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Dissolved and particulate metals (Fe, Zn, Cu, Cd, Pb) in two habitats from an active hydrothermal field on the EPR at 13 degrees N

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Abstract:

The distribution of Fe, Cu, Zn, Pb, Cd between the dissolved (< 2 μm) and the particulate (> 2 μm) fractions was measured after in-situ filtration in two hydrothermal habitats. The total metal concentration ranges exhibit a clear enrichment compared with the seawater concentration, accounting for the hydrothermal input for all the metals considered. Iron is the predominant metal (5–50 μM) followed by Zn and Cu. Cd and Pb are present at the nM level. At the scale studied, the behavior of temperature, pH and dissolved iron is semi-conservative whereas the other dissolved and particulate metals are characterized by non-conservative patterns. The metal enrichment of the > 2 μm fraction results from the settlement and accumulation of particulate matter close to the organisms, acting as a secondary metal source. The enrichment observed in the dissolved fraction can be related to the dissolution or oxidation of particles (mainly polymetallic sulfide) or to the presence of small particles and large colloids not retained on the 2 μm frit. SEM observations indicate that the bulk particulate observed is characteristic of crystalline particles settling rapidly from the high temperature smoker (sphalerite, wurtzite and pyrite), amorphous structures and eroded particles formed in the external zone of the chimney. Precipitation of Zn, Cu, Cd and Pb with Fe as wurtzite, sphalerite and pyrite is the main process taking place within the area studied and is semi-quantitative. The distribution of the dominant observed fauna has been related to the gradient resulting from the dilution process, with the alvinellids worms colonizing the hotter and more variable part of the mixing zone, but also to the metallic load of the mixing zone. Dissolved and particulate metal concentrations are therefore necessary abiotic factors to be studied in a multiparametric approach to understand the faunal distribution in hydrothermal ecosystems.

Keywords: Metals; Dissolved; Particulate; Habitats; Hydrothermal fauna

1. Introduction

Deep-ocean hydrothermalism is the meeting point of deep-seated geological processes with the ocean above resulting in the transfer of heat and chemicals from the earth's deep mantle and the crust, through volcanic and hydrothermal systems. The physical and chemical characteristics of vent emissions are initially the result

of complex rock seawater interactions in the subsurface that form high temperature hydrothermal fluids. These fluids can be subsequently modified by subseafloor and near surface mixing with background seawater leading to a variety of emitted fluids enriched with gases, metals and radionuclides (Bowers et al., 1985; Von Damm, 1988; 1998; Cherry et al., 1992).

Deep-sea hydrothermal communities occupy the interfacial zone where the hot and reduced hydrothermal fluid turbulently mixes with the cold and oxygenated seawater. The mixing zone is characterized by steep chemical gradients (Johnson et al., 1986; Sarradin et al., 1998; Le Bris et al., 2003) and produces mineral precipitation in the rising plume (Feely et al., 1990) or the chimney conduit (Metz and Trefry, 2000). This fluctuating environment provides a periodical access to reduced chemical species from the vent fluid (e.g. H_2S, CH_4) and seawater oxidized compounds which are both required for chemolithoautotrophic bacterial primary production and associated fauna (Childress and Fisher, 1992). Many authors have suggested links between the spatio-temporal distribution of hydrothermal species and the physical and chemical properties of the vent fluids: concentrations of hydrogen sulfide (Urcuyo et al., 2003), flow intensity and substratum type (Sarrazin et al., 1999), speciation and bioavailability of oxygen, iron and sulfur (Luther et al., 2001), particulate fluxes and variability in fluid composition (Desbruyères et al., 2000; 2001).

Hydrothermal species are also subjected to potentially toxic material, such as heavy metals, provided in large concentrations by the hydrothermal fluid (Douville et al., 2002). However, the main processes controlling the metal concentrations in these highly reactive areas and the influence of the metallic load of the mixing zone on species distribution have been poorly documented (Desbruyeres et al., 1998; Geret et al., 1998; 2002; Di meo-Savoie et al. 2004, Kadar et al., 2005). The main conclusion of ecotoxicological papers dealing with hydrothermal vent organisms (Cosson, 1996; Cosson-Manevy et al., 1988; Desbruyères et al., 1998; Ruelas-Inzunza et al., 2003) is the apparent contradiction between the large amounts of metals present in the organisms and the absence of recognizable deleterious effects. These organisms seem to have developed efficient adaptations and detoxification processes such as the sequestration of potentially toxic compounds into forms that are probably inactive: insoluble forms such as granules or concretions and soluble forms as metalloproteins (Cosson and Vivier, 1997; Geret et al, 2002). In consequence, there is a need to assess the metallic composition of these peculiar environments in order to understand its potential impact on the distribution of hydrothermal organisms and the efficiency of the detoxification processes involved.

This study was carried out at the Genesis hydrothermal vent field on the East Pacific Rise. Fe, Cu, Zn, Cd and Pb were analyzed in the environment surrounding hydrothermal organisms. Their distribution between the dissolved (<2µm) and particulate (>2µm) fractions is presented. The objectives were to document the content and behavior of these elements in this part of the mixing zone and to assess the potential link between the metallic load and the faunal distribution in two habitats dominated by alvinellids worms or giant tubeworms (*Riftia pachyptila*).

2. Material and methods

2.1. The Genesis site (13°N, EPR)

This study was conducted during the HOPE 99 cruise on the East Pacific Rise (1999, N/O L'Atalante/ submersible Nautile, Chief scientist F. Lallier) and focused on the Genesis vent field (12°48.63 N, 103°56.41W, depth 2645 m). This site is characterized by the presence of a black smoker emitting a medium temperature (230 – 290°C) hydrothermal fluid (Childress et al., 1993; Sarradin et al., 1998; Le Bris et al., 2003). The fluid may have undergone sub surface phase separation with a major contribution of vapor phase in the emitted fluid, enriched in CO_2, depleted in iron (300 µM) and chloride (Le Bris et al., 2003) compared to the range previously established at 13°N (Von Damm, 1995a). The black smoker (Fig. 1) is 9 m high and is built on the 5 m upright wall oriented NW-SW bordering the western side of the Genesis hydrothermal vent field. The dominant species encountered in the vicinity of the smoker were *Riftia pachyptila* tubeworms and alvinellid worms such as *Alvinella pompejana*, *Alvinella caudata*, *Paralvinella grasslei* and *Hesiolyra bergi* (Desbruyères et al. 1998). *R. pachyptila* tubeworms live in an area (zone R) where the temperature ranges from 3 to 25°C (Sarradin et al., 1998; Le Bris et al., 2003). This area is a vertical crack on the 5 m upright wall a few meters north of the main chimney. Alvinellid worms were found in zone A (temperature 9 to 70°C) 5 meters north of the crack and 2 meters away from the bottom of the wall.

2.2. Sampling and sample treatment

Water samples were collected in the environment surrounding hydrothermal organisms. This environment is formed by the fluctuating mixing of cold seawater with hot hydrothermal fluid. Reference samples were taken at the periphery (c.a. 500 m) of the active area. The sampling device (Fig. 2) consisted of four 200 ml titanium bottles with an autonomous temperature probe (Micrel®) and manipulated by the manned submersible Nautile. The bottles were helium purged and set under vacuum (<2*102 Pa) before use. Each sample inlet (PEEK tubing, 0.8 mm i.d.) was equipped with a PEEK polymer frit (UPCHURCH®, porosity 2 µm) to perform in-situ filtration of the sample. In-situ filtration should prevent/lower any modification of the sample by oxidation / reduction or precipitation phenomena with cooling. The frit porosity was set to 2 µm to overcome a potential explosion of the frit when opening the bottle with a pressure increase of c.a. 250 bars in a few msec. Titanium bottles, polymer frits and frit holders were rinsed with HCl 0.1M and ultrapure water before use. Subsamples devoted to the analysis of dissolved metal (~ 100 mL) were stored acidified (1/1000 V/V HNO3, Merck, suprapur) prior to on shore analysis. The 2 µm frits were dried for 24 h at 85°C and stored prior to on shore mineralization and analysis of the particulate fraction. The mineralization step was conducted in a 3 ml HCl 30% / 1 ml HNO3 65% / 1 ml HF 40 % mixture by heating for 3 hours at 80°C and 1 hour at 100°C. The volume of the obtained solution was corrected by weighing to take into account the loss during the mineralization step. Subsamples were analyzed after dilution in 2% HNO3 (ICP-MS) or suprapure water (Potentiometric Stripping Analysis).

2.3. Analytical methods

pH of the samples was measured on board after submersible recovery using a combined pH electrode (Ingold®) for a sulfide rich medium. Measurements were made at 25°C after calibration with NBS buffers (pH 4 and 7). Sample temperatures were derived from the data recorded by the autonomous temperature probe associated with the sample inlet. Fe concentrations were determined by Flow Injection Analysis according to the procedure detailed in Sarradin et al. (2005). Reproducibility and detection limits are 0.8 % (n=5, 50 µM) and 70 nM respectively. Cu, Pb and Cd levels were measured by using electrochemical methods. Cu measurement was performed by Constant Current Stripping Chonopotentiometry (CCSA) with a gold electrode. Pb and Cd were measured by Potentiometric Stripping Analysis (PSA) with a mercury film electrode. Reproducibilities are 2, 5 and 4% for Cu, Pb and Cd, detection limits are respectively 0.17, 0.01 and 0.01 nM. The procedures has been detailed in Riso et al. (1997a, 1997b) and one of their assets is that they need no UV irradiation to get the total dissolved metal concentrations. Certified sea waters were analysed for metal content prior to the samples (Table 1). Zn concentrations were determined by ICP-MS (Université de La Rochelle, Centre Commun d'Analyses, Varian Ultramass 700). The ICP-MS system operated in peak hopping mode with a dwell time of 40 ms per isotope. Instrumental conditions were: plasma power 1250 W, plasma flow 15 L/min, auxiliary plasma 1.50 L/min, nebulizer flow 0.90 L/min, 15 scans/replicate, 10 replicates/sample. The instrument was calibrated using commercially available aqueous standard solution (Astasol-Mix, Analytika Ltd, purity 99.999%). The quantification was done using the standard addition method to overcome the potential matrix effect. Three internal standards (Y, Rh, Eu) were also added to the samples to validate the results. Reproducibility and detection limits are 4% and 0.15 nM respectively. All reagents were prepared in a clean room.

In order to check whether the samples were free from contamination during sampling and handling, 2 samples were taken ca. 500 m away from the active area. The concentrations of dissolved metals obtained in sample SW1 (Fe < detection limit, Cu 0.01µM, Cd 1.3 nM, Pb 1.5 nM) are close to those currently reported for North Pacific deep waters (Fe 0.01 µM, Cu 0.005µM, Cd 1 nM, Pb 0.005 nM, Donat and Bruland, 1995) except for Pb. The values obtained in sample SW2 are relatively higher (Fe 1.9 µM, Cu 0.02µM, Cd 1.1 nM, Pb 1.5 nM) and can be linked to an input of hydrothermal material or to a limited contamination problem for lead. However, these levels remain below those found in the samples taken in the immediate surrounding of hydrothermal communities which were measured at the following ranges Fe: 1-33 µM, Cu 0.17-1.13 µM, Cd 1.8-8.56 nM, Pb 10.3-61.9 nM.

2.4. Scanning Electronic Microscope analysis and particle characterization

To determine the raw composition and the morphology of minerals on the particle samples, a Philips XL30 scanning electron microscope (SEM) equipped with an EDAX® detector was used at energy of 15 kV. The frits were gold coated prior to their analysis (Balzers® SCD 040). The characterization of the particulate matter was conducted on 2 frits (corresponding to samples R3, T= 4.3°C and A4, T=12.8°C). These 2 samples were supposed to give us a snapshot of the minerals present in the cold and warm part of the mixing zone. In the first stage, 6 analyses were performed on a frit transect at low magnification to establish the raw

composition of the particles and to evaluate the homogeneity of the samples. The second stage was conducted at higher magnification in order to recognize and list the dominant mineral structures and to establish their elemental composition. Elemental analysis was done using the EDAX® detector and was followed by a visual recognition of the structures (Y. Fouquet).

2.4. Statistical methods

Statistical treatments were done using the Statgraphics Plus 5.1 software. Principal components analysis (PCA) is a technique used to reduce multidimensional data sets to lower dimensions for analysis. PCA is mostly used as a tool in exploratory data analysis. A study of the rank correlation (Spearman) was performed prior the PCA to identify the independent variables.

3. Results and discussion

3.1. Temperature and pH

Temperature and pH measurements are shown in table 2. The samples were collected in the cold part of the mixing zone with temperature and pH ranging from 3.8 to 20°C and 7.1 to 5.9, respectively. The corresponding hydrothermal input estimated from the endmember concentrations is limited to 0.6 to 7.9%. The environment of the dominant species colonizing the Genesis vent site is thus not fully represented as the hot part of the mixing zone, preferred habitat of Alvinellid worms, with measured temperature up to 70 or 80°C (Sarradin et al., 1998; Le Bris et al., 2003; Di Meo-Savoie et al., 2004), has not been effectively sampled. However, the samples obtained (Fig. 3a) cover the whole temperature range encountered by the giant tubeworms *Riftia pachyptila* and the colder part of the microhabitat of Alvinellids as can be seen in Fig. 3b. Fig. 3b was obtained using temperature time series measured by autonomous probes (Micrel®) during 2 cruises on the EPR (HOT96 and HOPE99, n = 10478, unpublished data).

3.2. Fe, Zn, Cu, Cd and Pb concentrations

Total metal concentration ranges obtained are presented in table 3 along with those reported for other studies in environments surrounding hydrothermal organisms. Fe is the predominant element with concentrations in the range 5-63 µM. Cu and Zn exhibit levels varying from 0.18 to 1.6 µM and from 0.3 to 27.3 µM, respectively. Cd and Pb were measured at the nM level with values in the range 2-47 and 11-260, respectively. All these concentrations are well above those reported for deep North Atlantic waters (Donat and Bruland, 1995) illustrating a marked metallic enrichment due to hydrothermal inputs. The ranges obtained are comparable though lower than those presented by Desbruyères et al. (1998) in the same area and by Di Meo-Savoie et al. (2004) at 9°N. These two studies were performed in the hot part of the mixing zone colonized by *Alvinella pompejana*. Compared to the studies carried out on the colder habitats of the hydrothermal mussels *Bathymodiolus azoricus* and the shrimps *Rimicaris exoculata* on the mid Atlantic Ridge (Geret et al., 1998; 2002; Sarradin et al., 1999; Kadar et al., 2005), the ranges were again similar. High metallic concentrations were encountered around hydrothermal organisms, bearing in mind the heterogeneity

of the mixing zone and the chemical diversity of hydrothermal fluids (Von Damm, 1995a). The comparison between the dissolved (<2 µm) and particulate (>2µm) fractions indicates that Zn, Fe and Cd are mainly associated with particles whereas Cu and Pb are roughly equally distributed between both fractions. The use of a 0.45-µm filters should enhance the importance of the particulate fraction by incorporating the small particles and large colloids not retained on the 2 µm frits.

3.3. Metal behavior in the mixing zone

The possibility of using temperature or pH as dilution tracer of hydrothermal fluid by seawater (Le Bris et al., 2000) was tested using the T/pH relationship of our samples enriched by published data from Von Damm (1995a) and Le Bris et al. (2003) obtained at the same site. The linear trend obtained (confidence level 99%, Fig. 4) can explain 96% of the variability in the data. This result confirms that at the scale and in the range studied, pH and temperature can be assumed to follow a semi-conservative process and can be used as tracers of the dilution, even though conductive cooling in the subsurface can modify its conservative behavior during mixing.

The amount of dilution of hydrothermal fluid by seawater in the samples (table 2) was derived from this conservative behavior and by using the data published by Le Bris et al. (2003). Assuming a seawater temperature of 1.9°C with no hydrothermal input and a pure fluid temperature in Genesis of 230°C (Le Bris et al. 2003), the amount of hydrothermal fluid in the mixture was estimated as being % fluid = (Temperature -1.9)/2.281.

In order to check whether the concentrations of the metals studied in the mixing zone are controlled by a dilution-like process, dissolved and particulate metal concentrations were plotted against temperature (Fig. 5). Theoretical dilution lines were estimated using the two components of the mixture i.e. the concentrations and temperature of the pure fluid and seawater presented in Table 3. No endmember value was available for Cu. The endmember Fe concentration was taken at 300 µM, most recent value obtained by Le Bris et al. (2003) for this particular vent field.

No trends were observed between metal concentrations and temperature except for dissolved iron. Fed presents a weak but significant relationship with temperature (confidence level 95%) explaining only 30% of the variability. This lack of relationship between metal concentrations and temperature indicates that both dissolved and particulate forms are not controlled by a simple dilution process at the scale studied. By comparing the values obtained with the theoretical dilution line (Fig. 5), it can be noted that particulate metal concentrations are mainly located above the dilution line suggesting the presence of a secondary metal input. This enrichment, which is particularly important for all the metals studied, should result from the continuous settlement and accumulation of particulate matter close to the organisms. These observations are supported by the ability of Cu, Fe, Zn, Cd and Pb to form precipitates with sulfides in plumes, chimney or in conduits surfaces (Trefry and Trocine, 1985; Von Damm et al., 1995b). Iron will form preferentially fine grained sulfides particles that will be exported in the buoyant and neutrally buoyant plume. Cu and Zn sulfides will form large sized grains and more crystalline particles that will settle rapidly in the near field region (Feely et al., 1994). In the dissolved (<2µm) fraction, enrichments observed for Cd and Pb can be explained by the presence of small particles or large colloids not retained on the 2 µm filter. The particles accumulated

(mainly polymetallic sulfides) may also undergo dissolution and/or oxidation reaction in this cold part of the mixing zone which contains dissolved oxygen (Dunk and Mills, 2006). On the contrary, dissolved Fe and Zn are close to or below the theoretical dilution line as observed by Di Meo et al. (2004). This depletion should correspond to the precipitation of Fe and Zn sulfides (Di Meo et al. 2004, Luther et al. 2001). Sander et al. (2007) also evidenced the presence of organic ligands that will form strong complexes likely to play an important role in controlling the behavior of metal ions around hydrothermal vents.

3.4. Particle characterization and origin

Observations by SEM permitted the determination the elemental composition of particles and the investigation of their origin. Measurements indicated that the dominant elements were sulfur (26.2± 3 %), zinc (19.7± 2.2 %), iron (16.5 ± 3 %), sodium (7.1 ± 3.7 %), chloride (5.3 ± 1.4 %) and silica (7.7 ± 1.6 %). The presence of residual NaCl can be explained by an incomplete rinsing of the frits before analysis. These compositions can be compared to the data obtained by German et al. (2002) within the Totem site (EPR, 13°N) on particles sampled with traps a few meters from the vent. These authors found higher S composition (50 to 80%) and comparable Fe one (11-20%). The Fe/S, Zn/S and Fe/Zn atomic ratio obtained in this study are respectively 0.4, 0.4 and 1, leading to a relative stoechiometry of $FeZnS_{2.5}$. The mean composition of the particles was homogeneous along frit transects permitting the second stage of particle identification to be performed at a higher magnification. Table 4 lists the dominant structures observed and summarizes 32 individual observations (Y. Fouquet). No obvious difference was observed between the 2 frits studied. The particles were predominantly zinc – iron sulfides (wurtzite and sphalerite) and iron sulfides (pyrite). The minerals were present in their crystalline form, with large individual crystals (up to 50µm) and smaller crystals (1 to 5 µm) forming stacks reaching up to 20 µm. These crystalline structures were probably formed in an area where the chemical conditions are homogeneous, *i.e.* in the internal part of the chimney. The amorphous forms were also frequent. These forms should originate from precipitation in the external part of the chimney (cold part of the mixing zone, chimney walls). The presence of microcrystalline structures associated with a biofilm-like substance was nearly ubiquitous. This organic material could either be exopolysaccharids (J. Guezennec, com. pers.) or mucous excreted by organisms such as alvinellid worms. Laboratory experiments conducted by Loaec et al. (1997, 1998) reported the biosorption of lead, cadmium and zinc by an exopolysaccharid produced by hydrothermal strains through a chemical equilibrated and saturable mechanism. The presence of exopolysaccharids has to be verified as this organic material enriched in metals could be directly assimilated by organisms through dietary exposure. Particulate matter such as anhydrite which is a dominant constituent of the plume was seldom observed. The rare observation of anhydrite and the absence of observation of iron oxyhydroxide phases suggest that the plume has only a limited impact on the environment surrounding hydrothermal communities (Mottl and MacConachy, 1990). Zbinden et al. (2003) observed the presence of sphalerite, wurtzite, pyrite and marcasite minerals as major constituents of the solid phase associated with alvinellid tubes. These observations are strengthened by the statement of German et al. (2002). These authors indicated that sulfides particles carry 80-90% of the Fe fall out and also transport most of the Cu, Zn and Pb fluxes, whereas Fe oxyhydroxides were responsible for only

10-20 % of the Fe removal. Furthermore, the composition of vent particles in southern Juan de Fuca (Feely et al., 1987) was predominately sphalerite, wurtzite and pyrite whereas suspended and settling particles 100 m away from the vent were mainly sphalerite, anhydrite and Fe oxyhydroxide. The bulk particulate observed in our samples is composed of particles formed in the high temperature smoker which settle rapidly (sphalerite, wurtzite and pyrite) and of amorphous structures and eroded particles coming from external zones of the chimney. This last observation reinforces the hypothesis of particle accumulation in areas where hydrothermal organisms have settled.

According to Trocine and Trefy (1988) and German et al. (1991), we used particulate iron concentrations (Fep) as an indicator of the trends and factors influencing other particulate metals. High positive linear correlations (confidence level 99%, correlation coefficient ranging from 0.918 to 0.972) were obtained between particulate Cu, Zn, Cd, Pb vs. Fep (Fig. 6). These correlations can explain between 84 to 94 % of the variability observed in the data and indicate that Zn, Cu, Cd and Pb will co-precipitate with Fe as wurtzite, sphalerite and pyrite. Moreover, this high correlation underlines that this precipitation is the main process involved within the studied area and is nearly quantitative. Mottl and MacConachy (1990) also stated that the chalchophile elements released into the water column from vent fluids tend to be quantitatively removed from solution very close to their point of origin through precipitation with Fe as polymetallic sulfides. In the same way, Cd and Pb will be associated as trace elements, during the precipitation step to form Zn-Fe sulfides in the wurtzite and sphalerite phases precipitated below 200°C (Trocine and Trefy, 1988; Metz and Trefry, 2000).

3.5. Metallic load and faunal distribution

The data were submitted to Principal Component Analysis, a statistical exploratory method to highlight the links between the whole set of variables. Study of the rank correlation (Spearman) allowed the identification of the independent variables. Significant correlations were found at the 95% level i) between dissolved Cu, Cd and Pb, ii) between all the particulate concentrations as stated above (see 3-4) and iii) between pH and temperature (see 3-2). The remaining variables to be used for the PCA were temperature (T°C correlated with pH), dissolved iron (Fed), zinc (Znd) and cadmium (Cdd correlated with Cud and Pbd) and particulate iron (Fep correlated with all the particulate metals). Two components explaining 72% of the variability of the data were extracted by the analysis and are presented in Fig. 7. The use of a third axis explained 15% more of the variability but did not add new information. The first axis opposes the variables temperature (T°C) and dissolved iron (with respective coordinates on the correlation circle of -0.60 and -0.57) to the 3 other variables Cdd, Znd, Fep (with respective coordinates of 0.42, 0.29, 0.22). The second axis essentially confronts particulate iron (0.51 on the correlation circle) to dissolved Zn and Cd (-0.58 and -0.53 on the correlation circle) and temperature and dissolved Fe (0.22 and 0.25). The combination of the 2 axes illustrates the complexity of processes occurring in the mixing zone. T°C and Fed are linked to a dilution process between the cold seawater and the hot fluid. Particulate (>2µm) metal concentrations result from the precipitation and accumulation of polymetallic sulfides. The other dissolved metals (<2 µm) have different

behaviors and include the processes that may occur within the 0.45 – 2 µm phase constituted of small particles and large colloids.

A projection of the observations on the biplots highlights their position along the two axes as a function of the sampling zone. Samples obtained in areas dominated by the tubeworm *R. pachyptila* (R) are characterized by a limited variability along the 2 components corresponding to the cold part of the mixing zone (Childress et al., 1993; Sarradin et al., 1998; Le Bris et al., 2003). Samples collected in areas dominated by alvinellid worms (A) showed a higher variability associated with extreme values along the 2 axis (A1 16°C, Fed 33.7µM; A5 Znd 2µM, Cdd 7.84nM; A6 Fep 57.1µM). The microhabitat of alvinellid worms present steeper gradients but is not fully represented in our study. Faunal assemblages dominated by alvinellids are present in a temperature range between 9.6-81°C (Desbruyères et al., 1998; Le Bris et al., 2003; 2005; Sarradin et al., 1998; Di Meo-Savoie et al., 2004). The temperature range and therefore the fraction of the mixing zone sampled in this study is only 3.2 to 20°C, leaving the hot part of the mixing zone unsampled, even though *A. pompejana* has developed a specific strategy to cool and modify the fluid mixture within its tube (Le Bris et al. 2005).

The faunal distribution in hydrothermal environment has been shown to depend upon fluid dilution process, sulfide concentrations and speciation (Luther et al., 2001, Le Bris et al., 2003), flow intensity or substratum type (Sarrazin et al., 1999). From this multiparametric descriptive analysis, it can be hypothesized that the distribution of the observed dominant fauna is not only constrained by these variables, but also by the concentration and speciation of metals which are controlled by complex geochemical processes. Hydrothermal organisms will therefore colonize areas where the energy supply (H_2S and CH_4, flow intensity) is sufficient, where the substratum is convenient but also where the environment is suitable i.e. where the metals bioavailability can be sustained. This result is particularly important in this area where the two dominant species present different nutritional strategies and hence different sensitivity to dissolved and particulate metals. *Alvinella pompejana* (Desbruyères et al., 1998) is a grazer (particulate feeding consumer). This organism will ingest large quantities of metal rich particulate matter (Cosson and Vivier, 1997) that may serve as the main pathway for metal accumulation. Wang (2002) suggested that the metal desorption within the acidic gut may be important for the assimilation of easily exchangeable metals but not for metals that are bound tightly. Zbinden et al. (2003) observed the presence of mineral gradients between the inner and outer part of the alvinellids' tubes suggesting that the tube wall acts as an efficient barrier to external environment, including metal compounds. Conversely, *Riftia pachyptila* is a symbiotic tubeworm devoid of mouth and gut that will transfer hydrogen sulfide to its symbiotic bacteria harbored in the trophosome (Goffredi et al., 1997). The majority of the exchange of this organism with its environment will take place through the branchial plume: the metal entry route will be either by adsorption of the particles on the membrane or by direct crossing of the membrane by the dissolved fraction i.e. with no direct impact of metallic particles. Cu, Cd, Zn have been quantified in the blood and can be transported from the plume to the trophosome (Cosson-Manevy et al., 1988) yet with limited metallic bioaccumulations in the bacteria present in the trophosome (Truchet et al., 1998). Ruelas-Inzunza et al. (2005) observed that Cd and Fe concentrations in the vestimentum increased accordingly with the size of specimens and reported an extreme uptake in the case of

Cu and Zn. The metal compounds will be stored in the tissues as non toxic mineral compounds or associated to metallothioneins known to be involved in detoxication processes (Cosson, 1996).

Riftia clumps are present in zone R characterized by a limited variability, low temperature and metal concentrations whereas Alvinellids worms in zone A are exposed to stronger metal gradients and fluctuations than the tubeworms. This distribution along the chemical gradient can be linked both to the availability of energy sources but also to the ability of *Riftia pachyptila* or alvinellids worms to sustain the metallic load, alvinellids being potentially more adapted to tolerate harsher conditions. The possible metal entry routes may also be different as only the dissolved metal fraction will be available for *R. pachyptila*, whereas both dissolved and particulate fractions will be available for alvinellid worms. The role of metal speciation on bioavailability and toxicity towards the different faunal species has to be evaluated to examine if the metallic load is a key factor to explain faunal distribution in hydrothermal ecosystems.

4. Conclusion

This study tentatively describes the complexity of the hydrothermal biotope regarding the metal input and the relative distribution of Fe, Cu, Zn, Cd and Pb between a dissolved (<2µm) and particulate (>2 µm) fraction.

The total metal concentration ranges exhibit a neat metallic enrichment accounting for the hydrothermal input of this part of the mixing zone for all the metals considered compared with the seawater concentration. At the scale studied, temperature, pH and dissolved iron have a (semi-) conservative behavior whereas the other dissolved metals and the particulate metals are characterized by non-conservative behaviors involving different processes. The metal enrichment of the particulate fraction results from the settlement and accumulation of particulate matter close to the organisms. This accumulation can be seen as a significant secondary source of particulate metals. The enrichment observed in the dissolved fraction can be induced by the dissolution and/or oxidation of particles (mainly polymetallic sulfide) or by the presence of particles or colloids which size ranges from 0.45 to 2 µm.

The bulk particulate observed in our samples is characteristic of crystalline particles settling rapidly from the high temperature smoker (sphalerite, wurtzite and pyrite), amorphous structures and eroded particles formed in the external zone of the chimney. This observation reinforces the hypothesis of particle accumulation in areas where hydrothermal organisms have settled. Precipitation of Zn, Cu, Cd and Pb with Fe as wurtzite, sphalerite and pyrite is the main process involved within the studied area for the particles larger than 2µm.

The distribution of the observed dominant fauna can be related not only to the gradient resulting from the dilution process and hence the availability of energy sources but also to the metallic load and variability of the mixing zone. Distribution between dissolved and particulate fractions of the metals can be a first key to estimate metal bioavailability for organisms using different nutritional strategies such as *R. pachyptila* and alvinellid worms. Dissolved and particulate metal concentrations are therefore necessary abiotic variables in understanding the chemical reactivity of the environment. The study of faunal distribution in hydrothermal ecosystem should therefore be undertaken by a multiparametric approach taking into account both the necessary compounds available (O_2, H_2S, …) but also the potential stressors such as metals present in this

peculiar environment. The bioavailability of the metal compounds towards the different faunal species has to be evaluated to understand the strategies developed by hydrothermal fauna to cope with this environment.

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Figure
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Fig.1

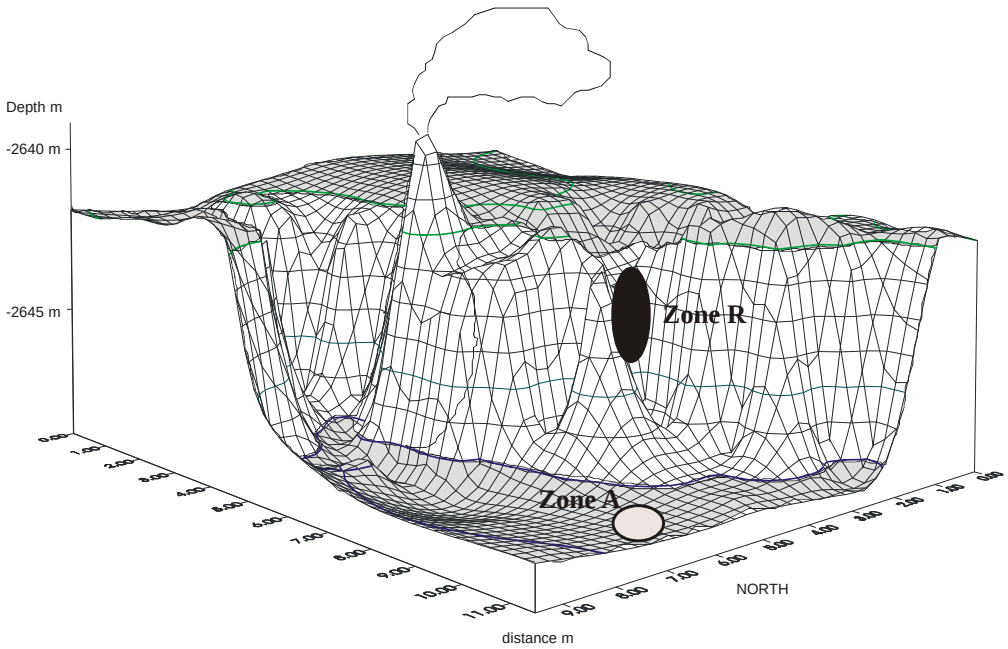
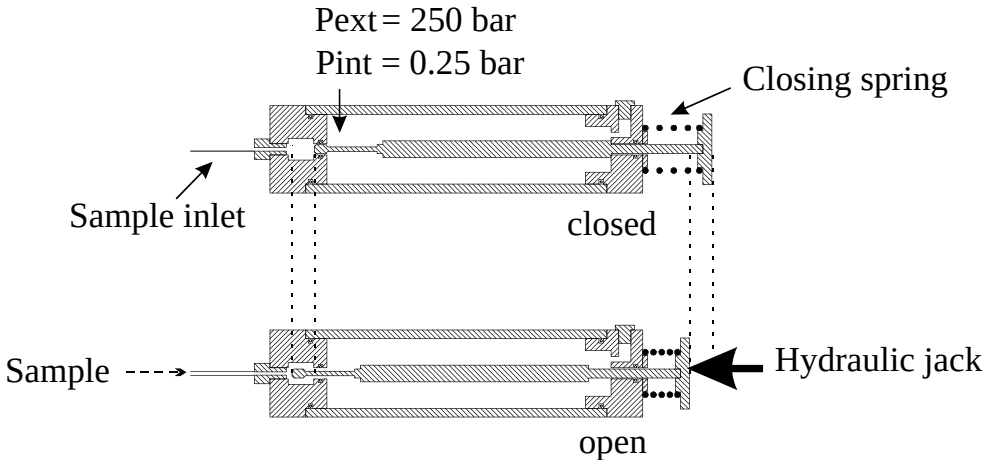


Fig 2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Pint = 0.25 bar
Closing spring
Sample inlet
closed
Sample
Hydraulic jack
open

Fig 3

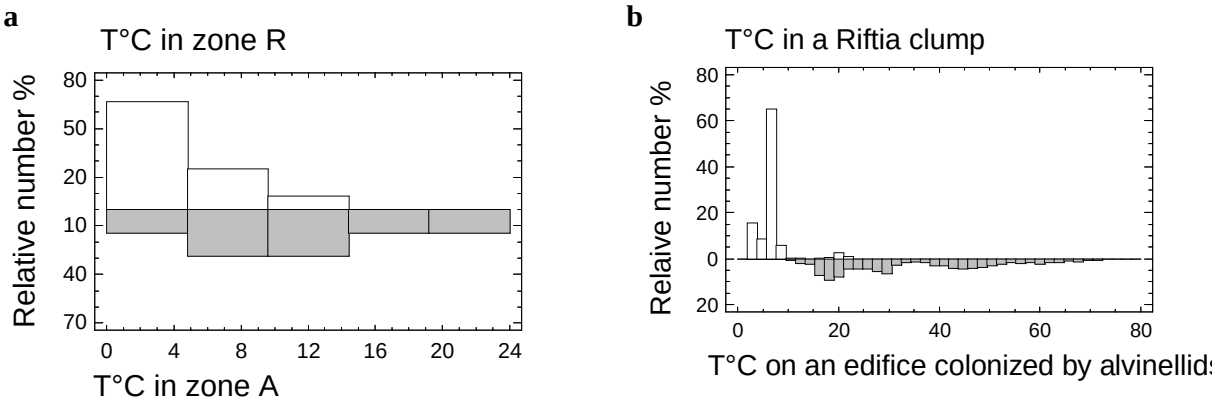

a
T°C in zone R
Relative number %
80
50
20
10
40
70
0
4
8
12
16
20
24
T°C in zone A
b
T°C in a Riftia clump
Relaive number %
80
60
40
20
0
20
0
20
40
60
80
T°C on an edifice colonized by alvinellid

Fig 4

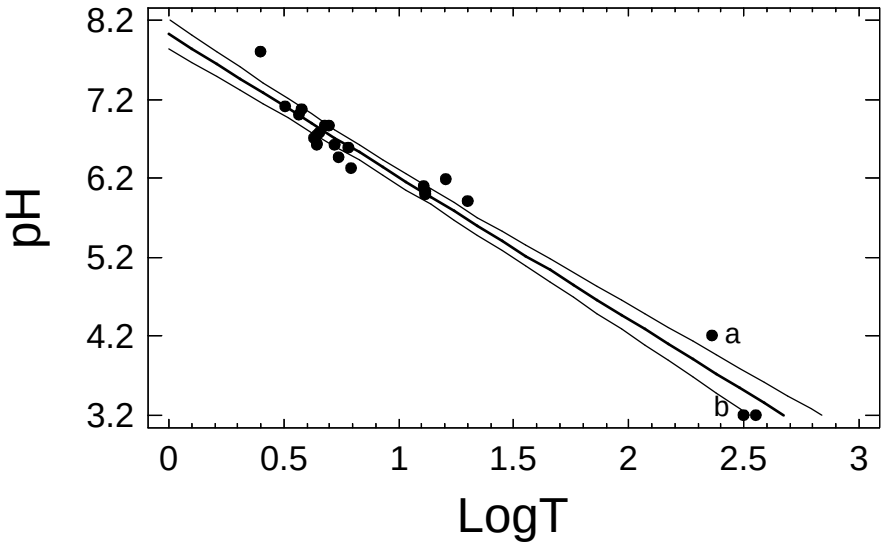
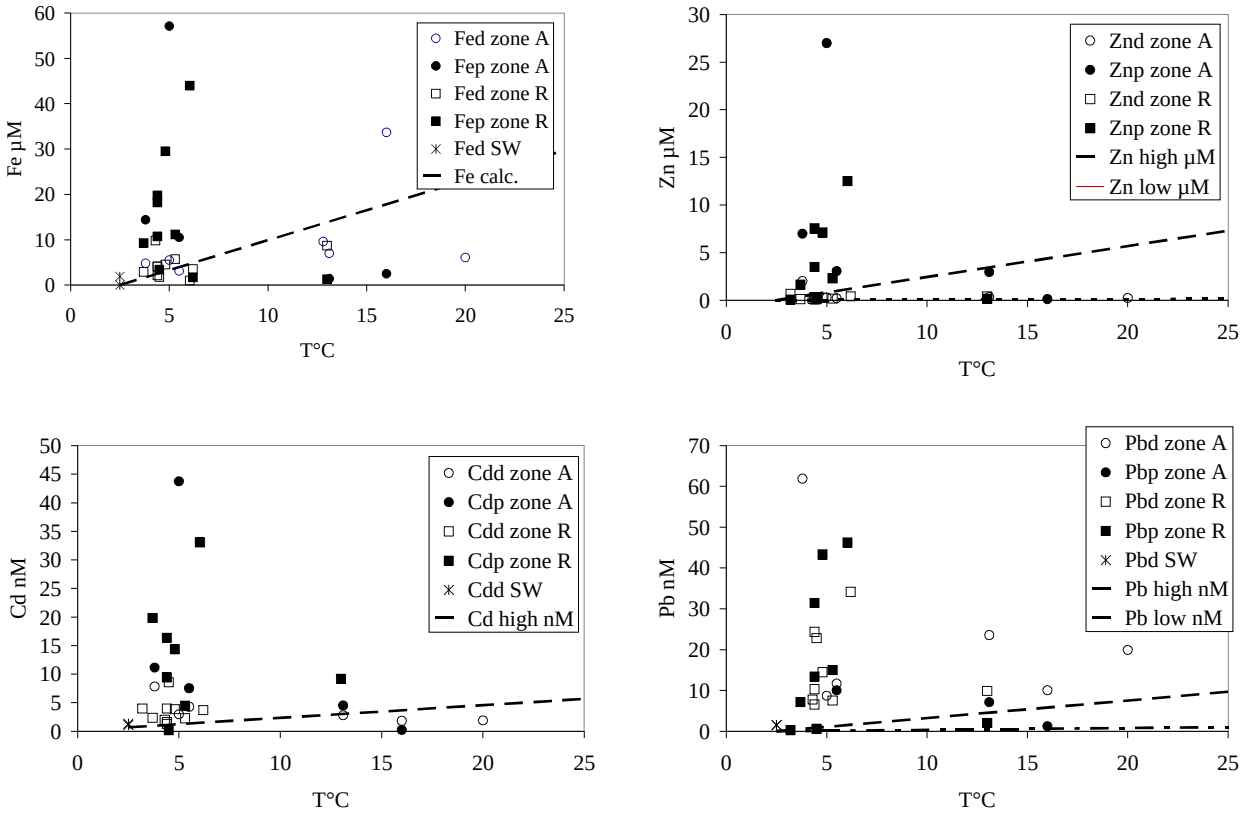


Fig 5


Fe µM
T°C
Fed zone A
Fep zone A
Fed zone R
Fep zone R
Fed SW
Fe calc.
Zn µM
T°C
Znd zone A
Znp zone A
Znd zone R
Znp zone R
Zn high µM
Zn low µM
Cd nM
T°C
Cdd zone A
Cdp zone A
Cdd zone R
Cdp zone R
Cdd SW
Cd high nM
Pb nM
T°C
Pbd zone A
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Pbd zone R
Pbp zone R
Pbd SW
Pb high nM
Pb low nM

Fig 6:

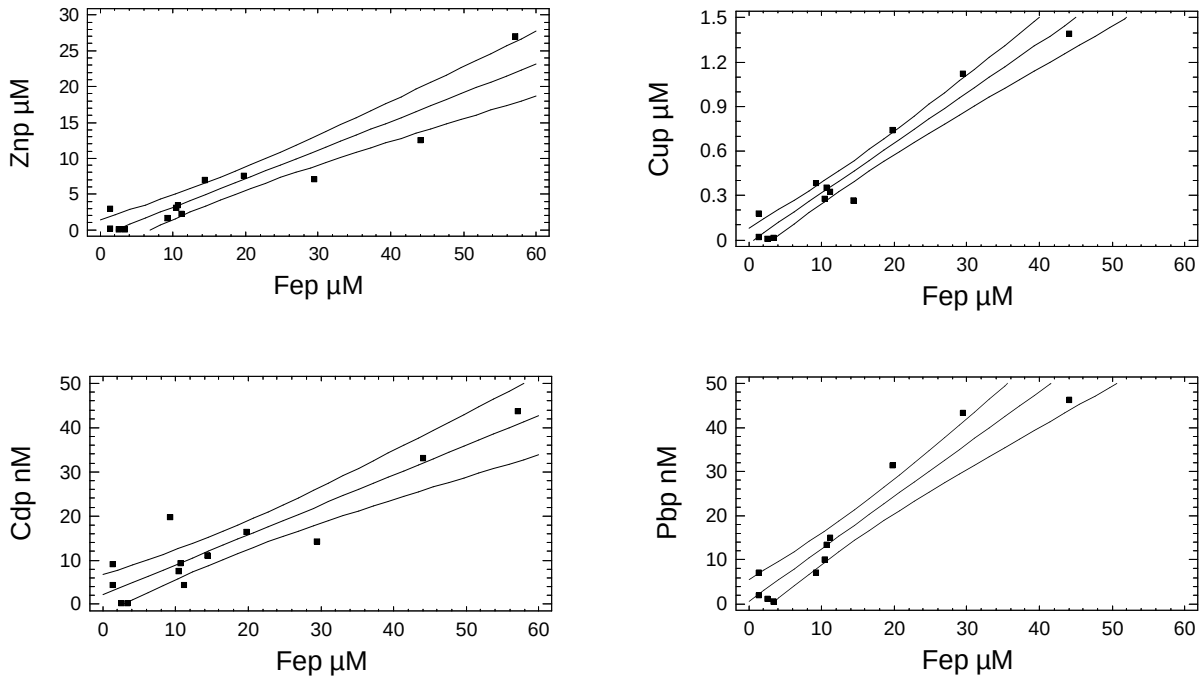

Fig 7

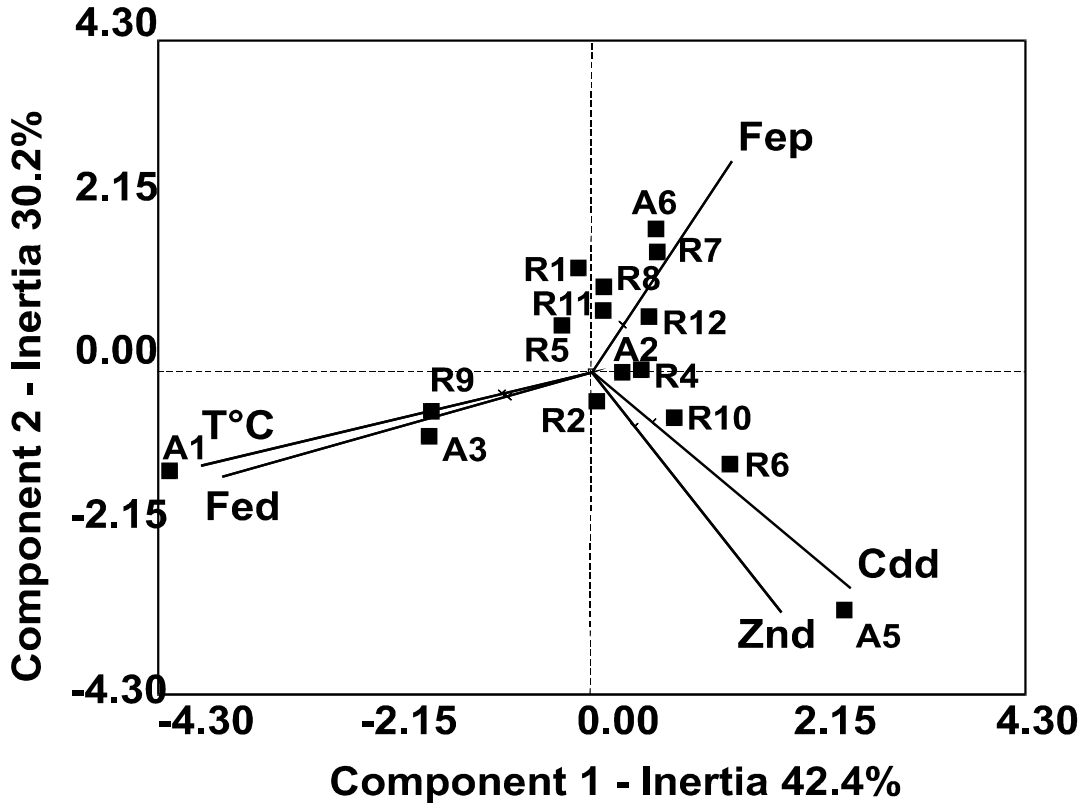
4.30
2.15
0.00
-2.15
-4.30
-4.30
-2.15
0.00
2.15
4.30
Component 2 - Inertia 30.2%
Component 1 - Inertia 42.4%
Fep
A6
R7
R1
R8
R11
R12
R5
A2
R4
R9
T°C
R2
R10
A1
A3
R6
Fed
Cdd
Znd
A5

Figure captions

Fig. 1: 3D schematic representation of the Genesis chimney (from Sarradin et al. 1998). The dark area corresponds to the sampling area “zone R”, the grey one to “zone A”.

Fig. 2: Schematic view of the vacuum based sampling device manipulated and actuated by the hydraulic jack of the submersible. The initial internal pressure is 0.25 bar (P_{int}). Temperature is recorded at the extremity of the sample inlet by an autonomous probe. The frit holder is connected to the sample inlet.

Fig 3: a), Distribution of the relative number (%) of temperature measurements of the samples in zones A and R, n=19. b) Distribution of the relative number (%) of temperature measurements within different microhabitats colonized by *Riftia pachyptila* or by alvinellid worms. This graph was obtained by gathering temperature data measured by autonomous probes (Micrel®) during 2 cruises on the EPR (HOT96 and HOPE99), n = 10478.

Fig. 4: Log Temperature vs. pH relationship. The dataset is enriched with endmember concentrations from a) Le Bris et al. (2003) and b) Von Damm (1995a). R^2 = 96.0 %, pH = 8.02 – 1.81*LogT. The dotted line represents the confidence interval at 95%.

Fig. 5: Dissolved (Md, <2µm) and particulate (Mp, >2µm) metal concentrations vs. temperature compared with theoretical dilution lines. Theoretical dilution lines were calculated from published high and low endmember concentrations simulating a simple dilution of hydrothermal fluid with seawater.

Fig. 6: Particulate metal (Mp) vs. particulate Fe (Fep) relationship. The correlation coefficients obtained for particulate Zn, Cu, Cd and Pb vs. particulate Fe are respectively 0.939, 0.972, 0.918, 0.957. The dotted line represents the confidence interval at 95%.

Fig. 7: Biplot of components 1-2 extracted from the PCA and explaining 72 % of the variability in the dataset. The samples (square symbols) are marked according to the sampling zone (R for Riftia clumps, A for colony of Alvinellids).

Table
Click here to download Table: SarradinTable1-4R2.doc

	Cu(nM)		Pb (nM)		Cd (nM)	
	Certified v.	Measured v.	Certified v.	Measured v.	Certified v.	Measured v.
NASS-5	4.7±0.7	4.4±0.2	0.04±0.02	0.05±0.02	0.20±0.03	0.17±0.02
CASS-3	8.1±1.0	7.6±0.5	0.06±0.02	0.07±0.01	0.27±0.04	0.26±0.04
SLEW-2	25.7±1.7	27.2±1.4	0.09±0.01	0.08±0.01	0.17±0.02	0.15±0.02

Table 1: Analysis of Cu, Pb and Cd in certified seawater samples: NASS-5 oceanic seawater, CASS-3 coastal seawater, SLEW-2 estuarine water. Values (v.) are expressed as mean ± confidence interval (95%)

Sampling zone	T°C	pH	% fluid	Fed µM	Fep µM	Cud µM	Cup µM	Znd µM	Znp µM	Cdd nM	Cdp nM	Pbd nM	Pbp nM
R1	4.4	6.6	1.1	4.1	19.8	0.18	0.742	0.07	7.54	1.62	16.34	10.29	31.43
R2	6.2	6.3	1.9	3.5	1.7	0.40	nd	0.40	nd	3.69	nd	34.13	nd
R3	4.3	6.7	1.1	9.8	nd	0.19	nd	0.13	nd	1.98	nd	7.77	nd
R4	4.4	6.6	1.1	4.1	10.7	1.13	0.353	0.30	3.49	3.96	9.41	24.32	13.39
R5	5.3	6.6	1.5	5.7	11.2	0.22	0.325	0.16	2.30	2.25	4.47	7.52	14.94
R6	4.5	6.8	1.1	1.8	3.4	0.49	0.016	0.21	0.09	8.56	0.20	22.82	0.56
R7	6.0	6.6	1.8	1.0	44	nd	1.393	nd	12.50	nd	33.04	nd	46.22
R8	4.4	6.8	1.1	2.1	18.2	0.36	nd	0.32	nd	1.26	nd	6.55	nd
R9	13.1	6.0	4.9	7.0	1.4	0.44	0.177	0.32	2.95	2.79	4.51	23.54	7.12
R10	3.2	7.1	0.6	nd	nd	0.80	0.004	0.65	0.06	3.96	nd	nd	0.26
R11	3.7	7.0	0.8	2.9	9.3	0.32	0.383	0.12	1.61	2.34	19.83	nd	7.13
R12	4.8	6.9	1.3	4.5	29.5	0.49	1.122	0.23	7.11	3.87	14.34	14.47	43.33
A1	16.0	6.2	6.2	33.7	2.5	0.17	0.009	nd	0.11	1.80	0.24	10.05	1.20
A2	5.5	6.5	1.6	3.1	10.5	0.24	0.280	0.21	3.06	4.28	7.52	11.70	10.03
A3	13.0	6.0	4.9	8.7	1.3	0.23	0.018	0.40	0.15	nd	9.19	9.85	2.03
A4	12.8	6.1	4.8	9.6	nd	nd	nd	nd	nd	nd	nd	nd	nd
A5	3.8	7.1	0.8	4.8	14.4	0.39	0.266	2.00	6.98	7.84	11.15	61.89	81.30
A6	5.0	6.9	1.4	5.5	57.1	0.34	0.873	0.29	27.00	2.97	43.7	8.69	251.53
A7	20.0	5.9	7.9	6.1	nd	nd	nd	0.23	nd	1.89	nd	19.90	nd
SW1	1.9	7.6	0.0	1.9	nd	0.02	nd	nd	nd	1.1	nd	1.5	nd
SW2	nd	7.6	nd	<LD	nd	0.01	nd	nd	nd	1.3	nd	1.5	nd

Table 2: Temperature, pH and metal concentrations measured in the water samples. d refers to dissolved concentration, i.e. not retained on a 2µm frit, p refers to particulate concentration, i.e. retained on a 2µm frit. The frits corresponding to samples R3 and A4 were used for the MEB studies. Samples SW1 and SW2 were taken ca. 500 meters away from the active area. % fluid is an estimation of the content of hydrothermal fluid in mixed with seawater (see § 3-3).

nd : not determined, <LD lower than the detection limit.

	References	T°C	Fe (µM)	Cu (µM)	Zn (µM)	Cd (nM)	Pb (nM)
Endmember, 13°N	Cosson (1996), Von Damm (1995a), Le Bris et al. (2003)	230-359	600-10800		2-102	55-70	14-135
Surrounding organisms							
EPR, 13°N	This study ***** R, A	3.8-20	5.2-62.6	0.18–1.6	0.3–27.3	2.0-46.7	11-260
EPR, 13°N	Desbruyères et al. (1998) A			1.38-3.27		16-133	
EPR, 9°N	Di Meo-Savoie et al. (2004) A	7.5-40	72-730	0.08-1.94	2.9-41.3	2.8-33	20-520
Mid Atlantic Ridge	Geret et al. (1998) Sarradin et al. (1999), Geret et al. (2002), M, S	4.7-25	58-1470	0.02-3.2			0.96-120
Mid Atlantic Ridge	Kadar et al. (2005) M, S	4.3-8	3.8–10.3	0.5-1.99	0.25-1.64		1.6-20
North Pacific deep waters	Donat and Bruland (1995)		0.001	0.005	0.008	1	0.005

Table 3: Temperature and composition of endmember vent fluids from 13°N, EPR, diluted fluids surrounding hydrothermal organisms and North Pacific deep waters.

* Total metal concentration (dissolved + particulate).

R: *Riftia pachyptila* clump, A: colony of alvinellids, M: mussel bed, S: shrimps

Structure	Composition	Size µm	n	Comment
Wurtzite	ZnFeS	1-10/50	9	hexagonal, individual or stacked crystals
Collomorphous (wurtzite)	ZnFeS	20	5	spheres, stacked
Sphalerite	ZnS/ ZnFeS	15	2	crystal + stacked
Pyrite	FeS_2	20	3	cubic
Collomorphous (pyrite)	FeS_2	5-20	5	sphere, stacked
Sulfur	S	2-5	1	globule, stacked
Anhydrite	$CaSO_4$	60	1	not eroded crystal
"Biofilm"			32	microcrystalline assemblage associated to biofilms
Organic fragments			4	

Table 4: Mineralogical composition of the particles, n is the number of observations for a total of 32.

*** Responses to Reviewers' Comments**

Sarradin
STOTEN-D-07-00750R1
We have followed all the reviewer's comments. Our modification start with a < in the following text

Reviewers' comments:

2. Unclear relationship between observed metal data and faunal association with the microhabitats; While I have no concern about using PCA for a statistical evaluation of the data, I am still not completely satisfied with the discussion and conclusion from the statistical results. It would be nice to have some concluding sentences about the different metal composition of the two microhabitats and possible explanations, why tubeworms may favor this environment and the alvinellid worms the other. Does the limited variability in area R indicate a more homogeneous microenvironment (and possibly lower tolerance limits for metals) of the tubeworms, and the higher variability of the values in area A that the alvinellid worms are exposed to stronger metal gradients and/or fluctuations than the tubeworms?

<Concluding sentences have been added to section 3.5
Riftia clumps are present in zone R characterized by a limited variability, low temperature and metal concentrations whereas Alvinellids worms in zone A are exposed to stronger metal gradients and fluctuations than the tubeworms. This distribution along the chemical gradient can be linked both to the availability of energy sources but also to the ability of Riftia pachyptila or alvinellids worms to sustain the metallic load, alvinellids being potentially more adapted to tolerate harsher conditions. The possible metal entry routes may also be different as only the dissolved metal fraction will be available for R. pachyptila, whereas both dissolved and particulate fractions will be available for alvinellid worms.

I also have a few specific comments:
Page 1, Line 39, 40, abstract: I doubt that coprecipitation with Fe is the correct expression for the formation of the listed mineral phases. Wurtzite, sphalerite and pyrite are discrete phases that precipitate from hydrothermal solutions, as well as Cu-bearing phases such as chalcopyrite.
< co precipitation has been replaced througout the text by precipitation

Page 1, Line 42,43: Say how the distribution of the fauna is related to the gradients.
<added in the text
The distribution of the dominant observed fauna has been related to the gradient resulting from the dilution process, with the alvinellids worms colonizing the hotter and more variable part of the mixing zone, but also to the metallic load of the mixing zone.

Page 3, Line 50: What is PSA?
<added in the text potentiometric stripping analysis

Page 4, Lines 28-29: Fed, Cud etc. need to be defined somewhere in the text. I would suggest to make the d for "dissolved" subscript.
< done in the text
The concentrations of dissolved metals obtained in sample SW1 (Fe < detection limit, Cu 0.01µM, Cd 1.3 nM, Pb 1.5 nM) are close to those currently reported for North Pacific deep waters (Fe 0.01 µM, Cu 0.005µM, Cd 1 nM, Pb 0.005 nM, Donat and Bruland, 1995) except for Pb.

Page 6, lines 4-18. Is the temperature really a conservative parameter? Has the calculation of the fluid percentage been checked with the endmember data of a conservative element? Comparative calculations by Koschinsky et al. (Geochimica et Cosmochimica Acta 66, 1409-1427) had shown that conservative elements such as Li and Rb are better parameters for endmember calculations than

temperature, because conductive cooling in the subsurface can make temperature behave non-conservative during mixing.
< a sentence has been added in the text
This result confirms that at the scale and in the range studied, pH and temperature can be assumed to follow a semi-conservative process and can be used as tracers of the dilution, even though conductive cooling in the subsurface can modify its conservative behavior during mixing.

Page 7, line 40: Cu sulfide is a typical high-temperature precipitate, and not something that forms in the cold part of the chimneys.
< deleted in the text
Line 58: delete one "settle"
<done

Page 8, line 17: correct "calchophile" to "chalcophile"
<done

Figure 5: Legends of the Zn and Pb graphs: what does "Zn high µM, Zn low µM, etc. mean?
< done in the legend

Figure caption, figure 6: Include "(Fep)" after "particulate Fe"
< done