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Interactions of metallic elements and organisms within hydrothermal vents

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Abstract: This study investigated the bioaccumulation of trace elements in species living within deep-sea hydrothermal vents. Trace elements were quantified in whole organs known to be involved in exchanges with the surrounding medium or in detoxification processes. The binding of heavy metals to specific metalloproteins was studied as well as their balance within subcellular fractions. Sequestration of trace elements under insoluble forms appears to be the major adaptive pathway for polychaetes. Crustaceans show a high capacity for metallothionein induction, as demonstrated by quantitative analyses.

Résumé : *Interactions entre éléments métalliques et organismes au niveau des sources hydrothermales océaniques.*
Le but de notre travail était de mettre en évidence la bioaccumulation des éléments traces chez différentes espèces inféodées aux sources hydrothermales profondes. Ces éléments traces ont été quantifiés dans des organes impliqués dans les échanges avec le milieu environnant ou dans les mécanismes de détoxication. La répartition des métaux entre les fractions subcellulaires et leur association avec des métalloprotéines spécifiques ont été étudiées. Le stockage des éléments traces sous des formes insolubles semble prédominer chez les polychètes. Les crustacés sont connus pour leur forte capacité à synthétiser des métallothionéines. Cette caractéristique est retrouvée dans cette étude via la quantification de ces protéines chez les crustacés hydrothermaux.

Keywords : Trace elements, Hydrothermal vents, Alvinellids, *Bythograea*, Metallothioneins.

Introduction

Our knowledge of biological communities within deep-sea hydrothermal vents has progressed considerably since their discovery (Corliss *et al.*, 1979). In particular, high concentrations of metals within these organisms have been found to bioaccumulate from vent fluids enriched through leaching out of magmatic rocks (Edmond and Von Damm, 1983, 1985). In this context, heavy metal detoxification is generally achieved by two major mechanisms: metallic ions are either trapped in insoluble granules or bound to specific metalloproteins whose synthesis is triggered by the intracytoplasmic level of heavy metals (Viarengo and Nott, 1993). Both detoxification mechanisms have been found in hydrothermal vent organisms with high tissue levels of heavy metals (Gaill *et al.*, 1984; Chassard-Bouchaud *et al.*, 1988; Cosson-Mannevy *et al.,* 1986, 1988). Metallothioneins are low-molecular-weight (6,000 Da) sulphur-rich proteins clearly implicated in heavy metal detoxification (Cosson *et al.*, 1991; Roesijadi, 1992, 1993), whose highest concentrations have generally been observed within tissues or organs characterized by the presence of symbionts. In a recent work, Jeanthon (1991) described several free bacterial strains collected in the vicinity of hydrothermal vents, which are able to accumulate and tolerate heavy metals. However, the implication of bacteria in heavy metal detoxification has not been clearly established.

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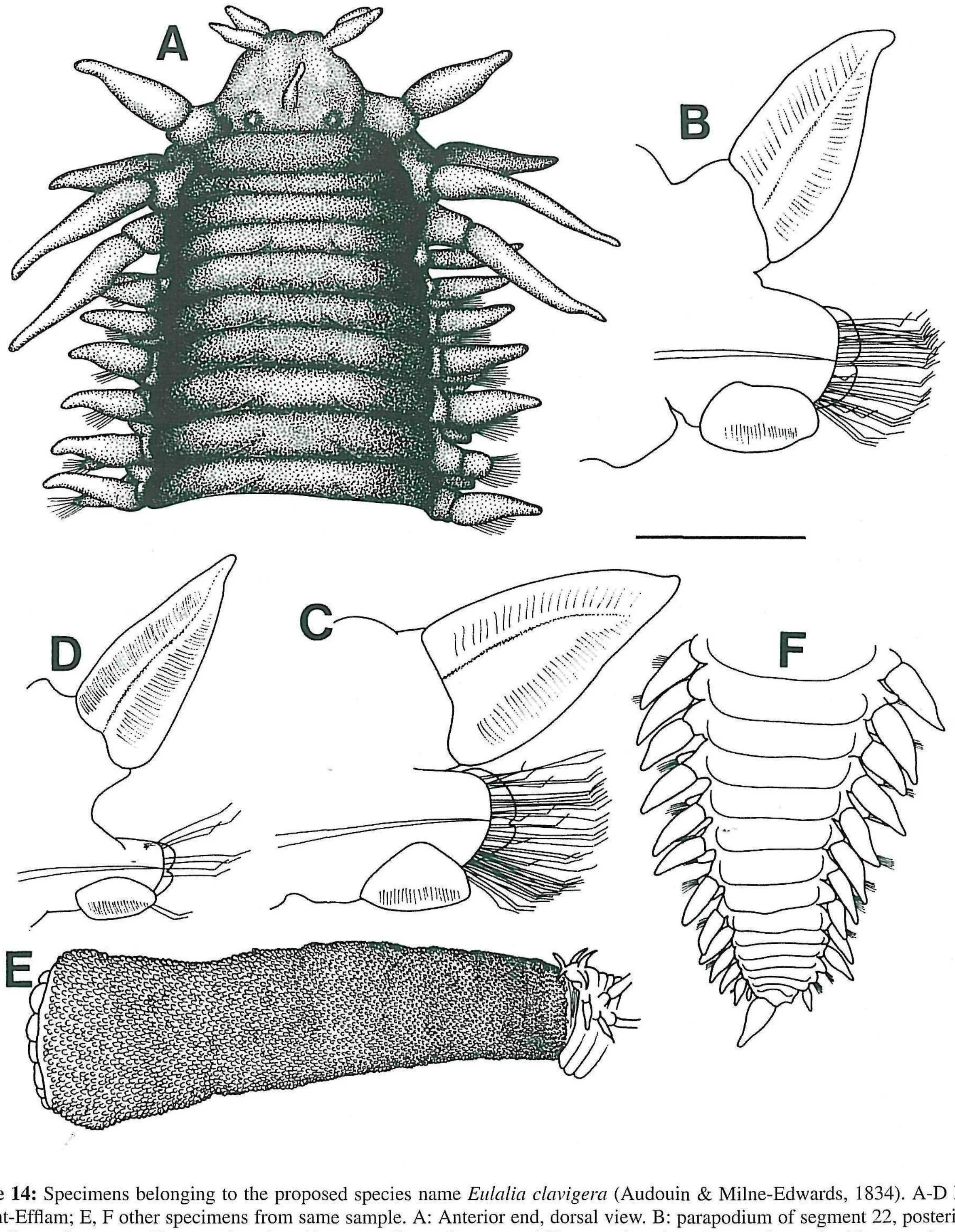

Figure 14: Specimens belonging to the proposed species name *Eulalia clavigera* (Audouin & Milne-Edwards, 1834). A-D Neotype from Saint-Efflam; E, F other specimens from same sample. A: Anterior end, dorsal view. B: parapodium of segment 22, posterior view. C: parapodium of segment 59, posterior view. D: parapodium of segment 108, posterior view. E: anterior end with everted proboscis, ventrolateral view. F: posterior end with anal cirri, dorsal view (right anal cirrus missing). Scale = 1 mm (A, F), 0.5 mm (B-D), or 4 mm (E).

Figure 14 : Spécimens attribués à l'espèce proposée *Eulalia clavigera* (Audouin & Milne-Edwards, 1834). A-D Neotype de Saint-Efflam ; E, F autres spécimens du même échantillon. A : extrémité antérieure, vue dorsale. B : parapode du segment 22 vue postérieure. C : parapode du segment 59, vue postérieure. D : parapode du segment 108, vue postérieure. E : extrémité antérieure avec la trompe sortie, vue ventro-latérale. F: extrémité postérieure avec un cirre pygidial, vue dorsale, (le cirre droit manquant). Echelle = 1 mm (A, F), 0.5 mm (B-D) ou 4 mm (E).

Table 1. Elemental composition of various Polychaetous annelids determined by atomic absorption spectrophotometry. Individuals (rinsed or not) or anatomic parts were pooled. The number of aliquots analysed from the same pool is given (n). The standard deviation (± S.D.) refers to the mean level of analysed aliquots. Elemental levels are expressed as $\mu g.g^{-1}$ dry weight.

Tableau 1. Composition en éléments de différents annélides polychètes déterminée par spectrophotométrie d'absorption atomique. Les individus entiers (rincés ou non) ou les différentes parties anatomiques ont été groupés. Le nombre d'aliquots analysés à partir des échantillons groupés est indiqué (n). L'écart-type (± S.D.) se rapporte à la moyenne des teneurs des aliquots analysés. Les teneurs en éléments sont exprimées en µg de métal par g de poids sec.

Element	*Alvinella pompejana*		*Alvinella caudata*			*Paralvinella grasslei*	HESIONIDAE		POLYNOIDAE
tissue	anterior parts (rinsed)	digestive tract plus gut contents	anterior parts (rinsed)	digestive tract plus gut contents	rinsed whole bodies	whole bodies plus mucus	rinsed whole bodies	not rinsed whole bodies	rinsed whole bodies
(n)	(2)	(3)	(2)	(1)	(2)	(3)	(3)	(3)	(3)
Cu	189 ± 4	577 ± 38	140 ± 7	93	108 ± 8	73 ± 8	116 ± 17	1137 ± 241	26 ± 4
Zn	1103 ± 680	16501 ± 474	7860 ± 329	7445	13780 ± 765	1552 ± 91	5997 ± 347	38602 ± 1454	5317 ± 131
Cd	22 ± 1	18 ± 1	13.7 ± 1.0	8.2	10.3 ± 1	8.6 ± 0.5	11.2 ± 1.0	38 ± 2	6.4 ± 0.1
Hg	131 ± 11	4.2 ± 1.0	89 ± 4	7.4	3.6 ± 1	73 ± 6	4.6 ± 1.0	7.4 ± 0.2	4.9 ± 0.2
As	3389 ± 103	92 ± 2	2288 ± 18	67	81 ± 2	1052 ± 15	20 ± 2	89 ± 2	38 ± 3

obvious. Hesionids, which are polychaetes not bearing symbiotic bacteria, are often found on smoker walls, whereas polynoids and alvinellids (especially *P. grasslei*) are more abundant within *Riftia pachyptila* bunches. With respect to Alvinellids, the zinc level in *P. grasslei* was lower than in other polychaetes, whereas mercury and arsenic levels were high. Juniper *et al.* (1986) have suggested that the mucus surrounding the body of *P. grasslei* plays a role in heavy metal detoxification processes. As we did not separate mucus from the body, our results may reflect the occurrence of trapped metallic particles within the mucus.

Pompei worms (*Alvinella pompejana* and *A. caudata*) harbouring epibiotic bacteria were sampled on the smokers. Analysis of the rinsed whole body of *A. caudata* indicated that metal concentrations were similar to those observed in the hesionid rinsed pool, except for arsenic and zinc which had higher levels. These polychaetes, which live very close to vents, are exposed to a continuous flow of particles from the precipitation of metallic sulphides in hydrothermal fluids. Essential metals (Cu, Zn) were equally *(A. caudata)* or more abundant *(A. pompejana)* in the digestive tract (gut wall and content) than in the anterior part (gills, feeding tentacles and first segments), which was directly exposed to mineral particles and thus contained more toxic metals (As, Hg). This condition noted in a previous study (Cosson-Mannevy *et al.*, 1989) could be explained by two non-exclusive hypotheses. First, as pompeii worms are known to "utilize their retractable grooved buccal tentacles to seize food particles and to move them to the mouth" (Desbruyères *et al.*, 1985), they may be able to identify and sort the particles, thereby discarding toxic metal-rich particles. Secondly, as the samples were rinsed, metal-rich particles may have been partially washed out from the surface of the anterior part but persisted within the digestive tract since the worms could not be starved prior to metal analyses.

Compartmentation of metals: Intracellular balance (Table 2).

Insoluble forms were predominant for copper, zinc and cadmium in all studied body parts. For *P. grasslei*, ratios of cytosolic zinc were higher than those of cytosolic copper, whereas this was true only for the ventral body wall of *A. pompejana*. When cadmium was detected, it was associated essentially with insoluble compounds.

Association with proteins within the cytosol (Table 3).

Tissues of *A. pompejana* displayed differences concerning the balance of soluble copper between heat-stable and heat-denaturable compounds. The anterior part and the ventral body wall exhibited a preferential association of cytosolic copper with thermolabile compounds. Conversely, cytosolic copper was mainly associated with heat-stable compounds within the digestive tract and dorsal body wall of *A. pompejana* and within all studied parts of *P. grasslei.* Cytosolic zinc and cadmium (when detected, data not shown) were associated essentially with heat-stable compounds for both species.

Abundance of metallothioneins (Table 4).

The dorsal body wall of *A. pompejana*, which bears epibiotic bacteria, was classified as the richest MT-containing tissue in an earlier study (Cosson-Mannevy *et al.*, 1986). Our findings here show that the ventral body wall had an MT level 3 to 6 times as high as that of the other

Table 2. Ratios of metal associated with the insoluble compounds within homogenates of alvinellid tissues. Results are expressed as percentages of the total amount of metal in the tissue. n. d.: not determined.

Tableau 2. Proportions de métal associé aux composés insolubles dans des homogénats de tissus d'avinellidés. Les résultats sont exprimés en pourcentages de la quantité totale de métal présente dans le tissu analysé. n. d. : non déterminé.

	Alvinella pompejana				*Paralvinella grasslei*		
	rinsed anterior parts	full digestive tract	rinsed ventral body wall	rinsed dorsal body wall	rinsed anterior part	full digestive tract	rinsed remainders
Copper	93	92	88	89	97	96	81
Zinc	80	99.5	80	93	82	84	74
Cadmium	99.6	99.8	90	n.d.	99.5	99.7	n.d.

Table 3. Ratios of metals associated with the heat-stable soluble compounds within homogenates of alvinelid tissues. Results are expressed as percentages of the total amount of metal within the cytosol (heat- stable + heat-denaturable compounds). All parts except digestive tracts were rinsed.

Tableau 3. Proportions de métal associé aux composés solubles thermostables au sein d'homogénats de tissus d'alvinellidés. Les résultats sont exprimés en pourcentage de la quantité totale de métal présente dans le cytosol (composés thermostables + composés thermolabiles). Tous les tissus sauf les tubes digestifs ont été rincés.

	Alvinella pompejana				*Paralvinella grasslei*		
	anterior parts	full digestive tract	ventral body wall	dorsal body wall	anterior parts	full digestive tract	remainders
Copper	24	80	37	76	92	81	77
Zinc	62	87	88	95	99	94	86

Table 4. Levels of total proteins, heat-stable proteins (HSP) and metallothioneins within alvinellids, expressed as mg.g-1 wet weight. The ratios of HSP vs total proteins, of metallothioneins (MT) vs total proteins, and of MT vs HSP are given as percentages. Two aliquots of pooled anatomic parts were analysed. The standard deviation (± S.D.) refers to the mean level of two analysed aliquots. All parts except digestive tracts were rinsed.

Tableau 4. Teneurs en protéines totales, protéines thermostables (HSP) et métallothionéines (MT) chez les alvinellidés, exprimées en mg.g^{-1} de poids frais. Les rapports de HSP aux protéines totales, de MT aux protéines totales et de MT aux HSP sont données en pourcentages. Deux aliquots d'échantillons groupés ont été analysés. L'écart-type (± S.D.) est relatif à la moyenne des deux aliquots analysés. Tous les tissus sauf les tubes digestifs ont été rincés.

	Alvinella pompejana				*Paralvinella grasslei*		
	anterior parts	full digestive tract	ventral body wall	dorsal body wall	anterior part	full digestive tract	remainders
Total Proteins	41.10 ± 0.02	7.5	13.3 ± 4.5	11.5 ± 0.2	35.2 ± 19.2	59.5	14.3 ± 3.4
Heat-Stable Proteins: HSP	7.34 ± 0.00	6.29	11.33 ± 3.83	3.91 ± 0.08	9.81 ± 5.35	10.0	6.0 ± 1.4
% vs tot. proteins	18	84	85	34	28	17	42
Metallothioneins	0.58 ± 0.00	0.337 ± 0.019	1.562 ± 0.148	0.262 ± 0.027	0.361 ± 0.103	0.518	0.364 ± 0.060
% vs tot. proteins	1.41	4.340	12.27	2.28	1.02	0.87	2.54
% vs HSP	7.90	5.36	13.79	6.70	3.68	5.19	6.09

tissues, which had levels of the same order of magnitude as those recorded in the earlier study. The anterior part displayed the highest level of total proteins, and the lowest level of MT when expressed relative to total protein content. The ventral body wall had the highest levels of heat-stable proteins and MT when expressed either in mg.g^{-1} ww. or in percentage versus total protein content. These last observations relate to the relative abundance of cytosolic zinc within these anatomic parts and the preferential association of zinc with heat-stable ligands.

In *P. grasslei*, the full digestive tract exhibited the highest MT level expressed with regard to wet weight, whereas the remainders had the highest level when MT was expressed versus total or versus heat-stable protein content. The remainders also possessed the lowest total protein level and the highest percentage of heat-stable proteins.

In addition to the low ratio of cytosolic metals observed in studied polychaetes, there was an important variation in metal balance among soluble intracellular ligands. The association of metal with heat-stable or heat-denaturable compounds differed according to the species and organs considered.

Crustaceans

Bioaccumulation of metals: Organotropism (Table 5).

Zinc levels were very high within the gill of *Bythograea thermydron*, and the highest level of copper was found within the digestive gland. Arsenic levels in both organs were moderate, as previously observed within tissues of the vestimentiferan tubeworm *Riftia pachyptila* (Cosson, 1996), but lower than those detected within alvinellid full digestive tract or annelid whole body, and considerably below those found in alvinellid anterior body parts. The high amount of copper in the digestive gland of *B. thermydron* can be related to the presence of both copper sulphur-containing granules accumulated within particular cells and haemocyanin. It has been reported that haemocyanin could represent 50% of the proteins synthesized in this organ and account for > 90% of haemolymph proteins (Viarengo and Nott, 1993; Brouwer *et al.*, 1995).

Compartmentation of metals: Intracellular balance (Table 6).

Copper and cadmium were very abundant in digestive gland cytosol, whereas zinc was associated with insoluble ligands. Within the gills, trace metals (Cd, Cu, Zn) were preferably bound to insoluble ligands.

Association with proteins within the cytosol (Table 7).

The high ratio of cytosolic metals bound to heat-stable compounds within the digestive gland was remarkable. Within the gill, copper was the only metal preferably bound to this class of compounds.

Table 5. Elemental composition of *Bythograea thermydron* tissues determined by AAS, and values determined for a coastal crab (Engel and Brouwer 1984). Levels are expressed as µg.g^{-1} dry weight. Twenty animals were pooled (n=20). The standard deviation (± SD) refers to the analyses of three aliquots from the pool.

Tableau 5. Composition en éléments de *Bythograea thermydron* déterminée par AAS et teneurs rencontrées chez un crabe côtier (Engel and Brouwer 1984). Les teneurs sont exprimées en µg.g^{-1} de poids sec. L'écart-type (± SD) correspond à la teneur moyenne obtenue par l'analyse de trois aliquots de vingt individus regroupés en un seul échantillon.

Element	*Bythograea thermydron*		*Callinectes sapidus*	
	gills	digestive gland	gills	digestive gland
Cu	493 ± 18	683 ± 16	76	246
Zn	1356 ± 69	442 ± 19	15	40
Cd	7.6 ± 0.2	3.8 ± 0.4	0.3	1
Hg	28.9 ± 1.3	1.6 ± 0.3		
As	14.6 ± 1.5	33 ± 1.3		

Table 6. Ratios of metal associated with insoluble compounds within homogenates of crab tissues. Results are expressed as percentages of the total amount of metal in the tissue.

Tableau 6. Proportions de métal associé aux composés insolubles dans des homogénats de tissus de crabe. Les résultats sont exprimés en pourcentages de la quantité totale de métal présente dans le tissu analysé.

	Bythograea thermydron	
	gills	digestive Gland
Copper	65	7
Zinc	87	67
Cadmium	60	37

Abundance of metallothioneins (Table 8).

There was a great difference in protein amounts between the gills and the digestive gland. Total and heat-stable protein levels were lower in the gills than in the digestive gland, whereas the ratio of heat-stable proteins to total proteins was higher. MT level was 2 to 10 times as high in the digestive gland as in the gills, depending on the units employed (respectively mg MT.g^{-1} of total cytosolic proteins and mg MT.g^{-1} ww). The differences between gill and digestive gland metal metabolism have already been pointed out (Brouwer *et al.*, 1984; Engel *et al.*, 1985). Our results emphasize the importance of the digestive gland with respect to copper metabolism and MT synthesis (Brouwer *et al.*, 1989). The levels of total or heat-stable proteins within

Table 7. Ratios of metal associated with the heat-stable soluble compounds within homogenates of crab tissues. Results are expressed as percentages of the total amount of metal within the cytosol (heat-stable + heat-denaturable compounds).

Tableau 7. Proportions de métal associé aux composés solubles thermostables au sein d'homogénats de tissus de crabe. Les résultats sont exprimés en pourcentage de la quantité totale de métal présente dans le cytosol (composés thermostables + composés thermolabiles).

	Bythograea thermydron	
	gills	digestive gland
Copper	90	98
Zinc	21	92
Cadmium	23	88

Table 8. Levels of total proteins, heat-stable proteins (HSP) and metallothioneins (MTs) of *Bythograea thermydron* tissues, expressed as $mg.g^{-1}$ wet weight. Ratios are expressed as percentages of HSP vs total proteins, of MTs vs total proteins, and of MTs vs HSP. Six animals were pooled (n=6). The standard deviation (±SD) refers to the analyses of three aliquots from the pool.

Tableau 8. Teneurs en protéines totales, protéines thermostables (HSP) et métallothionéines (MTs) des tissus de *Bythograea thermydron* exprimées en $mg.g^{-1}$ de poids frais. Les proportions de HSP par rapport aux protéines totales, de MTs par rapport aux protéines totales et de MTs par rapport aux HSP sont données en pourcentages. Trois aliquots d'échantillons groupés (n = 6) ont été analysés. L'écart-type (± S.D.) est relatif à la moyenne des trois aliquots analysés.

	Bythograea thermydron	
	gills	digestive gland
Total Proteins	10.96 ± 0.54	48.01 ± 13.30
Heat-Stable Proteins: HSP	9.67 ± 0.48	17.80 ± 4.94
% vs tot. proteins	88.27 ± 10.80	37.07 ± 9.10
MTs	0.24 ± 0.03	2.33 ± 0.51
% vs tot. proteins	2.19 ± 0.40	4.93 ± 0.58
% vs HSP	2.47	13.07

Bythograea thermydron tissues were higher than those found in other vent organisms.

Comparisons between the trace element levels of hydrothermal vent organisms and those of similar coastal organisms, used as indicators of trace metal contamination, have already been established. The hydrothermal clam, *Calyptogena magnifica*, exhibited metal body burdens considered to be very high and potentially toxic in other related species (Roesijadi and Crecelius, 1984). The levels of several trace elements in molluscs (the mussel *Bathymodiolus thermophilus* and the limpet *Neomphalus fretterae*) were comparable to or slightly higher than baseline levels in related intertidal species (Smith and Flegal, 1989). The highest concentrations encountered in coastal annelids (*Nereis diversicolor* and *Nephthys hombergii*) from polluted areas (Bryan *et al.*, 1985) were lower than those of some elements (As, Cd, Fe, Hg, Mn, Zn) found in alvinellids, *Alvinella caudata* and *Paralvinella grasslei* (Cosson-Mannevy *et al.*, 1989).

Prior to our work, no data were available concerning the levels and intracellular balance of heavy metals within *Bythograea thermydron*, although a considerable body of information existed on metal metabolism within crustaceans (Bryan *et al.*, 1985). Copper and zinc levels were found to be higher in *B. thermydron* than in *Callinectes sapidus* (Engel and Brouwer, 1984) for specimens collected from sites with elevated trace metal levels, although the level of cadmium was lower in *B. thermydron* digestive gland. However it is generally acknowledged that bioaccumulation of metal within organisms depends on several parameters, such as bioavailability, ambient concentration, uptake route, and physiological status, so that comparisons between coastal and hydrothermal organisms are only relative.

Our study was particularly concerned with the essential or toxic bioaccumulation of trace elements by hydrothermal vent organisms. Little is known about the duration of vent activity, and it is very difficult to determine the age of the organisms living in such ecosystems. Volume, intensity and the direction of hot vent mineral discharges in the surrounding medium are extremely difficult to determine. It was impossible to discriminate between samples of the same species collected at different locations at the same site because all were brought to the surface in the same container. This procedure precluded the establishment of any relationship between individual metallic impregnation and corresponding chemical or spatial parameters. Recently, 150-ml sampling bottles were developed and then tested during the HOT 96 French cruise at the E.P.R. 13°N by collecting water samples around organisms (Sarradin *et al.*, pers. com.). This procedure, together with a method of collecting corresponding organisms within individual containers, will be used to assess the relationship between the concentration of metals in seawater and their bioaccumulation.

The sequestration of trace elements under insoluble forms seems to be the major adaptive pathway for annelids. Metal detoxification and storage in membrane-bound vesicles in epidermal cells are well-documented for invertebrates (Bryan, 1984; Viarengo and Nott, 1993). Thus, the existence of such a detoxification process within hydrothermal fauna is not surprising.

The occurrence of low-molecular-weight cadmium- or copper-binding proteins in several annelids has already been reported (Kägi and Kojima, 1987). However, unlike the case of the oligochaete *Eisenia foetida* (see Suzuki *et al.*, 1980; Yamamura et al. 1981), these proteins did not possess the mammalian MT characteristics defined by Kägi and Nordberg (1979). MT isolated from *A. pompejana* and *R. pachyptila* have been partially characterized (Cosson-Mannevy *et al.*, 1986), but their amino acid composition or gene sequences are still unknown.

Crustaceans, in addition to metal excretion via molting, (Carney *et al.*, 1988; Martin, 1980; White & Rainbow, 1984) are known for their high MT induction capacity (Engel, 1987), a feature demonstrated by our MT quantitative analyses performed on hydrothermal vent crustaceans.

Investigations using the tools of biochemistry and molecular biology are now in progress to isolate and characterize metallothioneins within hydrothermal vent organisms.

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