Pergamon

Marine Pollution Bulletin Vol. 40, No. 8, pp. 688–696, 2000

Printed in Great Britain
0025-326X/00 $ - see front matter

PII: S0025-326X(00)00005-9

Bioaccumulation of 12 Trace Elements in the Tissues of the Nautilus *Nautilus macromphalus* from New Caledonia

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Soils in New Caledonia are particularly rich in metals among which Fe and Ni are intensively exploited. Due to important natural erosion in tropical latitudes and to mining activities, coastal waters are enriched in Co, Cr, Fe and Ni. In deeper waters lives a cephalopod species which is considered as a living fossil, the nautilus *Nautilus macromphalus*. In this study, 12 trace elements were analysed in the tissues of 4 nautilus specimens. Results showed high metal concentrations compared to data available for cephalopods from temperate waters. These concentrations were often in the same order as those encountered in bivalves or gastropods from contaminated areas. Relatively high concentrations of Ni and Cr in the haemolymph strongly suggest a high exposure of *N. macromphalus* to these metals. Among the tissues, the digestive gland has the highest concentrations of Cd, Co, Fe, V and Zn while for Ag, Al, As, Cr and Ni, renal and pericardial appendages exhibited the highest values. Despite this, the digestive gland contained the largest quantities of all metals with the exception of As and Mn which were mainly found in the body muscular remains. These results highlighted the major role of digestive gland and excreting organs in the metabolism of metals in these cephalopod species.

Keywords: New Caledonia; trace metals; cephalopods; digestive gland; *Nautilus*.

Introduction

Cephalopods play a key role in many marine ecosystems (Amaratunga, 1983; Rodhouse, 1989). Trace elements have been investigated extensively in many marine organisms but rarely in cephalopods. Some elements are essential, others toxic, and for a number of them the role is still not known. The studies concerning trace elements in cephalopods molluscs are limited to species targeted by commercial fisheries such as the cuttlefish *Sepia officinalis* (Decleir *et al.*, 1978; Schipp and Hevert, 1978; Miramand and Bentley, 1992; Bustamante, 1998), the octopuses *Octopus vulgaris* and *Eledone cirrhosa* (Ghiretti-Magaldi *et al.*, 1958; Rocca, 1969; Renzoni *et al.*, 1973; Froesch and Packard, 1979; Ueda *et al.*, 1979; Miramand and Guary, 1980; Miramand and Bentley, 1992; Barghigiani *et al.*, 1993; Rossi *et al.*, 1993; Bustamante, 1998), some Ommastrephid squids (Martin and Flegal, 1975; Ueda *et al.*, 1979; Smith, 1983; Smith *et al.*, 1984; Finger and Smith, 1987; Yamada *et al.*, 1997; Bustamante, 1998) and some Loliginid squids (Martin and Flegal, 1975; Yamada *et al.*, 1997; Bustamante, 1998). Among these species, high levels of metals have generally been recorded, the digestive gland of Coleidae concentrating many trace elements, such as cadmium, copper, silver or zinc (Martin and Flegal, 1975; Smith *et al.*, 1984; Miramand and Guary, 1980; Finger and Smith, 1987; Miramand and Bentley, 1992; Bustamante, 1998; Bustamante *et al.*, 1998a). The most striking feature is that the digestive gland stores most of the total cadmium, reaching up 98% in some species (Bustamante, 1998).

Present cephalopods comprise two subclasses: the Coleoidae, with ca 650 species and the Nautiloidae with a single genus (*Nautilus*) and an as yet undetermined number of species (3–7 according to the authors). *Nautilus* is the last representative of the ectocochleate cephalopods and is considered as a living fossil. This taxon shares a number of common anatomical structures with present Coleoidae, but some organs are specific to *Nautilus*.

Among the nautilus species, *Nautilus macromphalus* is endemic to the New Caledonian waters. In this area,

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natural erosion and mining activity have provoked an enrichment of several metals, mainly Co, Cr, Fe and Ni, of the New Caledonian waters and consequently in the coral reef food webs (Monniot *et al.*, 1994). These authors have described the metal enrichment in several species of filter-feeding ascidians from the shallow waters. But no data are available on species living off the New Caledonia coast.

For these reasons, levels of trace elements in the tissues of *N. macromphalus* from New Caledonia were investigated here and compared to previous results reported for Coleoid species.

Materials and Methods

Sampling and sample preparation

Two male and two female *N. macromphalus* were taken in baited traps from the barrier reef off Nouméa (New Caledonia). Samples were frozen upon arrival at the Nouméa Aquarium in individual plastic bags. Each individual was weighed and sexed. The digestive gland, gills, renal and pericardial appendages, digestive tract and genital tract were totally removed. In addition, pieces of muscle and crop were sampled to determine metal concentrations. The remains of the animals were thus composed of arms, the rest of the muscles and the rest of the crop. The stomach was emptied before metal analysis.

Analytical procedure

Tissue samples were dried for several days at 80°C to constant weight. Two aliquots of approx. 300 mg of each homogenized dry sample were digested with 4 ml of 65% HNO_3 and 1 ml of 70% $HClO_4$ at 80°C until the solution was clear. After evaporation, the residues were dissolved in 1 N nitric acid.

Cadmium, copper and zinc were determined both by atomic absorption spectrophotometry (AAS) and by induced coupled plasma mass spectrophotometry (ICP-MS). Other elements (Ag, As, Al, Co, Cr, Fe, Mn, Ni and V) were analysed only by ICP-MS.

Appliances used for metal 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), Orchard-Leaves (NBS) and MA-A-2 fish-flesh standard (IAEA), were treated and analysed in the same way as the samples. The results for the standard reference materials (Table 1) are in good agreement with certified values. The detection limits were (μg g^{-1} dry wt): 0.002 (Ag), 0.005 (Al), 0.15 (As), 0.005 (Cd), 0.005 (Co), 0.005 (Cr), 0.027 (Cu), 0.15 (Fe), 0.017 (Mn), 0.002 (Ni), 0.005 (V) and 0.15 (Zn). Metal concentrations in tissues are given relative to the dry weight (μg g^{-1} dry wt) while the distribution percentages were calculated for wet weight.

TABLE 1

Comparison of trace elements concentrations (μg g^{-1} dry wt) of dogfish liver DOLT-2 (NRCC), Orchard Leaves SRM 1571 (NBS) and fish-flesh homogenates MAA2 (IAEA) determined in the present study with certified values.

Standard	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	V	Zn
DOLT-2												
Present study	0.556 ± 0.041	26.9 ± 3.5	16.1 ± 0.2	20.4 ± 0.27	0.22 ± 0.01	0.39 ± 0.09	26.8 ± 0.3	1072 ± 17	6.26 ± 0.11	0.24 ± 0.04	–	86.3 ± 1.2
Certified values	0.608 ± 0.032	25.2 ± 2.4	16.1 ± 1.1	20.8 ± 0.05	0.24 ± 0.05	0.37 ± 0.08	25.8 ± 1.1	1103 ± 47	6.88 ± 0.56	0.20 ± 0.02	–	85.8 ± 2.5
Orchard-leaves												
Present study	–	–	–	0.13 ± 0.02	(0.15)	(2.3)	12 ± 1	290 ± 15	85 ± 6	1.5 ± 0.1	(0.5)	25 ± 1
Certified values	–	–	–	0.11 ± 0.02	(0.2)	(2.3)	12 ± 1	300 ± 20	91 ± 4	1.3 ± 0.2	(0.6)	25 ± 3
MA-A-2												
Present study	0.09 ± 0.03	–	–	0.063 ± 0.002	0.06 ± 0.01	1.1 ± 0.1	3.7 ± 0.2	51 ± 2	0.69 ± 0.09	1.2 ± 0.1	–	32 ± 3
Certified values	0.10 ± 0.01	–	–	0.066 ± 0.004	0.08 ± 0.01	1.3 ± 0.1	4.0 ± 0.1	54 ± 1	0.81 ± 0.04	1.1 ± 0.2	–	33 ± 1

Results

Metal concentrations in the organs of nautilus are reported in Fig. 1. The percentages of heavy metals in each tissue are shown in Fig. 2.

Mean metal concentrations in whole *N. macromphalus* were 2.4 µg Ag g^{-1}, 19.1 µg Al g^{-1}, 186 µg As g^{-1}, 16.0 µg Cd g^{-1}, 2.7 µg Co g^{-1}, 2.6 µg Cr g^{-1}, 73 µg Cu g^{-1}, 258 µg Fe g^{-1}, 17 µg Mn g^{-1}, 6.7 µg Ni g^{-1}, 3.2 µg V g^{-1} and 260 µg Zn g^{-1} (Fig. 1). Variation coefficients ranged from 10% for As to 45% for Co and Ni.

Metal levels in soft tissues

In the four sampled nautilus, the digestive gland was the major site of concentration for Cd, Co, Fe, V and Zn with 28.2–60.5 µg Cd g^{-1}, 4.4–12.5 µg Co g^{-1},

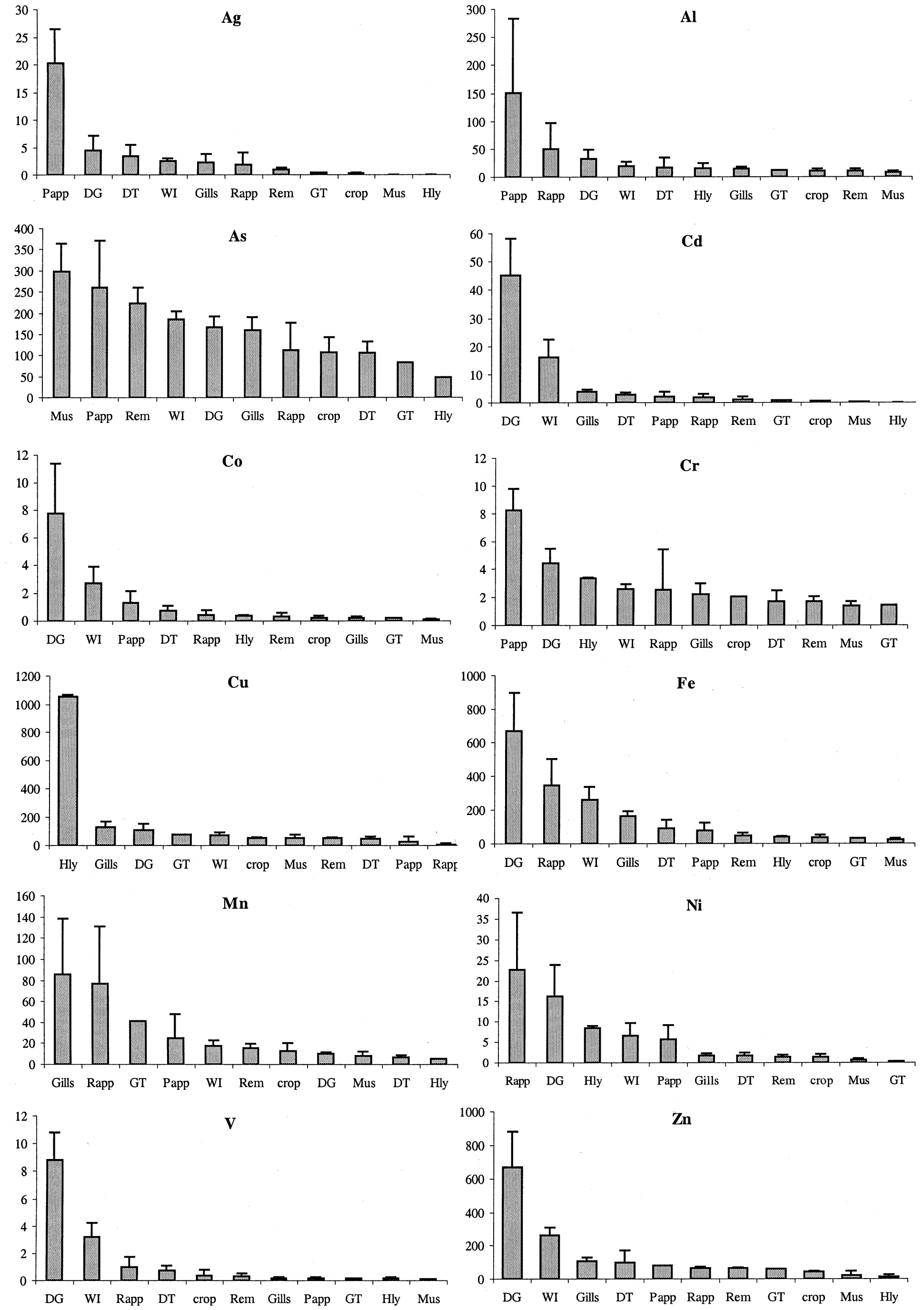

Fig. 1 Trace elements concentrations (µg g^{-1} dry wt) in the tissues of *Nautilus macromphalus* from New Caledonia. Scale bars represent 1 standard deviation. DG: digestive gland; DT: digestive tract; GT: genital tract; Hly: haemolymph; Mus: muscle; Papp: pericardial appendages; Rapp: renal appendages; Rem: remainders; WI: whole individual.

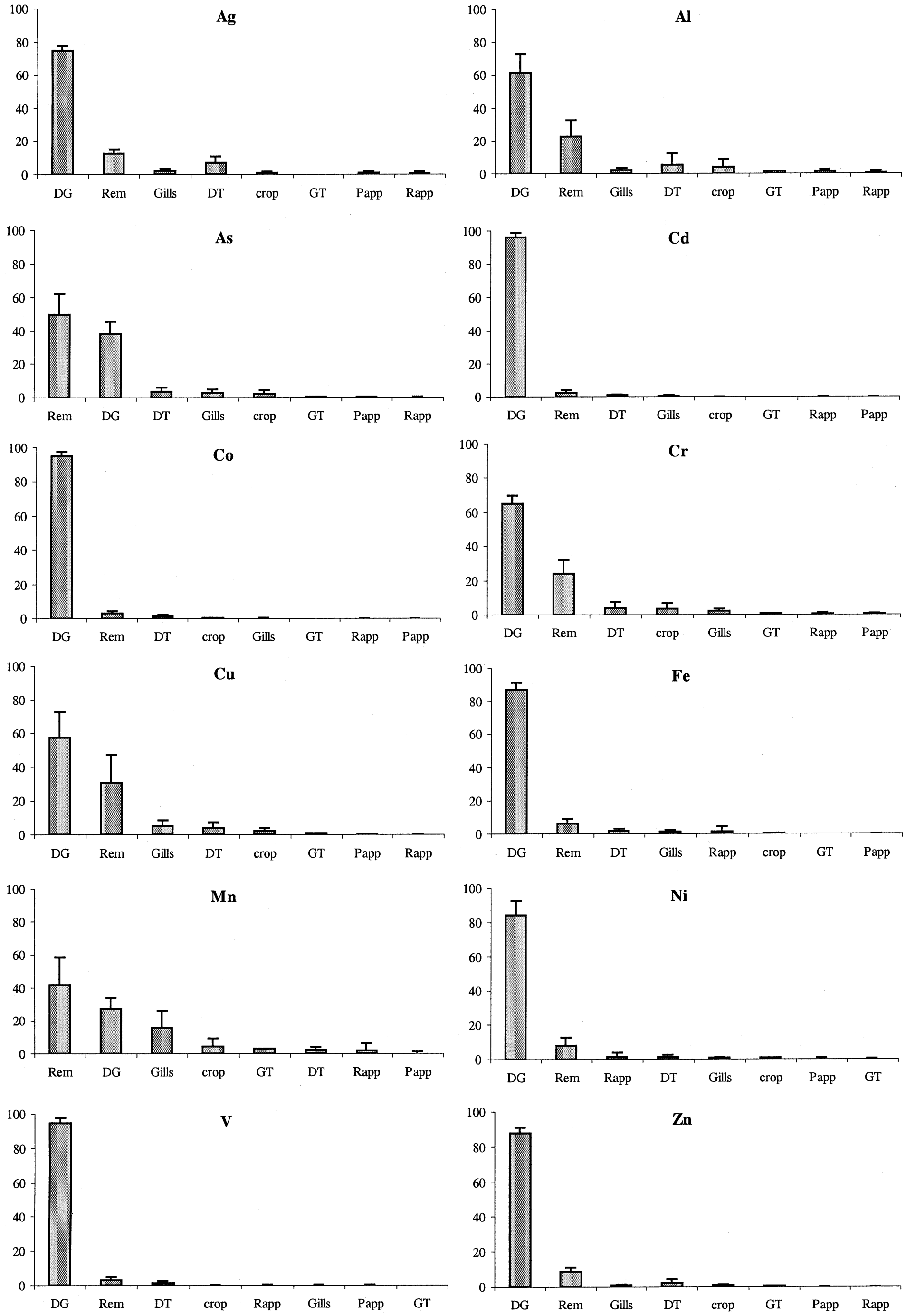

Fig. 2 Percentages distribution of trace elements in the tissues of *Nautilus macromphalus* from New Caledonia. Scale bars represent 1 standard deviation. DG: digestive gland; DT: digestive tract; GT: genital tract; Hly: haemolymph; Papp: pericardial appendages; Rapp: renal appendages; Rem: remainders; WI: whole individual.

477–953 μg Fe g^{-1}, 6.4–10.6 μg V g^{-1} and 515–963 μg Zn g^{-1} (Fig. 1). The digestive gland also concentrated Ag, As, Cr, Cu and Ni at levels close to the highest concentrations recorded in the other tissues with 1.1–6.8 μg Ag g^{-1}, 133–194 μg As g^{-1}, 3.4–5.5 μg Cr g^{-1} 78–174 μg Cu g^{-1} and 9.0–26.3 μg Ni g^{-1} (Fig. 1).

Ag, Al, As, Cr and Ni concentrations were also remarkable in excreting tissues, i.e. renal and pericardial appendages, which exhibited the highest concentrations for these metals. In fact, Ni concentrations ranged from 5.6 to 40.0 μg g^{-1} in renal appendages. Pericardial appendages concentrated Ag from 13.8 to 28.0 μg g^{-1}, Al from 45 to 305 μg g^{-1}, As from 155 to 412 μg g^{-1} and Cr from 6.0 to 9.4 μg g^{-1} (Fig. 1).

Muscles exhibited generally the lowest concentration for all metals except for As which exhibited the highest concentration in this tissue (203–354 μg As g^{-1}; Fig. 1). Mn was present in high concentration in the gills and the renal appendages (i.e. 42–151 μg g^{-1} and 16–152 μg g^{-1}, respectively) while most tissues exhibited concentrations lower than 20 μg g^{-1}. The highest Cu concentration was found in the haemolymph, with mean values of 1055 ± 12 μg g^{-1}. Thus, it is not surprising to find relatively high Cu concentrations in the gills (i.e. 127± 38 μg g^{-1}).

Percentage distribution of metals in soft tissues

With the exception of As and Mn which were mainly found in the body muscular remains (50 ± 12% and 42 ± 16%, respectively), the digestive gland contained the largest quantities of all metals: 75 ± 3% of Ag, 62 ± 11% of Al, 96 ± 3% of Cd, 95 ± 2% of Co, 57 ± 16% of Cu, 65 ± 5% of Cr, 87 ± 4% of Fe, 84 ± 8 of Ni, 95 ± 3% of V and 88 ± 3% of Zn (Fig. 2).

Although the concentration of some metals was high in the renal and pericardial appendages, these tissues contain in fact low amounts of metals because of their small mass (Fig. 2).

Discussion

The variability of metal concentration was relatively small among the four studied individuals. Low variation coefficients for toxic metals such as Ag, Cd or V (26%, 41% and 34%, respectively) are noteworthy. Nevertheless, coefficients of variation for Cd are similar to those found in two octopus species from the Southern Indian Ocean, *Benthoctopus thielei* and *Graneledone,* reported to have very high Cd levels in their tissues (Bustamante *et al*., 1998a). Such results suggest efficient regulation processes of toxic metals in Nautilidae even Cd levels were lower in *N. macromphalus* than in these octopuses. Some other toxic elements, Ag, Co, Cr, Ni and V, were more concentrated in nautilus than in temperate cephalopods such as the cuttlefish *S. officinalis* and the octopuses *E. cirrhosa* and *O. vulgaris* (Miramand and Guary, 1980; Miramand and Bentley, 1992). This might be due to accumulation during nautilus life span (10–15 years), much longer than Coleoid's (1–3 years). Moreover, several essential metals, such as Fe, Mn and Zn were also highly concentrated in *N. macromphalus*, but Cu concentrations almost the same as in Coleoidae species (Miramand and Guary, 1980; Miramand and Bentley, 1992; Bustamante *et al*., 1998a).

High metal concentrations might reflect the ambient life conditions of nautilus. Mineral extraction activity, mainly Ni, is important in New Caledonia : Ni enrichment in the waters and food webs might thus account for high levels of Ni in *N. macromphalus*. Although the dissolved Ni has not been measured, large amounts in coastal sediments suggest that abnormal concentration may occur in seawater (Bryan, 1976; Monniot *et al*., 1994). Indeed, Monniot *et al*. (1994) reported very high Ni concentrations in the body and the tunic of *Ascidia sydneiensis* from coastal waters, reaching 80.4 and 119.5 μg/g dry wt, respectively. Compared to other mollusc species, Ni concentrations in *N. macromphalus* were in the same order of magnitude as in several deposit feeder species such as *Scrobicularia plana* or *Macoma balthica* but higher than in filter feeder species such as *Pecten maximus* or *Mytilus edulis* (Table 2). It was also the case for Co and Cr. High levels of Ni, Co and Cr in deposit feeder bivalves were related to direct exposure through contaminated sediments (Bryan and Hummerstone, 1977). This strongly suggests that contamination is not only located on the New Caledonian coast but reaches deeper waters where nautilus lives. This hypothesis is reinforced by the relatively high concentrations of these metals encountered in the haemolymph. Indeed, in circulatory fluid as haemolymph, turnover of metals is supposed to be rapid and by the way, metal concentrations relatively low, with the exception of Cu which is a main component of haemocyanin. It is the case for most of the trace elements analysed but not for Ni and Cr (Fig. 1). This supposes a high exposure of nautilus to these metals.

Concerning Fe, *N. macromphalus* exhibits higher concentrations than in cephalopods from temperate waters that could also indicate contamination (Table 2). Nevertheless, Fe concentrations are lower than in bivalve and gastropod species from contaminated areas and relatively low in haemolymph (Table 2).

Table 3 compares the trace element concentrations in the digestive gland of *N. macromphalus* with those reported for other cephalopod species. The relative weight of the digestive gland in our samples is higher than in most of Coleoidae species. Indeed, the digestive gland consists of 20 ± 5% of the fresh weight of the soft tissues of *N. macromphalus* while in Coleoidae, it represents 6–10% of the total body weight. Thus, when metals were highly concentrated in the digestive gland (e.g. Cd), metal concentrations in the whole individuals were proportionally higher than in other cephalopod species.

Ag, Cd, Co, Cr, Ni and V levels in the digestive gland of *N. macromphalus* are higher than those reported for *E. cirrhosa* and *S. officinalis* from the English Channel. Nevertheless, several squid species, i.e. *Loligo opalescens*, *Ommastrephes bartrami* and *Sthenoteuthis oualaniensis* from the Pacific Ocean, exhibit higher concentrations of Ag in their digestive gland (Martin and Flegal, 1975). These authors explain these very high Ag concentrations by the release of the metal in the Californian waters. This supposes very high ability of cephalopods to concentrate Ag. For Cd, these squids and the octopuses from Kerguelen Islands (i.e. *Graneledone* sp. and *B. thielei*) have far higher concentrations in the digestive gland than nautilus (Bustamante *et al*., 1998a). Although Cd concentrations in *N. macromphalus* were higher than in squids and cuttlefish from temperate waters (Table 3, Bustamante *et al*., 1998b), it does not indicate a contamination as they are of the same order of

TABLE 2

Trace element concentrations (μg g^{-1} dry wt) in molluscs from this study and from the literature.[a]

Class / *Species*	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	V	Zn	Authors
Cephalopods													
Eledone cirrhosa	0.76 ± 0.48	–	–	5.36 ± 1.09	0.45 ± 0.09	0.6 ± 0.1	122 ± 11	81 ± 12	1.9 ± 0.5	0.8 ± 0.1	1.0 ± 0.2	234 ± 20	Miramand and Bentley (1992)
Nautilus macromphalus	2.4 ± 0.6	19 ± 7	186 ± 18	16.0 ± 6.5	2.7 ± 1.2	2.6 ± 0.3	73 ± 20	258 ± 79	17 ± 6	6.7 ± 3.0	3.2 ± 1.1	260 ± 49	Present study
Octopus vulgaris	–	–	–	1.2 ± 0.1	–	–	260 ± 70	140 ± 10	5 ± 0.5	–	0.7 ± 0.1	150 ± 50	Miramand and Guary (1980)
Sepia officinalis	0.66 ± 0.01	–	–	1.34 ± 0.03	0.39 ± 0.07	1.0 ± 0.1	59 ± 1	43 ± 4	1.6 ± 0.1	0.4 ± 0.1	0.7 ± 0.2	134 ± 6	Miramand and Bentley (1992)
Bivalves													
*Cerastoderma edule**	0.11–6.5	–	–	0.48–1.04	1.28–2.93	1.34–2.46	5.2–27.2	406–991	6.2–44.6	34–62	–	46–66	Bryan and Hummerstone (1977)
*Macoma balthica**	19–128	–	–	0.85–0.21	3.7–6.8	1.89–3.30	96–615	502–1540	19–24	6.9–7.9	–	510–1160	Bryan and Hummerstone (1977)
*Mytilus edulis**	0.10–0.55	–	–	0.84–2.64	0.02–1.07	0.94–2.74	3.9–13.6	152–401	5.2–35.4	0.9–3.5	–	57–199	Bryan and Hummerstone (1977)
Pecten maximus	2.7	55	–	32.5	0.25 ± 0.09	1.3	8.9 ± 4.5	196 ± 83	107 ± 60	0.73 ± 0.25	–	273 ± 95	Bryan (1973)
*Scrobicularia plana**	0.23–1.2	–	–	0.29–14.9	4.3–66	1.2–2.2	25–86	699–1240	19–87	3.4–11.9	–	353–2940	Bryan and Hummerstone (1978)
*S. plana**	–	–	5–190	–	–	–	–	–	–	–	–	–	Langston (1980)
Gastropods													
*Littorina littorea**	3.2–73	–	–	0.49–2.56	0.79–3.04	0.13–0.98	62–194	272–784	18–133	2.2–4.1	–	45–284	Bryan and Hummerstone (1977)
*Patella vulgata**	1.5–6.0	–	–	3.3–21.5	0.24–1.56	0.48–2.62	10–27	891–2330	5.4–36.0	1.7–3.7	–	83–224	Bryan and Hummerstone (1977)

[a] Values are mean ± 1 SD, except from species marked * which are minimum and maximum values; * also indicates species from contaminated areas.

TABLE 3

Trace element concentrations (μg g^{-1} dry wt) determined in the digestive gland of cephalopods from this study and from the literature.

Species	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	V	Zn	Authors
Nautilus macromphalus	4.45 ± 2.68	32.1 ± 16.3	166 ± 26	45.1 ± 13.2	7.8 ± 3.6	4.4 ± 1.1	106 ± 46	666 ± 231	10.1 ± 1.7	16.3 ± 7.8	8.8 ± 2.0	672 ± 208	Present study
Sepia officinalis	6.15 ± 1.75			12.67 ± 0.35	3.27 ± 0.6	1.1 ± 0.1	315 ± 3	244 ± 28	3.3 ± 0.1	1.3 ± 0.4	5.0 ± 1.3	571 ± 47	Miramand and Bentley (1992)
Loligo opalescens	251.1 ± 12.6			85.0 ± 51.6			5350 ± 3210	111 ± 73				247 ± 131	Martin and Flegal (1975)
L. opalescens	45.9 ± 19.0			121.5 ± 57.9			8370 ± 3130	87 ± 49				449 ± 201	Martin and Flegal (1975)
Nototodarus gouldi				33 ± 30			363 ± 238					830 ± 355	Finger and Smith (1987)
N. gouldi	3.3 ± 1.4	7.7 ± 4.0		50 ± 25			246 ± 298	745 ± 440	4.2 ± 1.1			696 ± 295	Smith *et al.* (1984)
Ommastrephes bartrami	12.1 ± 8.6			287 ± 202			195 ± 212	399 ± 204				163 ± 55	Martin and Flegal (1975)
Stenoteuthis oualaniensis	24.1 ± 10.9			782 ± 255			1720 ± 151	319 ± 67				513 ± 288	Martin and Flegal (1975)
Eledone cirrhosa	3.20 ± 1.74			24.00 ± 1.75	2.06 ± 0.0	0.8 ± 0.1	456 ± 11	287 ± 13	4.2 ± 1.6	2.5 ± 0.1	3.3 ± 0.5	646 ± 86	Miramand and Bentley (1992)
Benthoctopus thielei				215			42					416	Bustamante *et al.* (1998a)
Graneledone sp.				369			1092					102	Bustamante *et al.* (1998a)
Octopus vulgaris							2550	1920					Ghiretti-Magaldi *et al.* (1958)
O. vulgaris				50 ± 10			2500 ± 700	700 ± 130	7.0 ± 0.5		4.5 ± 1.0	1450 ± 400	Miramand and Guary (1980)

magnitude as in octopus species from these areas. Concentrations of Cu, Fe, Mn and Zn in the digestive gland of *N. macromphalus*, all essential elements, are in the same order of magnitude as those of other cephalopod species. Thus, these elements are properly regulated in the digestive gland of *N. macromphalus* as in the other cephalopod species. Results for As appear to be the first reported for the cephalopod digestive gland. For Al, concentrations can only be compared with those in the squid *Nototodarus gouldi* (Smith *et al.* 1984) which are ranged from 1.5 to 20 $\mu g\ g^{-1}$ (Table 3).

Globally, concentrations of the toxic elements in the digestive gland of *N. macromphalus* are generally higher than those reported for cephalopods from the French coasts, i.e. *S. officinalis*, *E. cirrhosa* and *O. vulgaris* (Table 3).

In the Coleoidae species, branchial hearts have been reported to concentrate essential elements such as Fe and Cu (Fox and Updegraff, 1943; Ghiretti-Magaldi *et al.*, 1958; Nardi and Steinberg, 1974; Schipp and Hevert, 1978; Miramand and Bentley, 1992). These organs also concentrate toxic and radioactive elements such as ^{241}Am, Co, Ni, $^{239-240}$Pu and V (Ueda *et al.*, 1979; Nakahara *et al.*, 1979; Miramand and Guary, 1980; Guary *et al.*, 1981; Guary and Fowler, 1982; Miramand and Bentley, 1992). Moreover, kidneys of cephalopods have been shown to store Cd, Cu, Fe, Mn, Ni and Pb (Miramand and Guary, 1980; Miramand and Bentley, 1992).

High concentrations of trace elements (Ag, Al, As, Cr, Fe, Ni and Mn) were found in organs concerned with excretion, i.e. the pericardial and renal appendages (Fig. 1). These results, and those concerning the digestive gland, suggest that these metals could follow another way of detoxification in nautilus than in the few Coleoidae species studied. The digestive gland would assume mainly the detoxification of metals such as Cd, V or Zn. The hypothesis is strengthened by the trace element levels in the circulatory fluid. Indeed, as the food can be considered as the main source of metals in cephalopods since they are carnivorous, the haemolymph would be a major vector for metal distribution among the different tissues. Metal levels in the haemolymph of *N. macromphalus* showed relatively low concentrations for most elements, except for Cu which is present in haemocyanin. This is particularly clear for Cd, V and Zn which exhibited the lowest concentrations in the haemolymph (Fig. 1).

The other tissues store trace element in much lower amounts than the digestive gland, the renal and the pericardial appendages. Nevertheless, muscles which generally exhibit the lowest metal concentrations, showed the highest As concentrations. This could be due to high consumption of crustaceans which are particularly rich in As.

Investigations on trace element concentrations in the tissues have highlighted the key role of the digestive gland of nautilus in the metabolism of metals, as is also the case in Coleoidae. This organ is indeed the major site of concentration of Cd, Co, Fe, V and Zn and, compared to other tissues, it shows also high levels of Ag, As, Cr, Cu and Ni (Fig. 1). The digestive gland contains the highest percentage of metals, with the exception of As and Mn (Fig. 2). Miramand and Bentley (1992) have classified some trace elements based on the ratio between the concentration in the digestive gland and in the muscle. Thus, in the digestive gland, three groups of elements can be evidenced in *N. macromphalus* : poorly concentrated elements, i.e. Al, As, Cr, Cu and Mn (ratio <10), moderately concentrated elements, i.e. Fe, Ni and Zn (ratio >10 to <50) and highly concentrated elements, i.e. Ag, Cd, Co and V (ratio >50). Similar ratios were found in Coleoidae (i.e. *S. officinalis* and *E. cirrhosa*) for Cr, Mn, Fe, Cd and Ag. On the contrary, V and Co are highly concentrated in the digestive gland of *Nautilus* whereas they are respectively poorly and moderately concentrated in Coleoidae. In the same way, Ni and Zn are moderately concentrated in the digestive gland of *Nautilus* but only poorly concentrated in *S. officinalis* and *E. cirrhosa*. High levels and storage of toxic metals in the digestive gland suggest efficient detoxification processes in this organ. Moreover, these high levels of toxic elements do not apparently disturb essential elements metabolism. Thus, it would be of particular interest to study detoxification processes in the digestive gland of nautilus.

Conclusion

Previous studies have demonstrated the ability of cephalopods to concentrate many trace elements. Data from nautilus species were particularly interesting on an evolutionary point of view. Indeed, as several elements are considered to be toxic for biota, marine animals would evolve to counteract their toxicity. It seems to be the case in cephalopods as they are able to grow and reproduce with very high metal concentrations. In their case, detoxification strategy involves storage mechanisms of these elements. This strategy appears to be efficient, and probably applied to minimize energetic cost, and is common among Nautilidae and Coleoidae cephalopods.

Enrichment of metals in New Caledonian waters appears to not be limited to coastal waters. Indeed, contamination by Co, Cr and Ni is supported: (1) by high levels in whole animals which are of the same order of magnitude than in bivalves and gastropods from contaminated areas, (2) by relatively high levels in excretory organs and in haemolymph that indicate a high exposure to these metals, (3) by higher ratios between concentrations in the muscle and in the digestive gland that show an enrichment in this organ. Further studies are needed to evaluate the scale of the contamination by these metals and possible effects on the ecosystems.

We thank Pascale Joannot, director of the Nouméa Aquarium, the ORSTOM Center of Nouméa and the crew of the O.V. Dawa for providing the nautilus specimen. This work was supported financially by the "Conseil Régional Poitou-Charentes" and Rhodia (Rhône-Poulenc).

Amaratunga, T. (1983) The role of cephalopods in the marine ecosystem. Advances in assessment of world cephalopod resources. In *FAO Fisheries Technical Paper* ed. IF Caddy, vol. 231, pp. 379–415.

Barghigiani, C., D'Ulivo, A., Zamboni, R. and Lampugnani, L. (1993) Interaction between selenium and cadmium in *Eledone cirrhosa* of the Northern Tyrrhenian Sea. *Marine Pollution Bulletin* **26** (4), 212–216.

Bryan, G. W. (1973) The occurrence and seasonal variation of trace metals in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.). *Journal of the Marine Biological Association of the United Kingdom* **53**, 145–166.

Bryan, G. W. (1976) Heavy metal contamination in the sea. In *Marine Pollution*, ed. R. Jonhston, pp. 185–302. Academic Press.

Bryan, G. W. and Hummerstone, L. G. (1977) Indicators of heavy metal contamination in the Looe estuary (Cornwall) with particular regard to silver and lead. *Journal of the Marine Biological Association of the United Kingdom* **57**, 75–92.

Bryan, G. W. and Hummerstone, L. G. (1978) Heavy metals in the burrowing bivalve *Scrobicularia plana* from contaminated and uncontaminated estuaries. *Journal of the Marine Biological Association of the United Kingdom* **58**, 401–419.

Bustamante, P. (1998) Etude des processus de bioaccumulation et de détoxication d'éléments traces (métaux lourds et Terres Rares) chez les mollusques céphalopodes et bivalves pectinidés. Implication de leur biodisponibilité pour le transfert vers les prédateurs. Thesis, University of La Rochelle, 290 p.

Bustamante, P., Cherel, Y., Caurant, F. and Miramand, P. (1998a) Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. *Polar Biology* **19**, 264–271.

Bustamante, P., Caurant, F., Fowler, S. W. and Miramand, P. (1998b) Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *The Science of the Total Environment* **220**, 71–80.

Decleir, W., Vlaeminck, A., Geladi, P. and Van Grieken, R. (1978) Determination of protein-bound copper and zinc in some organs of the cuttlefish *Sepia officinalis* L. *Comparative Biochemistry and Physiology* **60B**, 347–350.

Finger, J. M. and Smith, J. D. (1987) Molecular association of Cu, Zn, Cd and ^{210}Po in the digestive gland of the squid *Nototodarus gouldi*. *Marine Biology* **95**, 87–91.

Fox, D. L. and Updegraff, D. M. (1943) Adenochrome a glandular pigment in the branchial hearts of the octopus. *Archieves of Biochemistry* **1**, 339–356.

Froesch, D. and Packard, A. (1979) Octopus chromatophores accumulate nickel. *Experientia* **35**, 828–829.

Ghiretti-Magaldi, A., Giuditta, A. and Ghiretti, F. (1958) Pathways of terminal respiration in marine invertebrates. I. The respiratory system in cephalopods. *Journal of Cellular and Comparative Physiology* **52**, 389–429.

Guary, J. C., Higgo, J. J. W., Cherry, R. D. and Heyraud, M. (1981) High concentrations of transuranic and natural radioactive elements in the branchial hearts of the cephalopods *Octopus vulgaris*. *Marine Ecology and Progressive Series* **4**, 123–126.

Guary, J. C. and Fowler, S. W. (1982) Experimental studies on the biokinetics of plutonium and americium in the cephalopod *Octopus vulgaris*. *Marine Ecology and Progressive Series* **7**, 327–335.

Langston, W. J. (1980) Arsenic in U.K. estuarine sediments and its availability to benthic organisms. *Journal of the Marine Biological Association of the United Kingdom* **60**, 869–881.

Martin, J. H. and Flegal, A. R. (1975) High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. *Marine Biology* **30**, 51–55.

Miramand, P. and Bentley, D. (1992) Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis*, from the French coast of the English Channel. *Marine Biology* **114**, 407–414.

Miramand, P. and Guary, J. C. (1980) High concentrations of some heavy metals in tissues of the Mediterranean octopus. *Bulletin of Environmental and Contamination Toxicology* **24**, 783–788.

Monniot, F., Martoja, R. and Monniot, C. (1994) Cellular sites of iron and nickel accumulation in ascidians related to the naturally and anthropic enriched New Caledonian environment. *Annales de l'Institut Océanographique* **70** (2), 205–216.

Nakahara, M., Koyanagi, T., Ueda, T. and Shimizu, C. (1979) Peculiar accumulation of cobalt-60 by the branchial hearts of Octopus. *Bulletin of Japanese Society of Scientific Fish* **45**, 539.

Nardi, G. and Steinberg, H. (1974) Isolation and distribution of adenochrome (s) in *Octopus vulgaris*. *Comparative Biochemistry and Physiology* **48B**, 453–461.

Renzoni, A., Bacci, E. and Falciai, L. (1973) Mercury concentration in the water, sediments and fauna of an area of the Tyrrhenian coast. *Revue Internationale d'Océanographie Médicale* 31–32, 17–45.

Rocca, E. (1969) Copper distribution in *Octopus vulgaris* Lam. hepatopancreas. *Comparative Biochemistry and Physiology* **28**, 67–82.

Rodhouse, P. G. (1989) Antarctic cephalopods- a living marine resource ? *Ambio* **18** (1), 56–59.

Rossi, A., Pellegrini, D., Belcari, P. and Bavghigiani, C. (1993) Mercury in *Eledone cirrhosa* from the Northern Tyrrhenian Sea: contents and relation with life cycle. *Marine Pollution Bulletin* **26** (12), 683–686.

Schipp, R. and Hevert, F. (1978) Distribution of copper and iron in some central organs of *Sepia officinalis* (Cephalopoda). A comparative study by flamless atomic absorption and electron microscopy. *Marine Biology* **47**, 391–399.

Smith, J. D. (1983) Radio-nuclides and heavy metals in *Nototodarus gouldi*. *Memoirs of the National Museum Victoria* **44**, 201–203.

Smith, J. D., Plues, L., Heyraud, M. and Cherry, R. D. (1984) Concentrations of the elements Ag, Al, Ca, Cd, Cu, Fe, Mg, Pb and Zn, and the radionuclides ^{210}Pb and ^{210}Po in the digestive gland of the squid *Nototodarus gouldi*. *Marine Environmental Research* **13**, 55–68.

Ueda, T., Nakahara, M., Ishii, T., Suzuki, Y. and Suzuki, H. (1979) Amounts of trace elements in marine cephalopods. *Journal of Radiation Research* **20**, 338–342.

Yamada, H., Takayanagi, K., Tateishi. M., Tagata, H. and Ikeda, K. (1997) Organotin compounds and polychlorinated biphenyls of livers in squid collected from coastal waters and open ocean. *Environmental Pollution* **96** (2), 217–226.