



Trace elements and arsenic speciation in tissues of tube dwelling polychaetes from hydrothermal vent ecosystems (East Pacific Rise): An ecological role as antipredatory strategy?



Marta Di Carlo^a, Donato Giovannelli^{b,c,d}, Daniele Fattorini^{a,f}, Nadine Le Bris^e, Costantino Vetriani^b, Francesco Regoli^{a,f,*}

^a Dipartimento di Scienze della Vita e dell'Ambiente (DISVA), Università Politecnica delle Marche (UNIVPM), Ancona, Italy

^b Institute of Earth, Ocean and Atmospheric Sciences, Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ, USA

^c Istituto di Scienze Marine (ISMAR), Consiglio Nazionale delle Ricerche (CNR), Ancona, Italy

^d Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan

^e Sorbonne Universités, UPMC Univ. Paris 6, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques, Observatoire Océanologique, 66650 Banyuls-sur-Mer, France

^f CoNISMa, Consorzio Nazionale Interuniversitario Scienze del Mare, Italy

ARTICLE INFO

Keywords:

Hydrothermal vent system
Polychaetes
Bioaccumulation
Arsenic chemical speciation
Trace elements
Adaptation

ABSTRACT

Hydrothermal vent systems are inhabited by dense benthic communities adapted to extreme conditions such as high temperature, hydrogen sulphide (H₂S) and elevated fluxes of metals. In the present work, a wide range of trace elements (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, V and Zn) were measured in tissues of three tube dwelling annelids, *Alvinella pompejana*, *Alvinella caudata* and *Riftia pachyptila*, which colonize distinct habitats of the East Pacific Rise (EPR) at 2500 m depth. Metals concentrations in alvinellids were often 2–4 orders of magnitude higher than those commonly found in marine organisms, while much lower values were observed in the vestimentiferan polychaete. Mobility of trace elements was further characterized in tissues of *A. pompejana* where metals appeared mostly in insoluble forms, i.e. associated with hydrated oxides and sulphides. Arsenic was mainly present in a weakly insoluble form and with concentrations in the branchial tentacles of alvinellids, approximately 5–15 fold higher than those measured in the thorax. Chemical speciation of this element in tissues of the three polychaete species revealed a major contribution of methylated arsenic compounds, like dimethylarsinate (DMA) and, to a lower extent, monomethylarsonate (MMA) and trimethylarsine oxide (TMAO). Although the biotransformation of inorganic arsenic might represent a detoxification mechanism in polychaetes from hydrothermal vents, the elevated levels of methylated forms of arsenic in branchial tissues also suggest an ecological role of this element as an antipredatory strategy for more vulnerable tissues toward generalist consumers.

1. Introduction

Since the discovery of hydrothermal vents in 1977 on the Galapagos Rift, these environments revealed dense benthic communities mainly characterized by dominating groups of molluscs, crustaceans and polychaetes, which typically exhibit the most efficient adaptability to extreme environmental conditions (Wolff, 2005). Frequent symbiotic relationships between invertebrates and chemoautotrophic bacteria highlight complex trophic, toxicological and ecological interactions (Cavanaugh, 1983; Childress and Fisher, 1992), including the main trophic link in which bacteria oxidize the reduced compounds from the vents, mainly hydrogen sulphide and methane, and synthesize organic

compounds for host feeding (Stewart et al., 2005).

The vents communities dwell in the mixing zone, where reduced metal-rich hydrothermal fluids are mixed with oxidized deep ocean waters at approximately 2 °C. Depending on their degree of dilution below the seafloor vent fluids display a wide range of temperature, the hottest and less diluted ones resulting in the formation of large deposits rich in minerals and trace metals (Van Dover et al., 2001; Desbruyères et al., 2000). The capability of vent organisms to tolerate such elevated concentrations of metals led to discover specific adaptive defence mechanisms. Among these, the possibility to reduce cellular reactivity of toxic elements by confining them into granules or vacuoles, excreting via mucus, or binding to soluble ligands including metal-complexing

* Corresponding author. Dipartimento di Scienze della Vita e dell'Ambiente (DISVA), Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy.
E-mail address: f.regoli@univpm.it (F. Regoli).

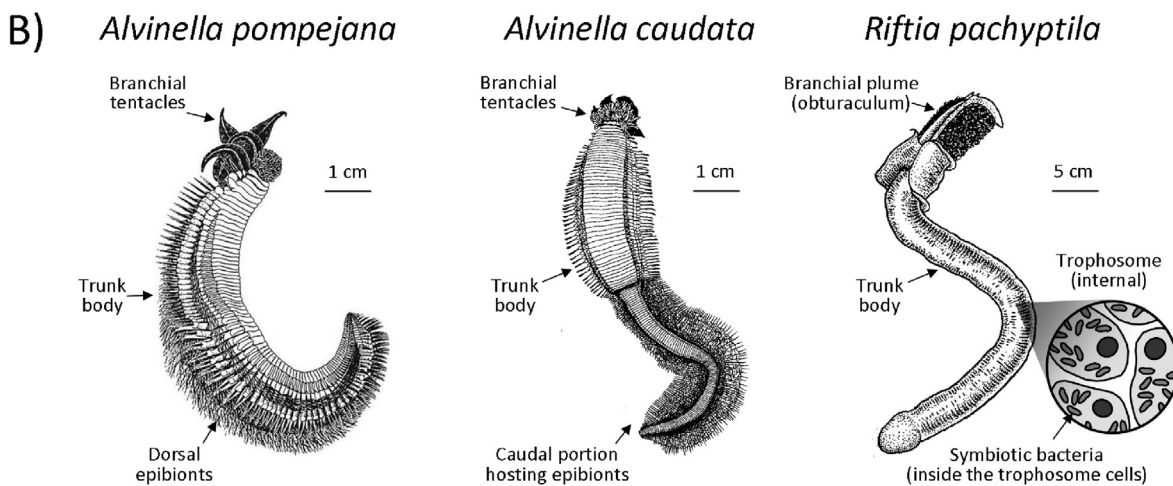
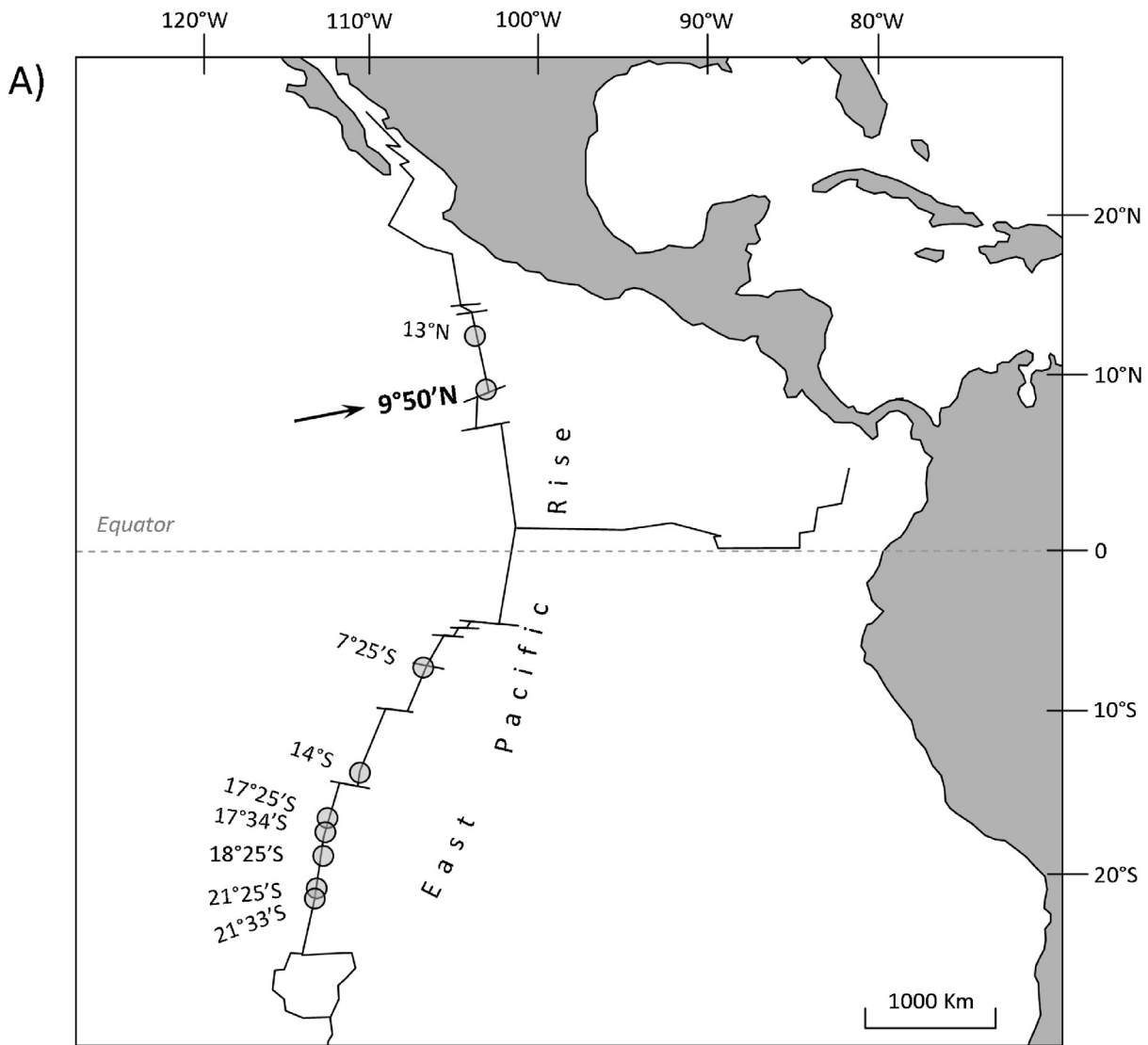


Fig. 1. A) Sampling site located at 9°50'N (arrow) on the East Pacific Rise (EPR). Circles represent the main sites of the area with documented benthic settlements of hydrothermal vent fauna. B) Schematic representation of the main anatomical regions of the tube dwelling polychaetes *A. pompejana*, *A. caudata* and *R. pachyptila* collected in this study.

proteins like metalloproteins and metallothioneins (Roesijadi and Crecelius, 1984; Desbruyères et al., 1998; Juniper et al., 1986; Kádár et al., 2005, 2006a; 2006b). The activities of symbiotic bacteria could

further contribute to metabolize and sequester trace metals, although a similar interaction has not been fully demonstrated and has been debated (Van Dover, 2000). Accumulation of toxic compounds has also

been suggested as an antipredatory strategy in deep sea hydrothermal vent communities which, representing large oases of biotic resources in a prey-poor surrounding background, can be exposed to heavy pressure by generalist consumers once the fluid source intensity declines and provide access to habitats previously protected by elevated temperature and toxicity (Kicklighter et al., 2004).

Among marine invertebrates, polychaetes represent one of the most abundant taxa with elevated tolerance to stressful conditions and capability to accumulate trace metals (Bocchetti et al., 2004; Ricevuto et al., 2016). The first objective of the present work was to characterize levels of a wide range of trace elements (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, V and Zn) in tissues of three tube dwelling polychaetes, the alvinellids *Alvinella pompejana* and *A. caudata*, and the vestimentifera *Riftia pachyptila* collected at the East Pacific Rise hydrothermal vent. The species are among the most abundant and common sessile organisms of the vent system, although they colonize distinct areas with different chemical and physical conditions in terms of pH, temperature, level of hydrogen sulphide and dissolved oxygen (Hessler et al., 1988; Desbruyères et al., 2000; Le Bris et al., 2003; Le Bris and Gaill, 2007). *A. pompejana* has a length of about 10 cm and inhabits the hottest places of the vent field, building the tube directly on the wall of mineral chimneys hosting high-temperature fluid flows, thus being exposed to a constant rain of mineral particles and fluids enriched in dissolved metals (Desbruyères et al., 1998). Measured temperature reaches 80 °C inside the distal parts of its tube that extend deeper in the chimney mineral wall and lies in the range 6–45 °C at the tube opening, confirming the exceptional thermo-tolerance of this species despite its upper thermal limit is still unknown (Le Bris and Gaill, 2007). *A. caudata* substantially shares the same habitat of *A. pompejana*, living on the walls of active sulphide chimneys, while *R. pachyptila* is a giant tube worm with a maximum length of about 1–2 m, living near in the mixing zone of diffuse mild temperature vents at a temperature of approximately 15–20 °C (Childress and Fisher, 1992; Van Dover et al., 2001). The comparison of trace elements content in polychaete species from the same extreme environment, but with different ecological habits, represents a novel aspect of the study providing new insights on bioavailability and bioaccumulation mechanisms at the vents systems.

Considering the peculiar environmental conditions of the hydrothermal vents habitats, previous studies highlighted the capability of some vent organisms to decrease reactivity and adverse effects of toxic elements through their precipitation in metal-binding mineral particles (Roesijadi and Crecelius, 1984; Cosson-Mannevy et al., 1988; Kádár et al., 2006b). In this respect, a second objective of this study was to investigate the mobility of trace elements in tissues of *A. pompejana* by evaluating the percentage of soluble and insoluble metals: those associated to insoluble forms were further characterized by sequential extraction to assess the chemical strength of such precipitates.

The last objective of this work was to characterize for the first time the chemical speciation of arsenic in tissues of these three vent polychaete species, comparing the distribution and occurrence of arseno-compounds previously reported in sabellid polychaetes from temperate environments (Fattorini and Regoli, 2004). Tube dwelling polychaetes usually extend branchial appendages outside of the protective tubes for respiration and filter-feeding activities and naturally elevated concentrations of toxic elements in the branchial appendages of some species have been suggested as a protection mechanism against predation (Gibbs et al., 1981; Fattorini et al., 2005), an hypothesis supported by unpalatability of these tissues for potential consumers (Kicklighter et al., 2004; Kicklighter and Hay, 2006, 2007).

Overall, the present study was expected to contribute in assessing the magnitude and biological significance of trace elements bioaccumulation in polychaete species living in such extreme environmental conditions.

2. Materials and methods

2.1. Species, sampling and site

The sampling sites are located at 9°50' N, 104°17' W on the East Pacific Rise (EPR) hydrothermal vent field discovered in 1989 in the axial rift zone of one of the faster-spreading basaltic ridge ($> 11 \text{ cm-yr}^{-1}$) (Carbotte and MacDonald, 1992), which experienced two massive volcanic eruptions in 1991 and 2006 (Fig. 1). The vent field is characterized by massive sulphide chimneys with high-temperature 'black smokers' vents colonized by various benthic invertebrates including the tubeworms *A. pompejana* and *A. caudata*; mild-temperature and diffuse vents spread over the basaltic seafloor colonized by *R. pachyptila*.

Polychaetes were sampled in March 2012 at approximately 2500 m depth, during the oceanographic expedition MESCAL-2 (R/V Atalante, Le Bris and Lallier, 2010). After collection by the robotic arm of the submersible *Nautilie* from the French oceanographic fleet, organisms were immediately placed in a pressurized sealed box, then brought to the surface and transferred to a cold room (4 °C) for sorting and identification. Thirty individuals of *A. pompejana* and 15 individuals of both *A. caudata* and *R. pachyptila* were selected among intact specimens. Organisms were gently removed from the external tubes, accurately washed with filtered seawater to remove any external residues or sediment particles and dissected to separate the filter-feeding appendages (branchial tentacles for alvinellids, branchial plume or obturaculum for *R. pachyptila*), from the rest of thoracic tissues (Fig. 1). Organisms were not depurated since they would not have survived to this treatment. For *A. pompejana* specimens, the epibionts filaments present on the dorsal surface of the thorax were also accurately separated.

To assess a possible distribution gradient of trace metals along the trunk body, these tissues were subdivided in three different regions, corresponding to the apical, median and distal portions for *A. pompejana* (Fig. 1); for *A. caudata* the apical thorax was separated from the caudal portion hosting epibionts which, however, could not be collected because not visible at naked eye (Fig. 1). In the vestimentiferan *R. pachyptila*, the internal trophosome containing endosymbiotic bacteria was removed from the rest of the animal tissues (Fig. 1). All the obtained samples were stored at -20 °C and analytical determinations were always carried out on five replicates ($n = 5$), each containing the tissues of singular individuals.

2.2. Determination of trace elements in tissues of polychaetes

Tissues were dried at 60 °C overnight to constant weight, pulverized and about 0.5 g digested with 5 mL of pure, analytic grade nitric acid and 1 mL of pure, analytic grade hydrogen peroxide (30% w/w) in a microwave digestion system (Mars CEM, CEM Corporation, Matthews, NC, USA), at 160 °C for 40 min (Fattorini et al., 2010). Quality assurance and quality control were assessed by processing blank samples and standard reference material (SRM NIST-2977, National Institute of Standards and Technology, Gaithersburg, MD, USA). Aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni), lead (Pb), selenium (Se), silver (Ag), and vanadium (V) were determined by atomic absorption spectrometry (AAS) using graphite furnace atomization and Zeeman effect (Varian SpectraAA 240Z); when necessary, a palladium solution (1 g/L, 10% nitric acid, 5% citric acid) was added as chemical matrix modifier and the standard addition technique applied for resolution of matrix effects (Fattorini et al., 2010). Iron (Fe), manganese (Mn), and zinc (Zn) were measured by flame atomization (Varian SpectraAA 220FS) while mercury (Hg) by specific analyser based on the formation of mercury cold vapours (Cetac Quick Trace Mercury Analyser M6100).

Concentrations were expressed as micrograms per gram of dry weight ($\mu\text{g}\cdot\text{g}^{-1}$ d.w.); values obtained for the standard reference material were always within the 95% confidence interval of certified values, while the CV% always fit between 5% and 10% analysing ten replicates.

2.3. Differential centrifugation of *A. pompejana* tissues and sequential extraction of insoluble elements

Tissues from 5 individuals of *A. pompejana* were subjected to differential centrifugation to separate soluble and insoluble forms of trace metals. Aliquots of branchial crowns and body portions (about 0.2 g of wet tissues) were homogenized (1:5 w:v) at 4 °C in phosphate buffer solution (50 mM K_2HPO_4 , 2.5% NaCl, pH = 7.4) (Fattorini and Regoli, 2004; Fattorini et al., 2010). Samples were then centrifuged at 800 \times g for 25 min (4 °C) to obtain the sedimentation of an “heavy” (H) pellet, enriched in insoluble particles, mineral concretions, nuclei and heterogeneous components. Due to the loss of organelles integrity during thawing of frozen tissues, the supernatants were directly spun at 100,000 \times g for 1 h (4 °C) to separate a pellet containing microsomes and subcellular debris (the “sub” fraction), from the soluble and cytosolic components (the “cyt” fraction). Each fraction was finally digested in concentrated nitric acid (pure, analytic grade) and analysed for trace elements as previously described.

To better characterize the association of trace elements with the insoluble fraction, the “heavy” pellets obtained after the first centrifugation (800 \times g, 25 min) were dried to constant weight (60 °C for 8 h) and then treated with a four-stage sequential extraction, slightly modified from a validated method for mineral particles (Tokalioğlu et al., 2000). The whole sequence of centrifugation and extraction steps, is summarized in Supplementary Material, SM1. Dried H pellets were digested with 5 mL acetic acid 1M (pH = 2.8) and maintained at 50 °C for 16 h. After centrifugation at 3000 \times g for 20 min, the resulting supernatants were digested with 2 mL of nitric acid and diluted with ultrapure water to a final volume of 10 mL, thus constituting the fraction F1. The pellet was washed and centrifuged three times with ultrapure water, before addition of 5 mL of hydroxyl ammonium chloride 0.1 M (pH = 2.0), and maintained at 50 °C for 16 h; after centrifugation (3000 \times g for 20 min), the supernatant was digested as above to obtain the fraction F2. The pellet was washed again three times with ultra-pure water, then 2 mL of hydrogen peroxide ($\geq 30\%$ w/w) were added, maintained in shaking at room temperature for 1 h, and at 85 °C for an additional hour. After centrifugation the supernatant (S1) was temporarily stored at 4 °C, while the resulting pellet was washed three times with ultrapure water, before addition of 5 mL of ammonium acetate 1 M (pH = 2.0) and maintained at 50 °C for 16 h: following this extraction, samples were centrifuged (3000 \times g for 20 min), the supernatant mixed with corresponding S1, and digested (fraction, F3). The last pellet residue was washed three times and finally digested (fraction F4).

With these procedures, fraction F1 contains acid soluble and easily exchangeable metals, including weakly adsorbed elements and those associated with carbonates (Okoro et al., 2014). F2 includes reducible metals, i.e. those adsorbed or complexed on the surface of Fe and/or Mn oxides. Fraction F3 contains oxidizable metals associated with mineral particles like insoluble sulphides, while F4 is the residual fraction in which metals are precipitated into mineral particles, concretions or granules. All the obtained fractions were analysed for the content of selected trace elements (As, Cd, Co, Cr, Cu, Fe, Hg, Pb, Zn) using the previously described procedures.

2.4. Chemical speciation of arsenic compounds

Chemical speciation of arsenic was performed on tissues of *A. pompejana*, *A. caudata* and *R. pachyptila*, according to previously validated techniques (Fattorini and Regoli, 2004). After microwave-pure methanol (HPLC grade) extraction at 55 °C for 25 min, samples (n = 5)

were centrifuged at 3000 \times g; the pellets, containing non extractable forms of arsenic (ne-As), were accurately washed and centrifuged three times with ultrapure water ($18.2\text{ M}\Omega\cdot\text{cm}^{-1}$), dried at 60 °C until constant weight, then digested and analysed by atomic absorption spectrometry as above. Separation of various arsenic compounds in supernatants was performed in isocratic conditions using a Supelcosil liquid chromatography-SCX column (25 cm, 4.6 mmID, 5 μm) and 2.5 mM pyridine (pH = 2.65) as the mobile phase at a flow-rate of 1 mL/min. A total of 40 fractions were collected, each every 30s from injection, digested with 0.5 mL of pure concentrated nitric acid, and analysed for the arsenic content. The certified reference standards DORM-2 (National Research Council Canada, Certified Reference Standard) and BCR627 (European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Belgium), containing certified levels of dimethylarsinate (DMA), tetramethylarsonium (TETRA) and arsenobetaine (AsB), and selected standards, including arsenate (AsV), DMA, trimethylarsine oxide (TMAO) and AsB were processed and analysed by the same procedures as controls for accuracy, precision, and recovery. The concentrations of As compounds for reference standards were always within the 95% of confidence interval of the certified values. Inorganic arsenic (iAs) was not resolved in terms of arsenite (AsIII) and arsenate (AsV), since both the extraction procedures and the chromatography separation induce oxidation of trivalent arsenicals, thus representing a confounding variable. Accuracy and precision of the arsenic determination by AAS were the same of those reported for the total arsenic analysis.

2.5. Statistical analyses

The *t*-student test or one way analyses of variance (ANOVA), followed by Student-Newman-Keuls *post-hoc* test, were applied at the 95% of confidence interval ($\alpha = 0.05$) to check for statistical differences of the trace metals levels between different portions or tissues of *A. pompejana*, *A. caudata* and *R. pachyptila*: the hypothesis to test was a major accumulation of some elements in specific tissues or body portions of the investigated organisms. ANOVA was also applied to check differences in the percentage distribution of trace metals along subcellular fractions and sequentially extracted insoluble fractions of *A. pompejana* ($\alpha = 0.05$), to verify a significant accumulation of elements into specific subcellular districts or the presence of insoluble associations. All the statistical analyses were performed after Levene's test on variances homogeneity, and data were normalized by log transformation when necessary.

3. Results

3.1. Characterization of trace elements content

Concentrations of trace metals did not reveal statistically significant differences in different portions of the trunk body both in *A. pompejana* and *A. caudata* (Supplementary Material, SM2 and SM3, respectively), and mean levels for the entire thorax are thus compared with values measured in the branchial tentacles (Table 1). *A. pompejana* exhibited typically elevated concentrations in branchial crowns for As ($1425 \pm 653\ \mu\text{g}\cdot\text{g}^{-1}$), Cd ($10.8 \pm 5.30\ \mu\text{g}\cdot\text{g}^{-1}$), Cu ($792 \pm 352\ \mu\text{g}\cdot\text{g}^{-1}$), Fe ($18118 \pm 11509\ \mu\text{g}\cdot\text{g}^{-1}$), Hg ($46.0 \pm 14.7\ \mu\text{g}\cdot\text{g}^{-1}$), Pb ($120 \pm 59.0\ \mu\text{g}\cdot\text{g}^{-1}$), Sb ($4.26 \pm 1.56\ \mu\text{g}\cdot\text{g}^{-1}$), Se ($8.96 \pm 2.96\ \mu\text{g}\cdot\text{g}^{-1}$) and Zn ($2275 \pm 1336\ \mu\text{g}\cdot\text{g}^{-1}$). Levels measured in the thorax were similar or lower, with marked and statistically significant differences for arsenic and mercury, which were 15 and 30 fold lower than in branchial appendages, respectively (Table 1). Concentrations of trace metals in isolated epibionts showed an elevated degree of variability for some elements, and mean levels were more than one order of magnitude higher than in branchial crown for Ag, Al, Cd, Co, Cu, Fe, Mn, Pb, V, Zn (Table 1). Concentrations of other elements in epibionts were comparable to branchial tentacles (e.g. Hg), while arsenic was significantly lower.

Table 1
Levels of trace metals in tissues (branchial appendages and trunk body) and associated symbionts of polychaetes (*A. pompejana*, *A. caudata* and *R. pachyptila*) from the 9°50'N East Pacific Rise (EPR) hydrothermal vent system field; concentrations are expressed as $\mu\text{g g}^{-1}$ dry weight (mean values \pm standard deviation, $n = 5$). Statistical differences between tissues for each species are tested by one way analyses of variance (ANOVA) or t-student test at the 95% of confidence interval (n.s. = not significative, $p \geq 0.05$) and different letters indicate significant differences between groups of means determined by Student-Newman-Keuls post-hoc test.

	Alvinella pompejana			Alvinella caudata			Riftia pachyptila						
	Branchial tentacles	Trunk body	Eplibionts	Branchial tentacles	Trunk body	Sig.	Branchial plume	Trunk body	Trophosoma	Sig.			
Ag	0.844 \pm 1.124	a	0.174 \pm 0.087	b	< 0.05	1.280 \pm 600	b	< 0.05	0.265 \pm 0.186	b	5.52 \pm 3.07	a	p < 0.01
Al	87.2 \pm 36.8	a	31.3 \pm 8.6	a	< 0.001	200 \pm 70	b	< 0.001	14.4 \pm 5.3	b	78 \pm 26	a	p < 0.01
As	1425 \pm 653	a	91.7 \pm 78.7	b	< 0.001	4.67 \pm 4.19	a	< 0.001	10.0 \pm 4.5	b	82.1 \pm 30.3	a	n.s.
Ba	1.08 \pm 0.54	a	1.36 \pm 0.42	a	< 0.005	7.33 \pm 4.28	b	< 0.005	0.959 \pm 0.502	b	0.959 \pm 0.502	a	p < 0.01
Cd	10.8 \pm 5.3	a	6.03 \pm 1.60	a	< 0.001	165 \pm 39	b	< 0.001	8.33 \pm 3.99	a	8.33 \pm 3.99	b	p < 0.01
Co	10.4 \pm 6.2	a	6.31 \pm 0.98	a	< 0.005	87.0 \pm 48.9	b	< 0.005	7.74 \pm 3.48	a	7.74 \pm 3.48	b	n.s.
Cr	4.63 \pm 1.16	a	3.22 \pm 2.02	a	< 0.05	9.54 \pm 5.27	b	< 0.05	1.90 \pm 0.77	a	1.90 \pm 0.77	a	p < 0.01
Cu	792 \pm 352	a	385 \pm 68	a	< 0.005	5621 \pm 3123	b	< 0.005	289 \pm 107	a	289 \pm 107	a	p < 0.001
Fe	18118 \pm 11509	a	14212 \pm 3125	a	< 0.001	199413 \pm 93599	b	< 0.001	19445 \pm 9067	a	19445 \pm 9067	a	p < 0.001
Hg	46.0 \pm 14.7	a	1.62 \pm 0.83	b	< 0.005	41.1 \pm 20.4	b	< 0.005	5.69 \pm 3.01	b	5.69 \pm 3.01	a	p < 0.05
Mn	19.2 \pm 6.2	a	26.1 \pm 5.8	a	< 0.001	163 \pm 72	b	< 0.001	39.2 \pm 11.5	ab	39.2 \pm 11.5	a	p < 0.001
Ni	1.23 \pm 1.10	a	0.620 \pm 0.184	a	< 0.005	4.08 \pm 1.67	b	< 0.005	1.85 \pm 0.94	b	1.85 \pm 0.94	a	n.s.
Pb	120 \pm 59	a	97.3 \pm 56.3	a	< 0.001	827 \pm 347	b	< 0.001	78.6 \pm 44.2	b	78.6 \pm 44.2	a	n.s.
Sb	4.26 \pm 1.56	a	2.06 \pm 0.37	a	< 0.001	18.8 \pm 3.2	b	< 0.001	< 1.0	< 1.0	< 1.0	< 1.0	n.s.
Se	8.96 \pm 2.96	a	2.72 \pm 1.38	b	< 0.001	26.2 \pm 5.7	c	< 0.001	2.66 \pm 0.66	a	2.66 \pm 0.66	a	p < 0.001
V	4.25 \pm 0.60	a	2.60 \pm 0.73	a	n.s.	19.1 \pm 16.9	b	< 0.05	2.39 \pm 0.58	a	2.39 \pm 0.58	a	n.s.
Zn	2275 \pm 1336	a	1605 \pm 310	a	< 0.005	27555 \pm 15759	b	< 0.005	2634 \pm 1182	b	2634 \pm 1182	a	p < 0.01

Trace metals in *A. caudata* showed similar levels to those of *A. pompejana* (Table 1), slightly lower for As and Hg in branchial crowns (410 ± 195 and $23.9 \pm 7.4 \mu\text{g g}^{-1}$) and higher for Cd ($17.5 \pm 13.7 \mu\text{g g}^{-1}$), Co ($21.3 \pm 6.4 \mu\text{g g}^{-1}$), Fe ($30367 \pm 12832 \mu\text{g g}^{-1}$) and Zn ($4665 \pm 2895 \mu\text{g g}^{-1}$). The tissue distribution was comparable in the 2 alvinellids, with higher values for all the elements in branchial tentacles of *A. caudata* than in trunk body, in particular for As, Hg, Cd, Co and Cu (Table 1).

On the other hand, concentrations of metals in both the tissues of the vestimentiferan *R. pachyptila* appeared up to 2 orders of magnitude lower than those measured in alvinellid polychaetes (Table 1). Also in this species, higher values were measured in branchial tissues than in body portions (with the exception of Cd), but the magnitude of such differences was never greater than 3 folds. Levels obtained for the trophosome were comparable to those measured in thoracic tissues of *R. pachyptila* with slightly higher concentrations for Cr, Cu, Fe and Hg (Table 1).

3.2. Trace elements distribution in tissues of *A. pompejana*

The differential centrifugation performed on both the tissues of *A. pompejana* indicated that all the elements were primarily associated with the heavy (H) pellet containing insoluble components (Table 2). This heavy fraction accounted for 90–100% of the total metal content for Cd, Cu, Fe, Hg, Pb, Zn (only in body portion). Soluble metals in the subcellular (sub) and cytosolic (cyt) fractions showed a maximum percentage of 15–30% for As, Co, Cr and Zn in branchial tissues (Table 2).

The results of the sequential extraction of insoluble H pellets are reported in Fig. 2. With a few exceptions, the contribution of different fractions to the insoluble metal content was generally comparable in branchial appendages and thoracic tissues. Arsenic was almost entirely associated with F1, indicating the presence of easily exchangeable forms in a weak acid, thus not precipitated in any mineral particles (Fig. 2). Cd, Cu, Fe, Pb and Zn were mainly concentrated (60–80%) in F3, which contains oxidizable metals likely as amorphous labile precipitates and ionic forms adsorbed to the surface of sulphides containing particles (Fig. 2). Co and Cr exhibited a different distribution pattern in various anatomical regions, being associated with fractions F1 and F3 in the branchial appendages, while occurring in the thorax for almost 80% within F2, enriched in reducible elements, i.e. adsorbed to Fe and Mn oxides. Finally, mercury was measured in fractions F3 and F4, the latter mainly containing insoluble particles and prevailing in branchial tentacles, while F3 was predominant in trunk body (Fig. 2).

3.3. Chemical speciation of arsenic compounds

Results on chemical speciation of arsenic compounds in tissues of the three polychaetes species are reported in Fig. 3. Elevated percentages of non-methanol-extractable arsenic (ne-As) were detected in branchial appendages (36–56%), corresponding to approximately $500 \mu\text{g g}^{-1}$ in *A. pompejana*, $300 \mu\text{g g}^{-1}$ in *A. caudata* and $4 \mu\text{g g}^{-1}$ *R. pachyptila*. The remaining extractable fractions of arsenic were mostly represented by methylated compounds, particularly DMA and, to a lower extent, monomethylarsonate (MMA) and TMAO; MMA and DMA together contributed for at least 90% of the extractable arsenic with concentrations of approximately $350 \mu\text{g g}^{-1}$ in *A. pompejana* and *A. caudata*, and much lower (approximately $5 \mu\text{g g}^{-1}$) in *R. pachyptila* (Fig. 3). Traces of inorganic arsenic were detected only in *R. pachyptila*.

Partially similar results were observed also for the thoracic tissues, with an elevated percentage of ne-As, and the prevalence of methylated compounds among the separated arsenic species: the most abundant molecules were MMA in *A. pompejana*, and DMA in *A. caudata* and *R. pachyptila* (Fig. 3). Some species-specific differences were observed also for the content of more complex organic As compounds in the trunk body: AsB represented from 3 to 22% of total arsenic in *A. pompejana*

Table 2

Distribution of selected trace elements in fractions obtained after differential centrifugation of *A. pompejana* tissues (branchial appendages and trunk bodies). Results are given as mean values ± standard deviation, n = 5. H: heavy pellets containing insoluble elements; sub: fraction containing microsomes and subcellular debris; cyt: cytosolic, soluble fraction. For each tissue, different letters indicate significant differences between groups of means, determined by one way analysis of variance (ANOVA) and *post-hoc* test (n.s. = not significant).

	Branchial tentacles				Trunk body			
	H	sub	cyt	sig.	H	sub	cyt	sig.
As	84.7 ± 5.79%	a 1.75 ± 0.02%	b 13.4 ± 5.78%	c p < 0.05	72.1 ± 5.58%	a 1.49 ± 0.09%	b 26.3 ± 5.55%	c p < 0.05
Cd	95.0 ± 3.30%	a 4.56 ± 3.56%	b 0.36 ± 0.26%	b p < 0.05	93.7 ± 1.17%	a 5.58 ± 1.24%	b 0.63 ± 0.42%	c p < 0.05
Co	74.7 ± 14.5%	a 3.24 ± 1.42%	b 21.9 ± 13.2%	b p < 0.05	66.6 ± 24.3%	a 3.98 ± 2.22%	b 29.3 ± 22.5%	ab p < 0.05
Cr	83.6 ± 3.06%	a 3.45 ± 0.69%	b 12.9 ± 3.40%	c p < 0.05	69.6 ± 3.76%	a 9.46 ± 2.49%	b 20.8 ± 2.17%	c p < 0.05
Cu	94.6 ± 6.52%	a 0.81 ± 1.41%	b 4.54 ± 7.01%	b p < 0.05	97.6 ± 2.50%	a 2.20 ± 2.55%	b 0.17 ± 0.29%	b p < 0.05
Fe	95.7 ± 1.23%	a 2.59 ± 0.62%	b 1.68 ± 1.82%	b p < 0.05	95.4 ± 3.38%	a 3.61 ± 2.12%	b 0.95 ± 1.37%	b p < 0.05
Hg	96.9 ± 2.62%	a 1.84 ± 1.09%	b 1.20 ± 1.52%	b p < 0.05	93.7 ± 0.77%	a 3.14 ± 0.65%	b 3.10 ± 0.67%	b p < 0.05
Pb	95.5 ± 1.54%	a 3.03 ± 1.81%	b 1.37 ± 0.26%	b p < 0.05	85.6 ± 18.1%	a 10.5 ± 12.2%	b 3.73 ± 5.99%	b p < 0.05
Zn	70.0 ± 33.8%	7.49 ± 0.78%	22.4 ± 33.0%	n.s.	89.4 ± 3.99%	a 5.76 ± 1.89%	b 4.82 ± 3.31%	b p < 0.05

and *A. caudata* respectively, while arsenocholine (AsC) was detected in these tissues of *R. pachytila* with a contribution of approximately 13% (Fig. 3).

4. Discussion

This study revealed the capability of *A. pompejana* and *A. caudata* to accumulate elevated levels of trace metals, while much lower concentrations were measured in tissues of *R. pachytila*: these organisms commonly colonize different areas of the chimneys where physico-chemical conditions might contribute to the observed effects. Alvinellids

live in the most extreme conditions of the vent, forming their tube directly in contact, with the chimneys walls where metal-rich and low pH fluids escape at high temperature and reducing conditions (Zbinden et al., 2003). At the chimney surface, interaction with seawater decreases the solubility of metals associated with vent fluids (Demina et al., 2013), plumes of mineral particles are formed from the main fluid outflows mixing with seawater, and the tubes become progressively incrustated in sulphide minerals. Despite this, high concentrations of dissolved metals are measured in waters surrounding the *Alvinella* and inside their tubes (Di Meo-Savoie et al., 2004; Sarradin et al., 2008). In addition to diffusive inputs of dissolved metals from the hot and porous

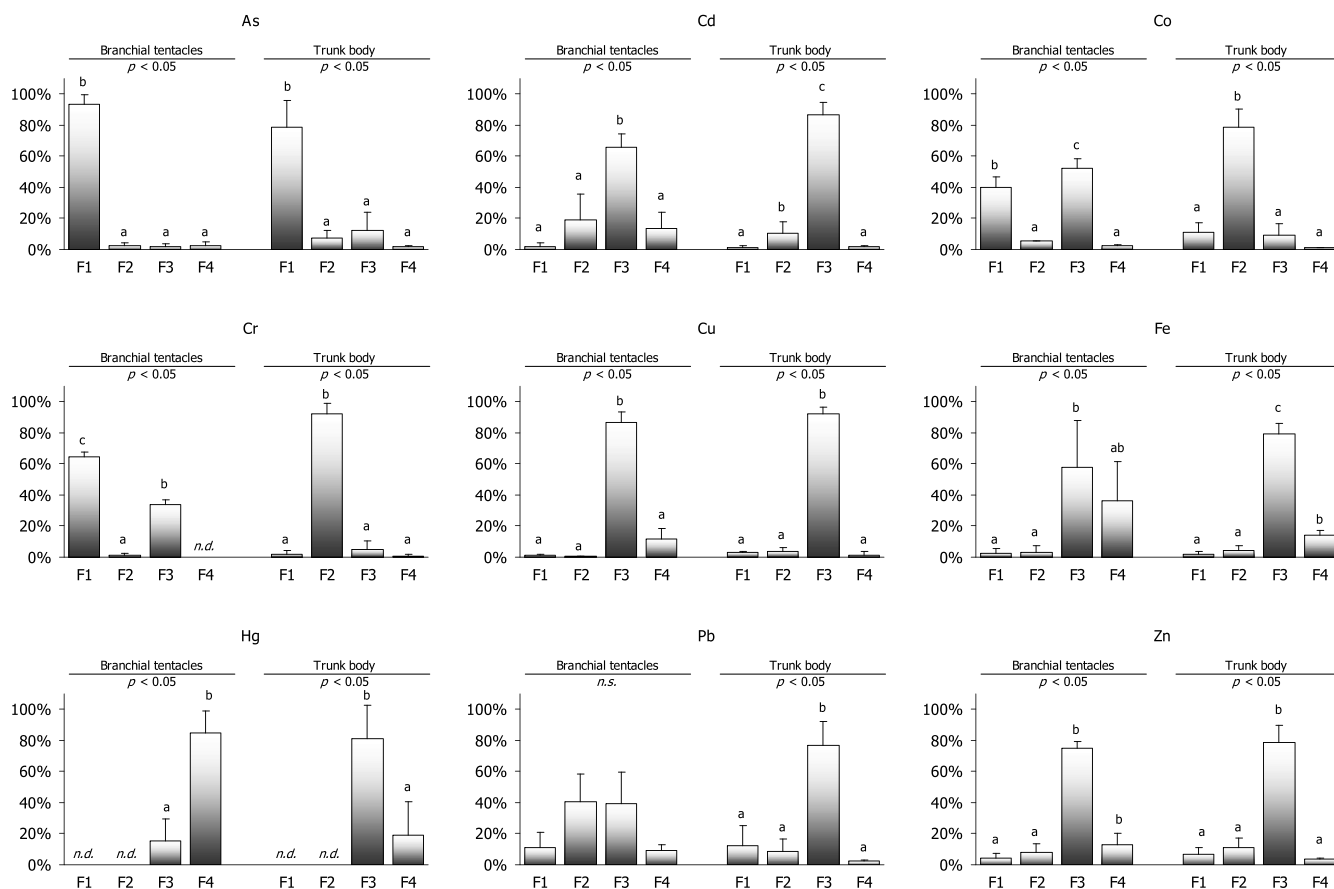


Fig. 2. Distribution of trace elements in insoluble fractions separated from branchial appendages and trunk body of *A. pompejana*. F1 = weakly insoluble and easily exchangeable metals; F2 = reducible trace elements, adsorbed to surface of Fe/Mn oxides containing particles; F3 = oxidizable metals associated with sulphides containing particles; F4 = residual insoluble fraction, constituted by mineral particles, concretions or granules. Data are expressed as percentage contribution of each fraction to the total metal burden (mean values ± s.d., n = 5). Different letters indicate statistical differences between groups (ANOVA and *post-hoc* test); n.s. = not statistically significant, p ≥ 0.05.

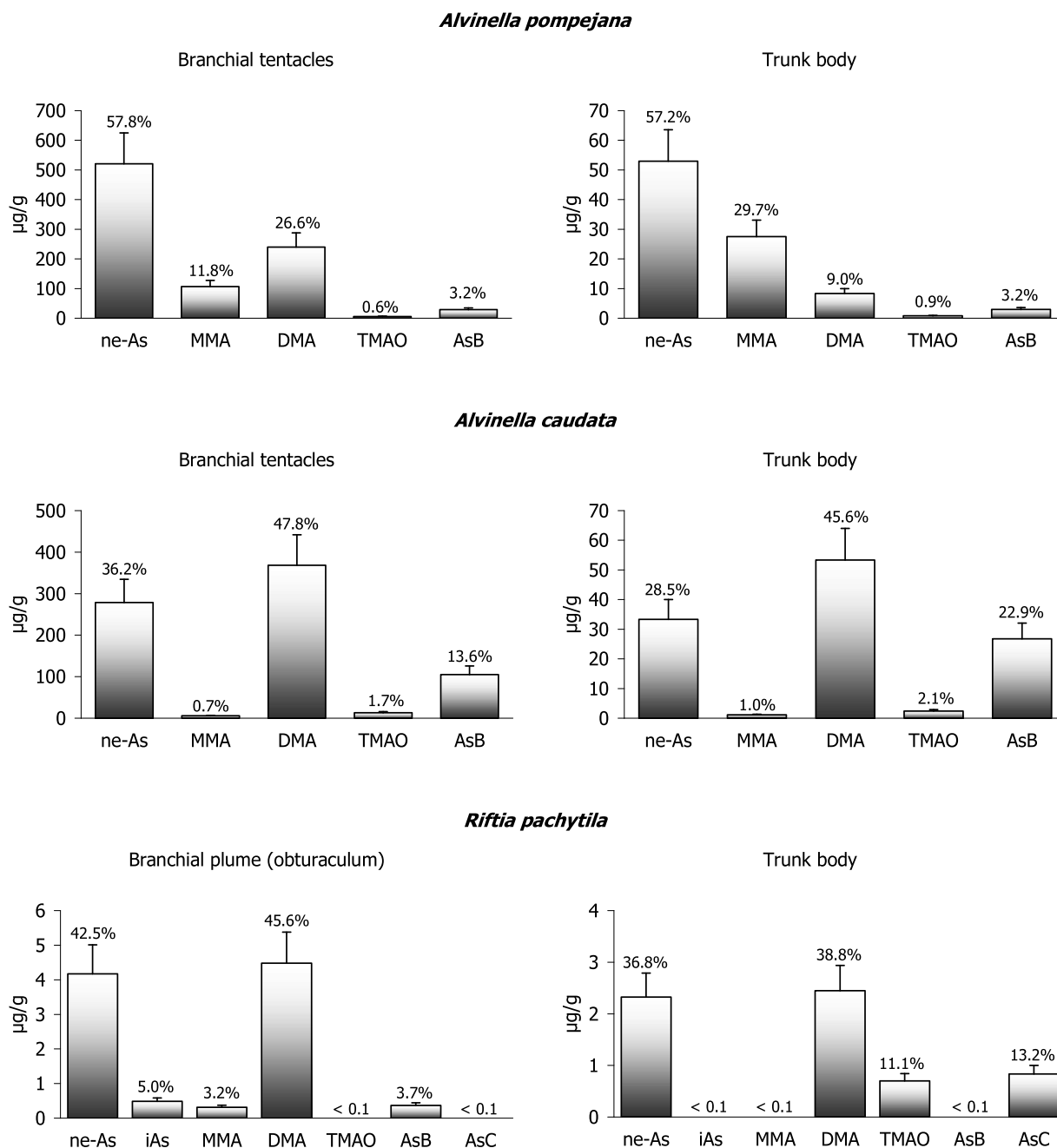


Fig. 3. Chemical speciation of arsenic in branchial appendages and trunk body of polychaetes (*A. pompejana*, *A. caudata* and *R. pachytila*). Determined arsenic compounds are: ne-As, not extractable and methanol insoluble arsenic; iAs, inorganic arsenic; MMA, monomethylarsenate; DMA, dimethylarsinate; TMAO, trimethylarsine oxide; AsB, arsenobetaine; AsC, arsenocholine. Data are expressed as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight (mean values \pm standard deviation, $n = 5$); the percentage contribution of each compound to the total arsenic content is given on each bar.

chimney wall, alvinellids constantly move inside out the tubes, thus facilitating the inflow of colder mixed fluids in these habitats, transporting oxygen and sulphide particles and further increasing the volume of labile metal forms to which these organisms are exposed (Le Bris et al., 2005). This characteristic can partially explain the elevated concentrations in the alvinellids tissues of metals such as Cu, Fe, Hg, Pb and Zn. These elements, typically abundant and occurring in soluble forms within the hottest vent fluids (Rona et al., 1986), are partly converted to labile sulphide precipitates at the interface with seawater, and can be remobilized close to the tube opening in seawater where more oxic conditions are found. Compared to alvinellids, *R. pachytila* colonizes the basaltic seafloor, where trace metals concentrations are

drastically decreased by subsurface dilution of vent fluids with seawater resulting in sulphide precipitates and oxidative reactions (Desbruyères et al., 2000). In these conditions and away from active hydrothermal chimneys, *R. pachytila* is not exposed to a constant rain of metal-rich particles and fluids as *Alvinella* spp., but rather to lower and chronic levels of dissolved metals in the surrounding mixed fluids with a large seawater component.

The higher values of trace elements in branchial appendages compared to thoracic tissues of the three polychaete species corroborate the influence of the direct contact with the external, metal enriched and weakly oxygenated environment. Similar characteristics of the medium inside the tube of alvinellids would also explain the high concentrations

Table 3
 Comparison between the trace metal concentrations in various polychaetes from hydrothermal vents. Data are expressed according to the original investigations as absolute levels, range of values or mean concentrations \pm s.d. ($\mu\text{g/g}$ d.w.).
 Acronyms: AP = anterior part; BA = branchial appendages; BL = blood; DG = digestive gland; MU = muscle; TB = trunk body; TR = trophosome; VE = vestimentum; WO = whole organisms; ATJ = Azores Triple Junction; EPR = East Pacific Rise; JFR = Juan de Fuca Ridge. References: (1) Desbruyères et al., 1998; (2) Ando et al., 2002; (3) Maher et al., 2016; (4) Shmelev et al., 2009; (5) Cosson, 1996; (6) Demina and Galkin, 2009; (7) Ruelas-Inzunza et al., 2005; (8) Colaço et al., 2006; (9) Káddár et al., 2007.

Species	Area	Tissue	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Se	V	Zn	Ref.		
<i>A. pompejana</i>	13°N EPR	AP	-	-	-	-	3.32-10.89	-	-	27-60	-	-	-	-	-	-	-	-	-	390-11-47	1	
		DG	-	-	-	-	0.79-11.49	-	-	30-205	-	-	-	-	-	-	-	-	-	-	8222-1-0523	
		WO	-	-	-	-	0.384-2.01	-	-	4-26	-	-	-	-	-	-	-	-	-	-	166-12-07	
<i>L. satsuma</i>	Kagoshima Bay	BA	-	-	-	-	-	-	-	-	-	0.016-0.073	-	-	-	-	-	-	-	-	2	
		BL	-	-	-	-	-	-	-	-	-	0.003-0.012	-	-	-	-	-	-	-	-	-	
		MU	0.087	-	8.108	-	1.750	0.126	3.626	10.21	-	-	0.062	6.062	-	0.248	0.04	0.093	0.257	-	-	
<i>P. palmiformis</i>	JFR	TB	-	-	-	-	-	-	-	-	-	0.044-0.399	-	-	-	-	-	-	-	-	-	
		TR	0.354	-	28.95	-	1.732	3.563	2.265	71.79	-	-	0.26	2.902	-	0.164	0.36	3.222	1.583	-	-	
		WO	-	-	125-321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
<i>P. sulfincola</i>	JFR	WO	1313	-	4820-77.22	-	-	11.9	154.9-5.947	-	40030-5.3630	-	-	-	-	12060-1.4970	-	-	-	1970-1-1640	4	
		WO	-	-	420-1417	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
		BA	0.4-4.6	-	3.2-15.0	-	0.1-1.9	-	-	-	4.0-28.5	167-389	0.93-5.9	4.7-26.8	0.5-5.9	0.4-0.6	-	0.8-2.7	-	213-506	5	
<i>R. pachypila</i>	13°N EPR	TB	0.6-1.5	-	1.9-5.6	-	0.2-3.1	-	-	10.2-14.0	122-240	2.1	5.4-6.3	0.5-0.7	0.3	-	1.0-1.2	-	-	79-198	-	
		TR	1.5-31.3	-	4.7-20.9	-	0.04-0.26	-	-	-	9.3-126	576-833	11.3-23.0	1.2-16.2	0.7-4.9	0.8-1.4	-	0.9-7.9	-	146-599	-	
		VE	0.6-3.7	-	2.0-8.1	-	0.1-1.9	-	-	-	6.7-28.1	137-188	1.5-6.6	4.2-15.8	0.7-3.5	0.3-0.4	-	1.6-3.4	-	108-230	-	
Guaymas Basin	Guaymas Basin	BA	0.62-0.74	-	6.30-46.35	-	42-1375	0.28-0.35	0.69-2.60	7.88-24.26	92-451	0.258-1.206	17.60-19.26	-	0.05-24.62	8.19-11.30	0.93-4.35	-	231-380	6		
		TR	0.92-3.46	-	7.12-17.75	-	0.34-3.26	1.10-3.14	15.44-3.463	1.10-3.14	15.44-3.463	463-785	1.659-7.779	10.0	0.14-1.10	4.95-6.30	0.41-1.84	-	100-255	-		
		VE	0.43	-	2.25-3.67	-	45-290	0.08-2.00	0.32-0.37	0.41-1.47	7.91-8.71	10-110	1.57-4.362	8.55-15.50	-	0.47-3.07	2.09-5.60	0.34-0.37	-	81.6-10.5	-	
<i>R. piscesae</i>	JFR	TR	-	-	-	-	1.7 \pm 1.1	2.2 \pm 0.8	3.4 \pm 0.6	26.4 \pm 1.4	5.21 \pm 2.35	22.2 \pm 1.2	4.1 \pm 2.3	5.3 \pm 1.9	1.9 \pm 0.5	-	-	-	-	245 \pm 2-4.4	7	
		VE	-	-	-	-	3.7 \pm 1.1	0.7 \pm 0.1	3.4 \pm 0.2	11.6 \pm 5.8	297 \pm 42	22.5 \pm 1.4	6.0 \pm 0.8	8.3 \pm 1.8	2.0 \pm 0.5	-	-	-	-	599 \pm 4-71	4	
		WO	36-112	-	24-296	-	-	-	3.0-15.4	14-87	-	1550-52.20	3.2-7.0	-	-	-	37.1-98.4	20.8	-	650-22-70	4	
<i>B. seepensis</i>	ATJ	WO	-	-	3.8-34.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	
		WO	18.6	-	143-194	-	-	-	2.3-22.8	79.6-22.59	-	2232-17.400	18	-	-	-	33.3-38.5	-	-	1520-6-700	4	
		WO	-	-	-	-	-	-	-	-	29 \pm 9	-	0.4 \pm 0.2	-	-	-	-	-	-	219 \pm 4-3	8	
<i>B. seepensis</i>	ATJ	WO	-	-	-	-	-	-	-	45 \pm 46	-	0.2 \pm 0.2	-	-	-	-	-	-	-	304 \pm 3-25	3	
		WO	-	-	-	-	-	-	-	140 \pm 26	834 \pm 1.25	0.6 \pm 0.1	-	-	-	-	-	-	358 \pm 3-6	9		

measured in external epibionts isolated from *A. pompejana*. Epibionts, not separated in *A. caudata* since not visible at the naked eyes, could not be analysed and their enhanced metal accumulation can only indirectly be hypothesized: in fact, despite concentrations of various elements not being statistically different in trunk body and caudal portion, mean levels were always higher in the region containing epibionts (SM3). Conversely, the internal trophosome of *R. pachyptila* contained metal concentrations generally comparable to those measured in thoracic tissues. The possible role of epibionts or endosymbiotic bacteria in modulating bioavailability, detoxification and excretion of metals cannot be excluded but this hypothesis still remains to be demonstrated (Ruelas-Inzunza et al., 2003, 2005).

Our results on bioaccumulation are comparable to those previously reported for *A. pompejana*, *R. pachyptila*, and other vent polychaetes (Table 3), other than various benthic invertebrates from hydrothermal vents systems (Table 4), including bivalve molluscs, gastropods and crustaceans (Roesijadi et al., 1985; Smith and Flegal, 1989; Cosson, 1996; Desbruyères et al., 1998; Ando et al., 2002; Ruelas-Inzunza et al., 2005; Colaço et al., 2006; Kádár et al., 2006a, 2007; Cunha et al., 2008; Demina and Galkin, 2008, 2009; Shmelev et al., 2009; Demina et al., 2013; Lee et al., 2015; Maher et al., 2016). These data highlighted higher metal concentrations in alvinellid polychaetes, but also an elevated variability as a function of geographical area, sampling period and tissues, even within specimens of the same population. Such variability is consistent with the large heterogeneity of vent habitats among mid-ocean ridges, among chimneys within a same vent field and even at micro-habitat scale (Desbruyères et al. 1998; Shmelev et al., 2009). On fast-spreading ridges like the EPR, hydrothermal vent ecosystems are evolving very rapidly: discontinuous geothermal activities, strong geophysical instabilities including eruptions and tectonic movements lead to rapid changes in the chemistry of the high temperature fluids and in the diversity of vent fauna communities, sometimes resulting in the complete removal of existing settlements and subsequent colonization by new communities (Van Dover, 2000), with changes in the pure fluid composition that are reflected in different metal enrichment in habitats (Le Bris and Gaill, 2007). Similar fluctuations of environmental conditions might thus reflect frequent and marked variations in metals bioavailability and accumulation processes.

Previous studies suggested that organisms from hydrothermal vents can regulate the amounts of trace elements in cells, compartmentalizing these into electron dense concretions, granules, vacuoles, or in specific anatomical region, to either facilitate the excretion or limit their biological reactivity (Roesijadi and Creelius, 1984; Cosson-Mannevy et al., 1988; Ruelas-Inzunza et al., 2003; Kádár et al., 2006b). In the present study frozen organisms were used, therefore it was not possible to perform transmission electronic microscopy (TEM), but the distribution of metals between soluble and insoluble forms was achieved by their analysis after differential centrifugation of tissues. This approach confirmed a certain detoxification of these elements, which appeared as mostly insoluble in both tissues of *A. pompejana*. Further insights on the mobility of metals were obtained by applying to the insoluble fraction the procedure typically used to sequentially extract metals from mineral granules of sediments as an indirect evaluation of their bioavailability (Tokalioglu et al., 2000). In our case, this approach was applied to evaluate the chemical strength of already accumulated and insoluble elements. The generally limited contribution of fraction F4 to the total content of various elements indicated that they were not primarily present in the form of mineral granules or concretions. Only branchial appendages contained elevated levels of Hg and Fe in the fraction F4 (80 and 40% of metal burden) and, to a slightly lower extent, of Cd and Cu (approximately 20%). This result could suggest that, branchial tentacles of *A. pompejana* may intercept, directly from the vent fluids, mineral particles like mercury sulphides (Hg_2S or HgS), insoluble Fe oxides, colloidal hydrated oxides and sulphides (FeO , Fe_2O_3 , Fe_3O_4 , $\text{Fe}_2\text{O}_3\cdot\text{H}_2\text{O}$, FeS), in addition to co-precipitated copper

and cadmium sulphides (CuS and CdS); these insoluble particles could not be transported to the trunk body, as revealed by the lower levels of such metals associated with F4 in this anatomical portion. Fraction F3 contained the majority of Cd, Cu, Fe, Pb and Zn accumulated in both the tissues, of Co and Cr in branchial appendages, and of Hg in the thorax, confirming their possibility to bind to sulphides. Iron and manganese oxides can further act as a sink for metals, and reducible elements extracted from fraction F2 were mostly Co and Cr in thoracic tissues and Pb in branchial filaments. Although the sequential extraction procedure does not clarify the nature of cellular ligands and mechanistic pathways of formation, our results highlighted the general tendency of accumulated metals to be in an insoluble form with different levels of sequestration and relative reduction of cellular reactivity. The fraction containing weakly insoluble or exchangeable forms included part of Co and Cr accumulated in the branchial appendages (40–60%), and the majority of arsenic measured in both the tissues (80–90%).

Chemical speciation of this element appeared of particular interest, since various arsenic compounds greatly differ in terms of environmental distribution, bioavailability and biotransformation, toxicological properties and potential biological role (Fattorini and Regoli, 2004; Notti et al., 2007). In the present study, the three polychaete species from hydrothermal vents contained an elevated percentage (30–60%) of arsenic not extractable in methanol. This fraction has typically a much lower contribution (less than 5%) to the total content of arsenic in marine species, while it can be higher in sediments where mineral particles adsorb various arseno-compounds (Fattorini et al., 2006, 2013).

However, levels of methanol-insoluble arsenic were shown to increase by about 5–7 folds in specimens of the Mediterranean tubeworm, *S. spallanzanii* transplanted in a naturally acidified vent system (Ricevuto et al., 2016): in this area, the frequent formation of poorly soluble compounds, such as Fe/Mn oxy-hydrates or Zn hydroxides could bind arsenical compounds, partly explaining the capability of these polychaetes to accumulate such mineral particles under peculiar environmental conditions. In our study, the elevated content of not methanol-extractable As, along with the marked occurrence of this element in a weakly insoluble and easily exchangeable form, could be compatible with the presence of arsenic (probably arsenite) leaching complexes, scarcely soluble in methanol solutions: the nature of similar compounds remains to be elucidated and it might be possibly related to intracellular carbonates or soluble sulphides (Lee and Nriagu, 2002).

Among methanol-soluble forms, the investigated polychaete species exhibited a similar pattern of arsenic chemical speciation. Methylated arsenic as DMA, MMA and TMAO largely dominated with slightly different proportions in various species and tissues, while levels of AsB and AsC ranged between 3 and 20% of total arsenic content. The sum of MMA, DMA and TMAO accounted for 40% of the total As in tissues of *A. pompejana*, a percentage higher than the soluble arsenic determined by differential centrifugation in subcellular and cytosolic fractions (about 15% in the branchial appendages and 25% in the trunk body). These results suggest that also part of methylated compounds can be precipitated into the weakly insoluble F1 fraction. In this respect, methylated arsenic compounds have been shown to adsorb to iron oxides (i.e. goethite, hematite, lepidocrocite, ferrihydrite, magnetite), with a binding affinity much lower than that of the inorganic forms, and thus being easily leachable at low pH (Campbell and Nordstrom, 2014).

Data on arsenic chemical speciation on organisms from hydrothermal vents are restricted to a very limited number of species (Larsen et al., 1997; Taylor et al., 2012; Maher et al., 2016). The shrimp *Rimicaris exoculata* forming swarms around active smoker walls and the scale worm *Branchiopolynoe seepensis* exhibited total arsenic concentrations lower than $30 \mu\text{g}\cdot\text{g}^{-1}$, in form of AsB and AsR, while bivalves of the genus *Bathymodiolus* presented an elevated contribution of unknown arsenic compounds (Taylor et al., 2012). These studies also described a limited methanol extraction efficiency for arsenic,

Table 4
Levels of trace elements in tissues of different benthic invertebrates, including bivalve molluscs, gastropods and crustaceans, from various hydrothermal vent systems. Data are expressed according to the original investigations as absolute levels, range of values or mean concentrations \pm s.d. ($\mu\text{g/g}$ d.w.). Acronyms: DG = digestive gland; GL = gills; MT = mantle; MU = muscle; WO = whole organisms; ATJ = Azores Triple Junction; EPR = East Pacific Rise. References: (1) Demina and Galkin, 2009; (2) Colaço et al., 2006; (3) Demina and Galkin, 2008; (4) Kádár et al., 2006a; (5) Kádár et al., 2006a; (6) Demina et al., 2007; (7) Smith and Flegal, 1989; (8) Roesijadi et al., 1985; (9) Ruelas-Inzunza et al., 2003.

Species	Area	Tissue	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Se	V	Zn	Ref.	
<i>A. gigas</i>	Guaymas Basin	GL	1.22	-	1.01	2.80	1.12	1.29	21.4	42.47	284	0.172	8.64	-	1.42	1.34	1.52	-	3110	1	
		MT	7.29	-	4.14	7.25	4.32	0.29	4.03	45.54	452	0.436	11.52	-	4.10	5.40	0.54	-	560		
<i>B. azoricus</i>	ATJ	DG	-	-	-	-	-	-	-	18-52	-	1.3-4.6	-	-	-	-	-	-	40-201	2	
		GL	-	-	-	-	-	-	-	47-70	-	1.0-4.4	-	-	-	-	-	-	106-555		
		MT	-	-	-	-	-	-	-	4-13	-	0.3-0.8	-	-	-	-	-	-	46-68		
		DG	3.69	-	0.49	-	-	-	0.58	0.37	29.5	107.5	0.47	1.91	2.2	7.0	3.67	0.62	-	62.3	3
		GL	3.19-3.75	-	6.6-50.0	-	1.24-1.37	-	0.44-7.5	1.05-4.6	124-424	233-424	0.41-0.99	5.06-25.4	22.5-39.0	16.3-21.0	1.19-2.0	1.53-3.81	-	162-229	
		MT	0.37-1.34	-	1.82-6.73	-	0.54-1.07	-	0.62-4.4	1.65-2.1	20.5-21.2	63-1513	0.075-0.080	10.1-21.9	10.1-24.9	5.9-15.3	0.36-0.6	0.84-2.52	-	54.8-13.2	
		MU	0.21	-	6.10	-	0.48	-	4.32	1.77	14.6	723.6	0.091	20.92	38.51	13.12	0.46	2.57	-	81.1	
		VM	0.50-1.49	-	1.07-12.5	-	1.33-2.25	-	0.24-3.9	0.34-2.5	19.5-24.0	124-123	0.15-0.33	2.8-16.5	20.3-32.7	5.9-17.0	0.39-0.8	0.82-3.72	-	76.7-93.5	
		DG	-	-	5.9-28.8	-	-	-	13.3-2.89	0.51-2.0	-	-	1	2.16-12.50	5.9-17.7	-	-	-	-	-	4
		GL	-	-	1.61-8.43	-	-	-	2.25-2.86	0.18-0.9	-	-	-	2.42-8.23	4.0-24.6	-	-	-	-	-	
<i>B. puteoserpentis</i>	ATJ	GL	0.46 \pm 0.15	-	29.4 \pm 6.7	-	2.62 \pm 1.50	3.91 \pm 1.30	4.09 \pm 0.83	380 \pm 69	6541 \pm 995	2.07 \pm 0.55	35.2 \pm 15.1	8.7 \pm 5	7.95 \pm 2.70	0.38 \pm 0.11	8.65 \pm 1.32	-	258 \pm 9	6	
		MT	0.23 \pm 0.08	-	2.25 \pm 1.05	-	0.10 \pm 0.04	4.42 \pm 1.63	0.76 \pm 0.37	27.1 \pm 5	1012 \pm 305	1012 \pm 305	0.240 \pm 0.115	8.0 \pm 3.6	2.0 \pm 0.6	7.42 \pm 2.78	0.24 \pm 0.11	3.02 \pm 1.62	95.2 \pm 18.3		
		VM	0.57 \pm 0.23	-	2.43 \pm 0.55	-	0.27 \pm 0.09	3.43 \pm 0.95	2.88 \pm 1.10	85.1 \pm 3	1495 \pm 438	1495 \pm 438	0.221 \pm 0.063	5.6 \pm 2.9	2.2 \pm 0.9	0.05 \pm 0.03	0.31 \pm 0.17	2.64 \pm 0.70	93.6 \pm 22.9		
		GL	54.43	-	145.8	-	63.92	5.02	2.35	975.1	37495	37495	0.185	102.5	4.5	407.7	37.3	1.49	-	57090	3
<i>B. thermophilus</i>	Galapagos Rift	MT	2.49	-	5.18	-	11.78	0.84	0.37	170.1	6092	0.031	11.1	3.7	17.7	2.04	0.61	-	4113		
		VM	1.77	-	9.82	-	8.98	0.22	0.26	134.9	5871	0.032	10.1	29	10.8	2.16	0.34	-	1781		
<i>C. magnifica</i>	EPR	WO	14.5 \pm 6.95	-	14.6 \pm 7.88	-	-	-	11.2 \pm 0.9	-	2500 \pm 5680	-	111 \pm 0.07	-	-	-	4.05 \pm 1.24	-	89.2 \pm 24.6	7	
		WO	20.4 \pm 2.0	-	24.8 \pm 2.8	-	9.8 \pm 0.8	-	16.6 \pm 1.3	148 \pm 10	760 \pm 40	0.41 \pm 0.08	-	-	6.0 \pm 2.4	-	3.6 \pm 0.9	-	2152 \pm 495	8	
<i>N. fretatae</i>	Galapagos Rift	WO	27.9 \pm 14.3	-	14.9 \pm 5.57	-	-	3.9 \pm 0.77	-	393 \pm 28	393 \pm 28	-	38.0 \pm 39.4	-	-	-	7.76 \pm 2.31	-	145 \pm 1.1	7	
		WO	64	-	56.31	-	1440	4.0	0.77	3.93	873	8904	1.574	6.84	-	24.3	13.1	7.3	-	580	1
<i>Alvinocaris</i> sp.	ATJ	WO	0.46 \pm 0.17	-	7.26 \pm 2.51	-	1.28 \pm 0.47	8.32 \pm 2.75	0.13 \pm 0.05	4982 \pm 182	6534 \pm 1435	0.185 \pm 0.12	104 \pm 7	6.6 \pm 3	10.78 \pm 2.85	0.29 \pm 0.05	5.07 \pm 1.45	-	565 \pm 81	6	
		MU	0.04 \pm 0.02	-	5.3 \pm 3.1	-	0.96 \pm 0.50	6.49 \pm 3.90	6.26 \pm 0.53	78.4 \pm 5.1	1700 \pm 521	0.02 \pm 0.01	7.6 \pm 3.8	2.6 \pm 1.4	4.24 \pm 1.43	0.57 \pm 0.27	9.89 \pm 5.00	-	41.8 \pm 15.2	3	
		VM	0.47 \pm 0.20	-	21.4 \pm 4.8	-	3.29 \pm 1.38	163 \pm 1.7	3.99 \pm 0.47	2535 \pm 896	16103 \pm 4308	0.041 \pm 0.013	21.3 \pm 8.4	2.1 \pm 0.3	12.08 \pm 2.65	1.47 \pm 0.43	3.11 \pm 0.48	-	47.9 \pm 18.3		

(continued on next page)

Table 4 (continued)

Species	Area	Tissue	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Sb	Se	V	Zn	Ref.	
<i>M. fortunata</i>	ATJ	DG	-	-	-	-	-	-	-	-	-	0.7-1.3	-	-	-	-	-	-	-	-	2
		MU	-	-	-	-	-	-	-	-	46	-	0.08-1.0	-	-	-	-	-	-	58	3
		MU	3.11	-	19.1	-	0.81	186.2	2.52	2.997	2227	2227	0.058	55.18	60.4	0.47	0.74	4.34	-	401	3
		WO	-	-	-	-	-	-	-	900-124	2461-10-713	2461-10-713	0.09-4.0	-	-	-	-	-	-	1249-3-700	5
		WO	-	-	-	-	-	-	-	-	3	713	-	-	-	-	-	-	-	-	-
<i>M. abisca</i>	Guaymas Basin	WO	1.83	-	3.92	125	4.32	0.36	1.72	122	107	0.245	367	-	7.06	0.36	1.07	-	52.8	1	
		DG	-	-	-	-	-	-	-	-	109 ± 6	-	0.14 ± -	-	-	-	-	-	-	207 ± 2-00	2
<i>R. exoculata</i>	ATJ	MU	-	-	-	-	-	-	-	52 ± 7	-	0.11	-	-	-	-	-	-	-	58 ± 10	10
		MU	-	-	-	-	-	-	-	-	52 ± 7	-	0.17 ± -	-	-	-	-	-	-	-	-
		MU	0.13-0-63	-	1.72-7-51	-	0.62-51	0.26-13-6	2.4-4.8	33.3-141	90-281	33.3-141	0.065-0-0.069	100-140	22.9-30-2	0.19-0-48	0.15-0.21	0.56-6-0.08	-	3.9-370	3
		VM	0.34	-	4.82	-	165.8	0.36	17.7	555.0	1250	1250	0.036	12.45	14.9	2.32	0.33	1.70	-	42.85	6
		WO	2.43 ± 1.05	-	3.44 ± 2.00	-	0.82 ± 0.30	4.42 ± 1.52	2.08 ± 1-0.0	636 ± 2-75	4580 ± 1734	4580 ± 1734	0.114 ± 0.010	50.0 ± 10.5	21.6 ± 10.3	7.33 ± 3.15	0.03 ± 0-0.01	4.93 ± 1.63	-	260 ± 8-7	6
<i>S. mesatlantica</i>	ATJ	WO	-	-	-	-	-	-	-	825 ± 70	33500-± 3600	0.07	-	-	-	-	-	-	-	2126 ± -459	5
		DG	-	-	-	-	-	-	-	150-285-0	-	0.20-1-40	-	-	-	-	-	-	-	108-439	2
		MU	-	-	-	-	-	-	-	47-125	-	0.22-0-67	-	-	-	-	-	-	-	111-271	3
		GL	2.67	-	28.42	-	3.68	0.42	4.26	789.2	3000	16.59	0.080	6.00	101.2	1.07	0.42	-	-	1321	3
		MU	0.68	-	27.50	-	0.008	0.15	0.34	29.6	460.0	29.30	0.043	17.97	5.14	0.76	1.38	-	-	480.5	3
<i>V. gigas</i>	Guaymas Basin	VM	2.98	-	12.65	-	0.20	0.51	0.69	247.4	150.4	0.046	103.1	6.26	9.78	0.27	0.94	-	-	290.3	5
		WO	-	-	-	-	-	-	-	7.0-3658	190-170-0	1.4-3.0	-	-	-	-	-	-	-	144-869	5
		GL	-	-	-	-	115 ± -196	-	-	-	8.3 ± 5.8	403 ± 2-42	4.96 ± -2.60	18.0 ± -17.0	2.9 ± 2-5	-	-	-	-	845 ± 1-042	9
		MT	-	-	-	-	12.3 ± -5.5	-	-	-	29.7 ± 1-1.1	278 ± 27	1.1 ± 0-2	10.6 ± -1.0	3.7 ± 1-8	-	-	-	-	419 ± 7-9	9
		MT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

suggesting the presence of precipitated inorganic compounds, originated from vent fluids, similarly to our previous hypothesis on tube-dwelling polychaetes (Larsen et al., 1997; Taylor et al., 2012). An elevated contribution of methanol-insoluble arsenic compounds (30–70%) was also detected in tissues of the alvinellids *Paralvinella sulfincola*, *P. palmiformis* and of the vestimentiferan *Ridgeia piscesae* from Juan the Fuca Ridge hydrothermal vent system, Northwest Pacific (Maher et al., 2016). Although the investigation was carried out on the whole tissues, not highlighting possible differences between branchial appendages and the trunk body, more elevated arsenic levels were observed in the alvinellids (up to about 1400 $\mu\text{g}\cdot\text{g}^{-1}$) compared to *R. piscesae* (lower than 35 $\mu\text{g}\cdot\text{g}^{-1}$). Xanes spectroscopy allowed to hypothesize the presence of inorganic arsenic associated to glutathione (GSH) and arsenopyrite into the insoluble fraction, while extractable arsenic was dominated by inorganic compounds in *P. sulfincola* and *P. palmiformis*, and by methylated arsenicals in the vestimentiferan species (Maher et al., 2016).

The presence of inorganic arsenic or methylated compounds in hydrothermal vents polychaetes differs from the general tendency of marine organisms to accumulate arsenic mostly as AsB, AsC or arsenoribosides (Fattorini and Regoli, 2004). In particular, the predominance of DMA observed in this study in tissues of *A. pompejana*, *A. caudata* and *R. pachyptila* suggests an efficient biomethylation of inorganic arsenic occurring in these organisms, where MMA could represent an intermediate reaction product. Methylation of arsenic usually involves an initial reduction of pentavalent arsenicals (AsV) to arsenite (AsIII) (Vahter, 2002) but, considering the reducing conditions at the vent systems, the occurrence of already available arsenite might facilitate this process. The capability to produce DMA from methylation of inorganic arsenic or degradation of more complex arsenic compounds has been demonstrated for the first time in the Mediterranean sabellid *S. spallanzanii*, which contains approximately 85% of the total arsenic as DMA (Notti et al., 2007). DMA is a relatively toxic molecule and its elevated bioaccumulation in branchial crowns (approximately 800 $\mu\text{g}\cdot\text{g}^{-1}$) has been explained as a deterrence mechanism against predation in more exposed tissues, which are unpalatable for typical consumers of *S. spallanzanii* (Fattorini and Regoli, 2004; Fattorini et al., 2005).

The antipredatory function of DMA in branchial tissues may not be necessary in the thoracic portion of the body, which is protected from predation by the tube, thus explaining the more limited content of DMA in this tissue observed both in the vent polychaetes analysed in the present study and in *S. spallanzanii* (Fattorini and Regoli, 2004). Unpalatable branchial appendages have been demonstrated in several tube-dwelling polychaetes (Kicklighter and Hay, 2006, 2007), suggesting that chemical deterrence might be a quite common strategy against predatory pressure especially in those species that are sedentary, overt and occurring on hard substrates (Kicklighter and Hay, 2007). In this respect, other sabellids exhibited much more elevated levels of toxic chemicals in unpalatable branchial crowns compared to thoracic tissues: *Branchiommabairdi* and *B. luctuosum* from Mediterranean also accumulate high levels of arsenic (Giangrande et al., 2016), while *Pseudopotamilla ocellata* from Japanese coasts, *Perkinsiana littoralis* from Antarctica, *Myxicola infundibulum* and *Megalomma lanigera* hyper-accumulated vanadium, up to 10,000 $\mu\text{g}\cdot\text{g}^{-1}$ (Ishii et al., 1994; Kicklighter and Hay, 2007; Fattorini et al., 2010; Giangrande et al., 2016).

Although the biotransformation of inorganic arsenic to methylated compounds might represent a detoxification mechanism in polychaetes from hydrothermal vents, the high levels of arsenic concentrated in a relatively toxic form within the branchial appendages of alvinellids may support the possibility that also these species have developed a chemical defence strategy against predation, representing an interesting hypothesis for these extreme environments. Predatory fish and crabs occur in high densities around hydrothermal vents communities, and the constraining environmental conditions have been thought to be the primary factors limiting the impact by generalist consumers on such dense patches of biotic resources (Kicklighter et al., 2004). Adapted

vent endemic species like the crabs *Cyanagrea praedator* and *Bythograea thermydron* can nevertheless exert a significant pressure, when milder conditions are encountered on hydrothermal chimneys with declining activities. Palatability experiments on deep-sea hydrothermal vent species evidenced the rejection of some polychaete tissues by shallow water consumers, suggesting the presence of chemical deterrents responsible for such distastefulness (Kicklighter et al., 2004): hydrogen sulphide did not appear to prevent feeding, while bacteria were suggested to possibly produce some metabolites to defend the host and its associates. Despite the occurrence and significance of chemical defence against predation remains to be fully elucidated in hydrothermal vents organisms, our results would provide new insights on the role of toxic metals concentrated in more exposed tissues.

In conclusion, this study provided new insights on metal accumulation in tissues of tube-dwelling polychaetes from hydrothermal vents, highlighting interesting differences that might be related to either environmental or species-specific peculiarities. Based on our personal knowledge, the chemical speciation of the arsenic compounds in such organisms represents a first contribution on the possible biological and ecological role of methylated arsenicals as antipredatory mechanism in hydrothermal vents polychaetes.

Acknowledgments

The study was financially supported by NSF grants OCE 11-36451 and OCE 11-24141 to CV. Special thanks to captain and crew of the R/V Atalante and to the operation group of Nautile (Ifremer/GENAVIR) for the MESCAL cruise.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.marenvres.2017.10.003>.

References

- Ando, T., Yamamoto, M., Tomiyasu, T., Hashimoto, J., Miura, T., Nakano, A., Akiba, S., 2002. Bioaccumulation of mercury in a vestimentiferan worm living in Kagoshima Bay, Japan. *Chemosphere* 49, 477–484. [http://dx.doi.org/10.1016/S0045-6535\(02\)00291-6](http://dx.doi.org/10.1016/S0045-6535(02)00291-6).
- Bocchetti, R., Fattorini, D., Gambi, M.C., Regoli, F., 2004. Trace metal concentrations and susceptibility to oxidative stress in the polychaete *Sabella spallanzanii* (Gmelin) (Sabellidae): potential role of antioxidants in revealing stressful environmental conditions in the mediterranean. *Arch. Environ. Contam. Toxicol.* 46, 353–361. <http://dx.doi.org/10.1007/s00244-003-2300-x>.
- Campbell, K.M., Nordstrom, D.K., 2014. Arsenic speciation and sorption in natural environments. *Rev. Mineral. Geochem.* 79, 185–216. <http://dx.doi.org/10.2138/rmg.2014.79.3>.
- Carbotte, S.M., MacDonald, K.C., 1992. East Pacific Rise 8°10'30"N: evolution of ridge segments and discontinuities from SeaMARC II and three-dimensional magnetic studies. *J. Geophys. Res.* 97, 6959–6982. <http://dx.doi.org/10.1029/91JB03065>.
- Cavanaugh, C.M., 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats. *Nature* 302, 58–61. <http://dx.doi.org/10.1038/302058a0>.
- Colao, A., Bustamante, P., Fouquet, Y., Sarradin, P.M., Serrão-Santos, R., 2006. Bioaccumulation of Hg, Cu, and Zn in the Azores triple junction hydrothermal vent fields food web. *Chemosphere* 65, 2260–2267. <http://dx.doi.org/10.1016/j.chemosphere.2006.05.034>.
- Cosson, R.P., 1996. Bioaccumulation of mineral elements within the vestimentiferan tube worm *Riftia pachyptila* (Jones): a review. *Oceanol. Acta* 19, 163–176.
- Cosson-Mannevy, M.A., Cosson, R.P., Gail, F., Laubier, L., 1988. Transfer, accumulation and regulation of minerals in hydrothermal vent organisms. *Oceanol. Acta*, sp 219–226.
- Childress, J.J., Fisher, C.R., 1992. The biology of hydrothermal vent animals: physiology, biochemistry, and autotrophic symbioses. *Oceanogr. Mar. Biol. Ann. Rev.* 30, 337–441.
- Cunha, L., Amaral, A., Medeiros, V., Martins, G.M., Wallenstein, F.F.M.M., Couto, R.P., Neto, A.I., Rodrigues, A., 2008. Bioavailable metals and cellular effects in the digestive gland of marine limpets living close to shallow water hydrothermal vents. *Chemosphere* 71, 1356–1362. <http://dx.doi.org/10.1016/j.chemosphere.2007.11.022>.
- Demina, L.L., Galkin, S.V., 2008. On the role of abiogenic factors in the bioaccumulation of heavy metals by the hydrothermal fauna of the Mid-Atlantic Ridge. *Oceanology* 48, 847–860. <http://dx.doi.org/10.1134/S0001437008060040>.
- Demina, L.L., Galkin, S.V., 2009. Geochemical features of heavy metal bioaccumulation in

- the Guaymas Basin of the Gulf of California. *Oceanology* 49, 697–706. <http://dx.doi.org/10.1134/S0001437009050117>.
- Demina, L.L., Holm, N.G., Galkin, S.V., Lein, A.Yu, 2013. Some features of the trace metal biogeochemistry in the deep-sea hydrothermal vent fields (Menez Gwen, Rainbow, Broken Spur at the MAR and 9°50'N at the EPR): a synthesis. *J. Mar. Syst.* 126, 94–105. <http://dx.doi.org/10.1016/j.jmarsys.2012.09.005>.
- Desbruyères, D., Almeida, A., Biscoito, M., Comtet, T., Khripounoff, A., Le Bris, N., Sarradin, P.M., Segonzac, M., 2000. A review of the distribution of hydrothermal vent communities along the northern Mid-Atlantic Ridge: dispersal vs environmental controls. *Hydrobiologia* 440, 201–216. http://dx.doi.org/10.1007/978-94-017-1982-7_19.
- Desbruyères, D., Chevaldonné, P., Alayse, A.-M., Jollivet, D., Lallier, F.H., Jouin-Toulmond, C., Zal, F., Sarradin, P.-M., Cosson, R., Caprais, J.-C., Arndt, C., O'Brien, J., Guézennec, J., Hourdez, S., Riso, R., Gaill, F., Laubier, L., Toulmond, A., 1998. Biology and ecology of the 'Pompeii worm' (*Alvinella pompejana* Desbruyères and Laubier), a normal dweller of an extreme deep-sea environment: a synthesis of current knowledge and recent developments. *Deep-Sea Res. Pt II* 45, 383–422. [http://dx.doi.org/10.1016/S0967-0645\(97\)00083-0](http://dx.doi.org/10.1016/S0967-0645(97)00083-0).
- Di Meo-Savoie, C.A., Luther III, G.W., Cary, S.C., 2004. Physicochemical characterization of the microhabitat of the epibionts associated with *Alvinella pompejana*, a hydrothermal vent annelid. *Geochim. Cosmochim. Ac.* 68, 2055–2066. <http://dx.doi.org/10.1016/j.gca.2003.10.039>.
- Fattorini, D., Regoli, F., 2004. Arsenic speciation in tissues of the Mediterranean polychaete *Sabella spallanzanii*. *Environ. Toxicol. Chem.* 23, 1881–1887. <http://dx.doi.org/10.1897/03-562>.
- Fattorini, D., Notti, A., Halt, M.N., Gambi, M.C., Regoli, F., 2005. Levels and chemical speciation of arsenic in polychaetes: a review. *Mar. Ecol. Prog. Ser.* 26, 255–264. <http://dx.doi.org/10.1111/j.1439-0485.2005.00057.x>.
- Fattorini, D., Notti, A., Nigro, M., Regoli, F., 2010. Hyperaccumulation of vanadium in the Antarctic polychaete *Perkinsiana littoralis* as a natural chemical defense against predation. *Environ. Sci. Pollut. Res.* 17, 220–228. <http://dx.doi.org/10.1007/s11356-009-0243-0>.
- Fattorini, D., Notti, A., Regoli, F., 2006. Characterization of arsenic content in marine organisms from temperate, tropical, and polar environments. *Chem. Ecol.* 22, 405–414. <http://dx.doi.org/10.1080/02757540600917328>.
- Fattorini, D., Sarkar, S.K., Regoli, F., Bhattacharya, B.D., Rakshit, D., Satpathy, K.K., Chatterjee, M., 2013. Levels and chemical speciation of arsenic in representative biota and sediments of a tropical mangrove wetland, India. *Environ. Sci. Process. Impacts* 15, 773–782. <http://dx.doi.org/10.1039/c3em30819g>.
- Giangrande, A., Licciano, M., Del Pasqua, M., Fanizzi, F.P., Migoni, D., Stabili, L., 2017. Heavy metals in five Sabellidae species (Annelida, Polychaeta): ecological implications. *Environ. Sci. Pollut. Res.* 24, 3759–3768. <http://dx.doi.org/10.1007/s11356-016-8089-8>.
- Gibbs, P.E., Bryan, G.W., Ryan, K.P., 1981. Copper accumulation by polychaete *Melinna palmata*: an antipredation mechanism? *J. Mar. Biol. Assoc. U. K.* 61, 707–722. <http://dx.doi.org/10.1017/S0025315400048153>.
- Hessler, R.R., Smithey, W.M., Boudrias, M.A., Keller, C.H., Lutz, R.A., Childress, J.J., 1988. Temporal change in megafauna at the Rose Garden hydrothermal vent (Galapagos Rift; eastern tropical Pacific). *Deep-Sea Res.* 35, 1681–1711. [http://dx.doi.org/10.1016/0198-0149\(88\)90044-1](http://dx.doi.org/10.1016/0198-0149(88)90044-1).
- Ishii, T., Otake, T., Okoshi, K., Nakahara, M., Nakamura, R., 1994. Intracellular localization of vanadium in the fan worm. *Pseudopotamilla Ocellata*. *Mar. Biol.* 121, 143–151. <http://dx.doi.org/10.1007/BF00349483>.
- Juniper, S.K., Thompson, J.A.J., Calvert, S.E., 1986. Accumulation of minerals and trace elements in biogenic mucus at hydrothermal vents. *Deep-Sea Res.* 33, 339–347. [http://dx.doi.org/10.1016/0198-0149\(86\)90095-6](http://dx.doi.org/10.1016/0198-0149(86)90095-6).
- Kádár, E., Costa, V., Martins, I., Santos, R.S., Powell, J.J., 2005. Enrichment in trace metals (Al, Mn, Co, Cu, Mo, Cd, Fe, Zn, Pb and Hg) of macro-invertebrate habitats at hydrothermal vents along the Mid-Atlantic Ridge. *Hydrobiologia* 548, 191–205. <http://dx.doi.org/10.1007/s10750-005-4758-1>.
- Kádár, E., Costa, V., Santos, R.S., 2006a. Distribution of micro-essential (Fe, Cu, Zn) and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. *Sci. Total. Environ.* 358, 143–150. <http://dx.doi.org/10.1016/j.scitotenv.2005.09.003>.
- Kádár, E., Santos, R.S., Powell, J.J., 2006b. Biological factors influencing tissue compartmentalization of trace metals in the deep-sea hydrothermal vent bivalve *Bathymodiolus azoricus* at geochemically distinct vent site of the Mid-Atlantic Ridge. *Environ. Res.* 101, 221–229. <http://dx.doi.org/10.1016/j.envres.2005.08.010>.
- Kádár, E., Costa, V., Segonzac, M., 2007. Trophic influences of metal accumulation in natural pollution laboratories at deep-sea hydrothermal vents of the Mid-Atlantic Ridge. *Sci. Total. Environ.* 373, 464–472. <http://dx.doi.org/10.1016/j.scitotenv.2006.12.022>.
- Kicklighter, C.E., Hay, M.E., 2006. Integrating prey defensive traits: contrasts of marine worms from temperate and tropical habitats. *Ecol. Monogr.* 76, 195–215. [http://dx.doi.org/10.1890/0012-9615\(2006\)076\[0195:IPDTCO\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9615(2006)076[0195:IPDTCO]2.0.CO;2).
- Kicklighter, C.E., Hay, M.E., 2007. To avoid or deter: interactions among defensive and escape strategies in sabellid worms. *Oecologia* 151, 161–173. <http://dx.doi.org/10.1007/s00442-006-0567-0>.
- Kicklighter, C.E., Fisher, C.R., Hay, M.E., 2004. Chemical defense of hydrothermal vent and hydrocarbon seep organisms: a preliminary assessment using shallow-water consumers. *Mar. Ecol.-Prog. Ser.* 275, 11–19. <http://dx.doi.org/10.3354/meps275011>.
- Larsen, E.H., Quérel, C.R., Munoz, R., Fiala-Medioni, A., Donard, O.F.X., 1997. Arsenic speciation in shrimp and mussel from the Mid-Atlantic hydrothermal vents. *Mar. Chem.* 57, 341–346. [http://dx.doi.org/10.1016/S0304-4203\(97\)00041-8](http://dx.doi.org/10.1016/S0304-4203(97)00041-8).
- Le Bris, N., Gaill, F., 2007. How does the annelid *Alvinella pompejana* deal with an extreme hydrothermal environment. *Rev. Environ. Sci. Biotechnol.* 6, 197–221. http://dx.doi.org/10.1007/978-1-4020-6285-8_20.
- Le Bris, N., Sarradin, P.M., Caprais, J.C., 2003. Contrasted sulphide chemistries in the environments of 13°N EPR vent fauna. *Deep-Sea Res. Pt I* 50, 737–747. [http://dx.doi.org/10.1016/S0967-0637\(03\)00051-7](http://dx.doi.org/10.1016/S0967-0637(03)00051-7).
- Le Bris, N., Zbinden, M., Gaill, F., 2005. Processes controlling the physico-chemical microenvironments associated with Pompeii worms. *Deep-Sea Res. Pt I* 52, 1071–1083. <http://dx.doi.org/10.1016/j.dsr.2005.01.003>.
- Le Bris, N., Lallier, F., 2010. MESCAL-LEG1 Cruise, RV L'Atalante. <http://dx.doi.org/10.17600/10010030>.
- Lee, J.S., Nriagu, J.O., 2002. Arsenic carbonate complexes in aqueous systems. In: biogeochemistry of environmentally important trace elements. *Am. Chem. Soc. chapter* 3, 33–41. <http://dx.doi.org/10.1021/bk-2003-0835.ch003>. ISBN: 9780841219373.
- Lee, S., Kim, S., Ju, S., Pak, S., Son, S., Han, S., 2015. Mercury accumulation in hydrothermal vent mollusks from the southern Tonga Arc, southwestern Pacific Ocean. *Chemosphere* 127, 246–253. <http://dx.doi.org/10.1016/j.chemosphere.2015.01.006>.
- Maher, W.A., Duncan, E., Dilly, G., Foster, S., Krikowa, F., Lombi, E., Scheckel, K., Girguis, P., 2016. Arsenic concentrations and species in three hydrothermal vent worms, *Ridgeia piscesae*, *Paralvinella sulficola* and *Paralvinella palmiformis*. *Deep-Sea Res. Pt I* 116, 41–48. <http://dx.doi.org/10.1016/j.dsr.2016.07.009>.
- Notti, A., Fattorini, D., Razzetti, E.M., Regoli, F., 2007. Bioaccumulation and biotransformation of arsenic in the Mediterranean polychaete *Sabella spallanzanii*: experimental observations. *Environ. Toxicol. Chem.* 26, 1186–1191. <http://dx.doi.org/10.1897/06-362R.1>.
- Okoro, H.K., Fatoki, O.S., Adekola, F.A., Ximba, B.J., Snyman, R.G., 2014. Fractionation, mobility and multivariate statistical evaluation of metals in marine sediments of Cape Town Harbour, South Africa. *Chem. Speciat. Bioavailab.* 26, 126–138. <http://dx.doi.org/10.3184/095422914X14038001068544>.
- Ricevuto, E., Lanzoni, I., Fattorini, D., Regoli, F., Gambi, M.C., 2016. Arsenic speciation and susceptibility to oxidative stress in the fanworm *Sabella spallanzanii* (Gmelin) (Annelida, Sabellidae) under naturally acidified conditions: an in situ transplant experiment in a Mediterranean CO2 vent system. *Sci. Total Environ.* 544, 765–773. <http://dx.doi.org/10.1016/j.scitotenv.2015.11.154>.
- Roesijadi, G., Crecelius, E.A., 1984. Elemental composition of the hydrothermal vent clam *Calyptogena magnifica* from the East Pacific Rise. *Mar. Biol.* 83, 155–161. <http://dx.doi.org/10.1007/BF00394723>.
- Roesijadi, G., Young, J.S., Crecelius, E.A., Thomas, L.E., 1985. Distribution of trace metals in the hydrothermal vent clam *Calyptogena Magnif.* *Bull. Biol. Soc. Wash* 6, 311–324.
- Rona, P.A., Klinkhammer, G., Nelsen, T.A., Tefry, J.H., Elderfield, H., 1986. Black smokers, massive sulphides and vent biota at the Mid-Atlantic Ridge. *Nature* 321, 33–37. <http://dx.doi.org/10.1038/321033a0>.
- Ruelas-Inzunza, J., Soto-González, L., Páez-Osuna, F., 2003. Heavy metal accumulation in the hydrothermal vent clam *Vesicomya gigas* from Guaymas basin, Gulf of California. *Deep-Sea Res. Pt I* 50, 757–761. [http://dx.doi.org/10.1016/S0967-0637\(03\)00054-2](http://dx.doi.org/10.1016/S0967-0637(03)00054-2).
- Ruelas-Inzunza, J., Páez-Osuna, F., Soto, L.A., 2005. Bioaccumulation of Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn in trophosome and vestimentum of the tube worm *Riftia pachytila* from Guaymas basin, Gulf of California. *Deep-Sea Res. Pt I* 52, 1319–1323. <http://dx.doi.org/10.1016/j.dsr.2005.03.001>.
- Sarradin, P.M., Lannuzel, D., Waeles, M., Crassous, P., Le Bris, N., Caprais, J.C., Fouquet, Y., Fabri, M.C., Riso, R., 2008. Dissolved and particulate metals (Fe, Zn, Cu, Cd, Pb) in two habitats from an active hydrothermal field on the EPR at 13 degrees N. *Sci. Total Environ.* 392, 119–129. <http://dx.doi.org/10.1016/j.scitotenv.2007.11.015>.
- Shmelev, I.P., Kuznetsov, A.A., Galkin, S.V., 2009. Heavy metals in the benthic animals from hydrothermal vents: results of neutron activation analysis. *Oceanology* 49, 429–431. <http://dx.doi.org/10.1134/S0001437009030151>.
- Smith, D.R., Flegal, A.R., 1989. Elemental concentrations of hydrothermal vent organisms from the Galapagos Rift. *Mar. Biol.* 102, 127–133. <http://dx.doi.org/10.1007/BF00391330>.
- Stewart, F.J., Newton, I.L.G., Cavanaugh, C.M., 2005. Chemosynthetic endosymbioses: adaptations to oxic-anoxic interfaces. *Trends Microbiol.* 13, 439–448. <http://dx.doi.org/10.1016/j.tim.2005.07.007>.
- Taylor, V.F., Jackson, B.P., Siegfried, M.R., Navratilova, J., Francesconi, K.A., Kirshtein, J., Voytek, M., 2012. Arsenic speciation in food chains from mid-Atlantic hydrothermal vents. *Environ. Chem.* 9, 130–138. <http://dx.doi.org/10.1071/EN11134>.
- Tokalioglu, S., Kartal, S., Elçi, L., 2000. Determination of heavy metals and their speciation in lake sediments by flame atomic absorption spectrometry after a four-stage sequential extraction procedure. *Anal. Chim. Acta* 413, 33–40. [http://dx.doi.org/10.1016/S0003-2670\(00\)00726-1](http://dx.doi.org/10.1016/S0003-2670(00)00726-1).
- Van Dover, C.L., 2000. *The Ecology of Deep-sea Hydrothermal Vents*. Princeton University Press ISBN: 0691049297, 424 pages.
- Van Dover, C.L., Humphris, S.E., Fornari, D., Cavanaugh, C.M., Collier, R., Goffredi, S.K., Hashimoto, J., Littey, M.D., Reysenbach, A.L., Shank, T.M., Von Damm, K.L., Banta, A., Gallant, R.M., Götze, D., Green, D., Hall, J., Harmer, T.L., Hurtado, L.A., Johnson, P., McKiness, Z.P., Meredith, C., Olson, E., Pan, I.L., Turnipseed, M., Won, Y., 2001. Biogeography and ecological setting of Indian Ocean hydrothermal vents. *Science* 294, 818–823. <http://dx.doi.org/10.1126/science.1064574>.
- Vahter, M., 2002. Mechanisms of arsenic biotransformation. *Toxicology* 181–182, 211–217. [http://dx.doi.org/10.1016/S0304-483X\(02\)00285-8](http://dx.doi.org/10.1016/S0304-483X(02)00285-8).
- Wolff, T., 2005. Composition and endemism of the deep-sea hydrothermal vent fauna. *Cah. Biol. Mar.* 46, 97–104.
- Zbinden, M., Le Bris, N., Compère, P., Martinez, I., Guyot, F., Gaill, F., 2003. Mineralogical gradients associated with alvinellids at deep-sea hydrothermal vents. *Deep-Sea Res. Pt I* 50, 269–280. [http://dx.doi.org/10.1016/S0967-0637\(02\)00161-9](http://dx.doi.org/10.1016/S0967-0637(02)00161-9).