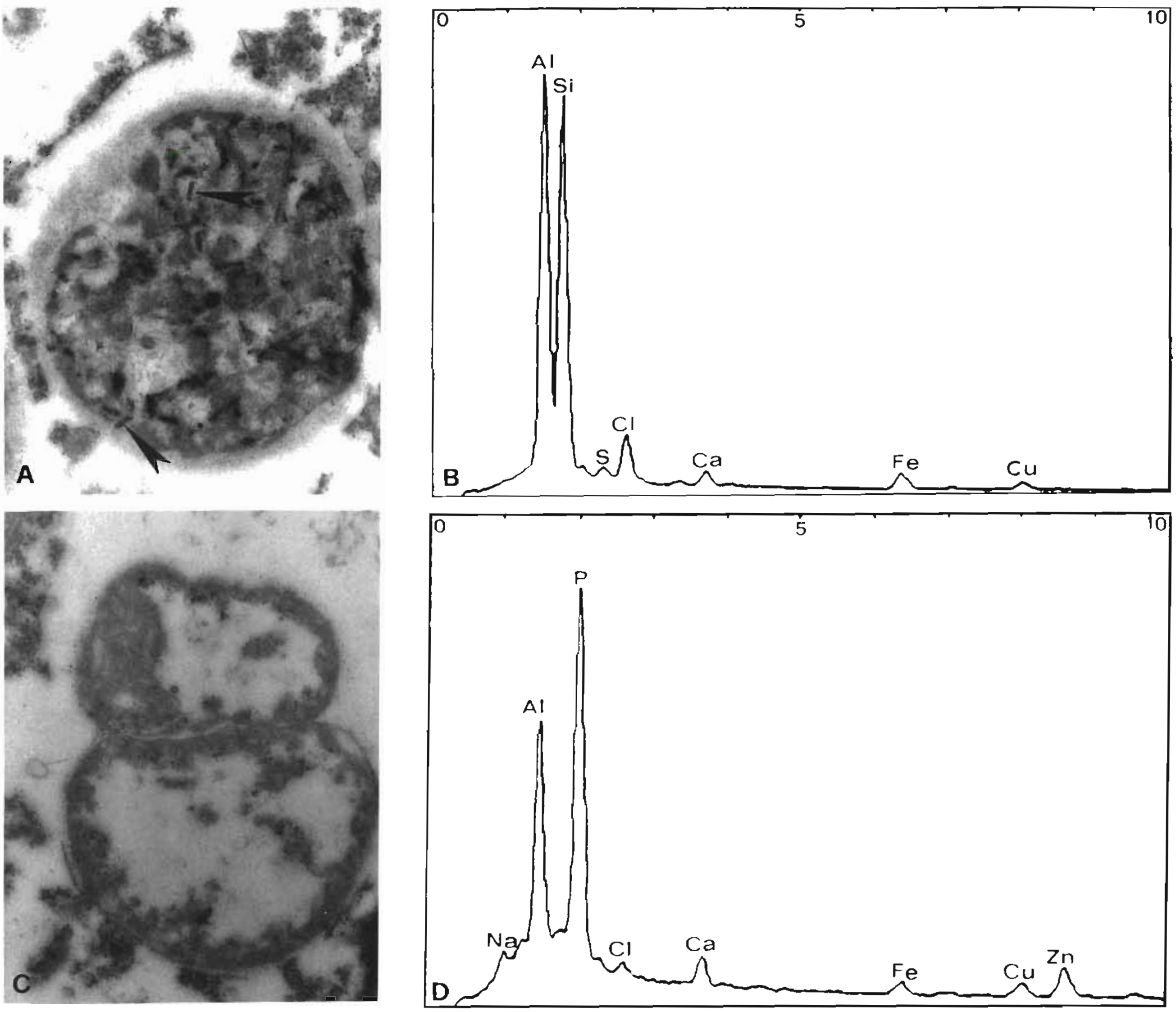

Fig. 5. *Adamussium colbecki*. Digestive gland. (A) Heterophagosome showing needle-like particles (arrowheads) (× 20 000); (B) typical X-ray spectrum from a heterophagosome; (C) residual body (× 20 000); (D) typical X-ray spectrum from a residual body. Al peak originates from the grid; horizontal scale = X-ray energy in keV; vertical scale = X-ray counts

George et al. 1980, Mauri & Orlando 1982, Lucas & Hignette 1983). These granules consist of phosphates and calcium with small amounts of other metals such as Mn, Zn, and Fe. In dissecting the scallops to isolate the kidneys for metal analysis large yellow-brown renal concretions were visible in almost every individual of *P. jacobaeus*, whereas the kidneys of *A. colbecki* did not show evident nephrolithes. Therefore, the difference in renal levels of Mn and Zn between the 2 scallops might depend on the amount of concretions present in the kidney.

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