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# Effect of shallow-water venting in Azores on a few marine biota

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**Abstract:** The 1000 m high D. João de Castro seamount lies in the middle of the Azores Archipelago (Portugal) on the hyperslow-spreading Terceira rift in the Atlantic. Hydrothermal vents were found near the top of the seamount and occurred in two distinct colour zonations, white and yellow, 50 metres apart from each other. The two zones are characterized by different physical and chemical properties. The macro-species composition at the shallow vents on top of the seamount was similar to the coastal and seamount area of the Azorean Archipelago. Preliminary data on the influence of the shallow water hydrothermal activity on the marine biota is presented here. This study suggests presence of protists and bacteria adapted to metal rich waters of this ecosystem. Several - algae and animals are also inhabiting this shallow water vent system. The heterogeneity of the vent fluids in the two contrasted areas is reflected by the differences found in the organisms collected and in accumulated metals in their tissues. Nevertheless, as differences have been also evidenced between species and the metals, more work needs to be carried out to understand the role of the different metal fractions in the environment and particularly in the food-web.

*Keywords*: Shallow-water venting • Hydrothermal vents • Trace metal concentrations • Biosorption • Bioaccumulation • Azores

## Introduction

The D. João de Castro seamount is located in the North Atlantic, between the Azorean islands São Miguel and Terceira (38º13.3’N, 26º36.2’W). The seamount lies on the Terceira Rift, which is considered to be the world’s slowest spreading plate boundary, 4 mm $y^{-1}$ at this location (Vogt & Jung, 2004). The top of the seamount today lies 13 m below sea level. Work carried out in D. João de Castro was recently reviewed by Cardigos et al. (2005).

The existence of shallow water submarine hydrothermal venting is well known off volcanic islands and provinces. Such sites are commonly detected by the presence of streams of gas bubbles such as observed offshore from White Island in the Bay of Plenty, New Zealand (Lyon et al., 1977); Kagoshima Bay, Japan (Hashimoto et al., 1993); Milos Island, Greece (Dando et al., 1995) among others (see revision in Tarasov et al., 2005). At deep-sea hydrothermal vents on Mid-Ocean Ridges (MORs) there is usually a high biomass of largely endemic, but species poor, fauna that depends on chemosynthesis-based production (Tunnicliffe, 1991). By contrast, at less than 100 m depth, shallow water vents tend to have a low biomass of a more diverse fauna with few, or no, endemic species (see revision in Tarasov et al., 2005).

Shallow water venting on the Mid-Atlantic ridge (MAR) has been described at a number of sites around Iceland (Fricke et al., 1989). However no endemic vent fauna has been found at these sites, which extend to depths of 406 m (Tarasov et al., 2005).

According to Cardigos et al. (2005), the D. João de Castro seamount today shoals to within 13 m depth of the sea surface. The summit of the volcanic cone is a caldera, 300 x 600 m, 0.18 km$^2$, at 40 m depth inside. The area of the D. João de Castro seamount shallower than 50 m is approximately 0.35 km$^2$, with 0.011 km$^2$ showing hydrothermal activity. Venting on the seamount can be divided in two main types; 'white' vents and 'yellow' vents, according to the bottom colour around the vents. The venting fluids from the white zone are significantly richer than those from the yellow zone in dissolved sulphide, $H_2$ and $CH_4$ and the trace metal Pb and are poorer in Fe and Mn (Table 1). The fluids from the yellow zone are warmer than the ones from the white zone. These venting fluids have much higher trace metal and gas concentrations than the ambient Atlantic seawater. No typical hydrothermal vent fauna was found at these shallow vents. The macro-species composition at the shallow vents on top of the seamount is similar to the coastal and seamount areas of the Azorean Archipelago.

The present paper reports the first studies on the trace metal concentrations of dominant species of macroalgae and macroinvertebrates and the tolerance of microorganisms to trace metals in the D. João de Castro (DJC) seamount. The ultimate aim of these studies was to identify algae and microorganisms with high biosorption/bioaccumulation capacity for application in bioremediation of contaminated sites.

**Table 1.** Physical and chemical differences between the two zones (adapted from Cardigos et al., 2005).

**Tableau 1.** Différences physiques et chimiques entre les deux zones étudiées (adapté de Cardigos et al., 2005).

| Parameters | White zone | Yellow zone |
|---|---|---|
| Depth (m) | 25.8 | 19.9 |
| Temperature °C | 35.8 | 61.3 |
| pH | 5.01 | 5.09 |
| S (µM) | 81.6 | 1.2 |
| $H_2$ (µM) | 3183.4 | 84.4 |
| $CH_4$ (µM) | 29.8 | 12.8 |
| $H_2S$ (µM) | 22.5 | 29.0 |
| Cu (µM) | 0.04 | 0.03 |
| Pb (µM) | 3.6 | 1.9 |
| Ba (µM) | 0.12 | 0.29 |
| Fe (µM) | 8.8 | 89.2 |
| Mn (µM) | 0.5 | 6.8 |
| Co (µM) | 0.04 | 0.03 |
| Cd (µM) | 0.006 | 0.005 |

## Materials and methods

### *Sample collection*

During the mission "Bancos 2004", 5 dives were performed at D. João de Castro hydrothermal vents. During these dives several species of the dominant algae and macroanimals that were near (< 1.5 metres) or adjacent to the vents, were collected in order to estimate and study heavy metal accumulation in higher organisms and tolerance in microorganisms. During some dives at the coasts of the S. Miguel and Santa Maria islands outside hydrothermal influence, algae were collected for comparison.

### *Identification of benthic diatoms*

Sediment samples from yellow and white zone were observed under the microscope, and the diatom species were identified.

### *Trace metal analyses*

The macroalgae and macroinvertebrates were washed in seawater at the sampling site, stored into precleaned polyethylene bags and frozen.

Upon their arrival at the laboratory they were thawed and thoroughly cleared of any epiphyta and sediments by using nylon brushes. Macroalgal material was rapidly rinsed in Milli-Q water. These were frozen and freeze dried until further analysis.

Organic material was digested on a pressurized microwave (© MARS 5) with 5 ml of ultra pure grade nitric acid. The solutions were filtered with a Whatman filter 42 and diluted accurately to 25 ml with Milli-Q water. Sediments were digested over more than 24 hours with a mixture of nitric acid, perchloric acid and hydrofluoric acid on a hot plate. Both types of samples were analyzed on an ICP-AES (PERKIN ELMER optima 2000 DV sequential) at NIO, Goa.

The accuracy of measurements was tested using certified reference materials DOLT2, TORT from NRC, Canada. One sample of reference material and blanks were included in each analytical batch. Results were in agreement with certified values, and the standard deviations were low, proving good repeatability of the method (Table 2).

**Table 2.** Trace metal concentrations of the dominant organisms at the shallow water hydrothermal vents (in $\mu g.g^{-1}$ dry weight).
**Tableau 2.** Concentrations métalliques dans le milieu et les organismes principaux des sources hydrothermales (en $\mu g.g^{-1}$ poids sec).

| | Species | Location (number of samples) | Mg Avg ± Std *Min-Max* | Fe Avg ± Std *Min-Max* | Al Avg ± Std *Min-Max* | Cu Avg ± Std *Min-Max* | Mn Avg ± Std *Min-Max* | Zn Avg ± Std *Min-Max* | Cd Avg ± Std *Min-Max* |
|---|---|---|---|---|---|---|---|---|---|
| Algae | *Cladostephus spongiosus* | DJC white (n = 3) | 9549 ± 1371 | 3947 ± 2008 | 210 ± 141 | 8 ± 5 | 26 ± 26 | 17 ± 10 | 1.2 ± 1.2 |
| | (Hudson) C. Agardh | | *8688-11130* | *2684-6263* | *108-372* | *2-10* | *10-56* | *8-28* | *0.3-2.6* |
| Algae | *Sargassum vulgare* C. Agardh | DJC white (n = 2) | 7733 | 6385 ± 7035 | 912 ± 1173 | 201 ± 8 | 25 ± 13 | 115 ± 88 | 4.1 ± 4.7 |
| | | | *9474-20410* | *1411-11360* | *82-1741* | *17-27* | *16-34* | *52-177* | *0.8-7.4* |
| | | DJC yellow (n = 5) | 7069 ± 4033 | 13855 ± 11368 | 121 ± 198 | 7 ± 7 | 39 ± 25 | 89 ± 52 | 4.7 ± 1.8 |
| | | | *88-10010* | *3828-31310* | *4-471* | *tr-13* | *15-63* | *40-144* | *2.8-7.4* |
| Algae | *Lithothamnion* sp. Heydrich | DJC white (n = 2) | 24500 ± 28 | 426 ± 40 | 4 ± 1 | 3 ± 3 | 18 ± 3 | 39 ± 15 | 2.2 ± 0.1 |
| | | | *24480-24520* | *397-454* | *4-5* | *1-5* | *16-20* | *29-50* | *2.1-2.2* |
| | | DJC yellow (n = 4) | 23597 ± 2391 | 4565 ± 7503 | 86 ± 137 | 8 ± 12 | 30 ± 30 | 33 ± 26 | 2.8 ± 4.3 |
| | | | *20170-25330* | *206-15780* | *tr-287* | *tr-26* | *8-74* | *8-37* | *0.1-9.3* |
| Sponge | *Cliona viridis* (Schmidt. 1862) | DJC white (n = 1) | 12620 | 3290 | 3072.0 | 5.7 | 39.0 | 59.5 | 9.9 |
| Sponge | *Cliona viridis* (Schmidt. 1862) | DJC yellow (n = 1) | 7343 | 8047 | 500.0 | 13.4 | 44.8 | 88.4 | 6.0 |
| | Polychaete | DJC white (n = 1) | 1903 | 1053 | 78.4 | 59.2 | 73.9 | 238.4 | 3.6 |
| | Polychaete | DJC yellow (n = 1) | 3689 | 8394 | 188.8 | 143.9 | 151.5 | 251.7 | 69.3 |
| | diatom films | DJC yellow (n = 1) | 96 | 194 | 39.5 | 0.0 | 3.6 | 0.7 | 0.0 |
| | diatom films + sediment | DJC yellow (n = 2) | 8093 ± 2398 | 67090 ± 19601 | 2732 ± 982 | 10 ± 0.3 | 66 ± 28 | 37 ± 10 | 8.1 ± 5.6 |
| | | | *6397-9789* | *53230-80950* | *2037-3426* | *10-10* | *47-86* | *31-44* | *4.1-12* |
| Algae | *Zonaria tournefortii* (J.V. | Caloura (n = 1) | 8615 | 4249 | 1239 | 9 | 88 | 46 | 1.8 |
| | Lamouroux) Montagne | Sta Maria (n = 1) | 6622 | 778 | 323 | 20 | 14 | 23 | 0.9 |
| | Sediment | DJC yellow (n = 2) | 12065 ± 1888 | 206750 ± 17041 | 3059 ± 798 | 4 ± 0.3 | 568 ± 373 | 4±4 | 9.0 ± 0.9 |
| | | | *10730-13400* | *194700-218800* | *2495-3623* | *4-4* | *304-832* | *159-162* | *8.4-9.7* |
| | DOLT2 | Certified | | 1103 ± 47 | 25.2 | 25.8 ± 1.1 | 6.88 ± 0.56 | 85.8 ± 2.5 | 20.8 ± 0.5 |
| | TORT | | | 105 ± 13 | 106 ± 1 | 13.6 ± 1.2 | 180 ± 6 | | 26.7 ± 0.6 |
| | DOLT2 | Presented | | 1037 ± 17 | 25.2 ± 5 | 30.2 ± 1.9 | 5.5 ± 0.1 | 111.3 ± 5.7 | 18.9 ± 0.08 |
| | TORT | | | 93 ± 1 | | 103 ± 1.9 | 12.9 ± 1.3 | 161 ± 2 | 25.2 ± 0.1 |
| | DOLT2 | % Recovery | | 94 | 100 | 115 | 80 | 130 | 91 |
| | TORT | | | 89 | | 97 | 95 | 89 | 94 |

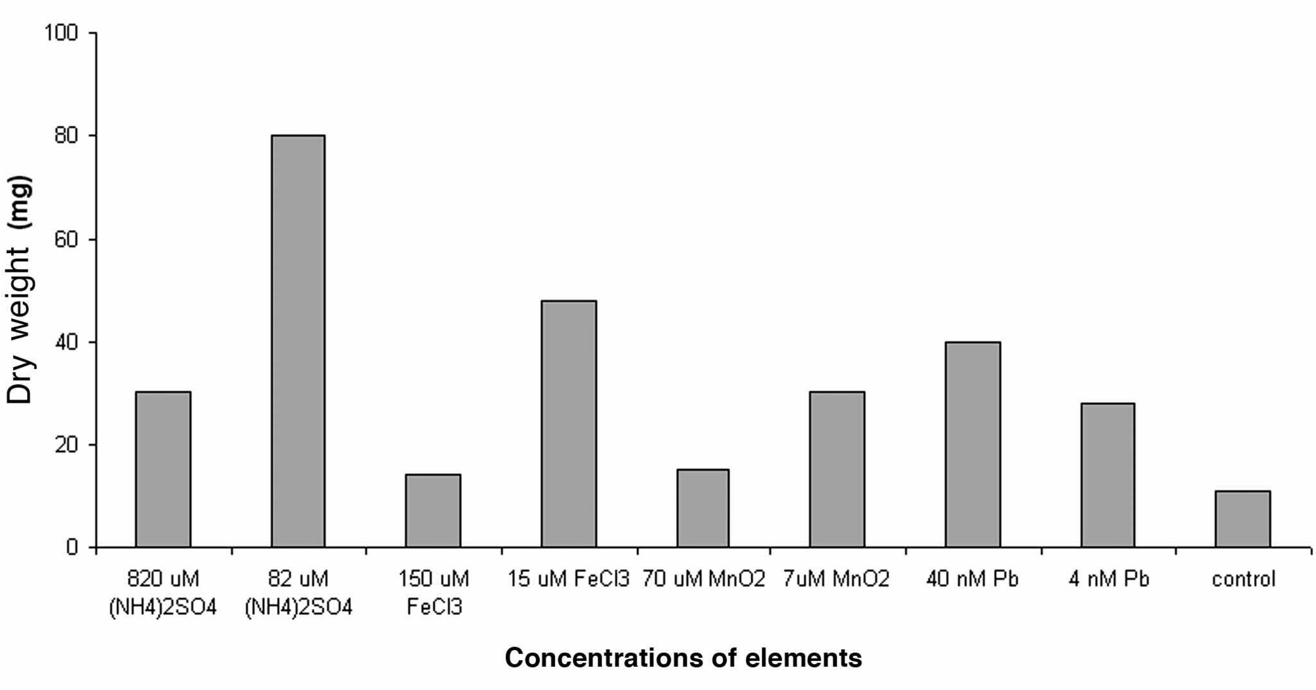


**Figure 1.** Effect of elements on growth of the thraustochytrid isolate #2a.
**Figure 1.** Effet des éléments traces sur la croissance de l'isolat n° 2a de thraustochytridae.

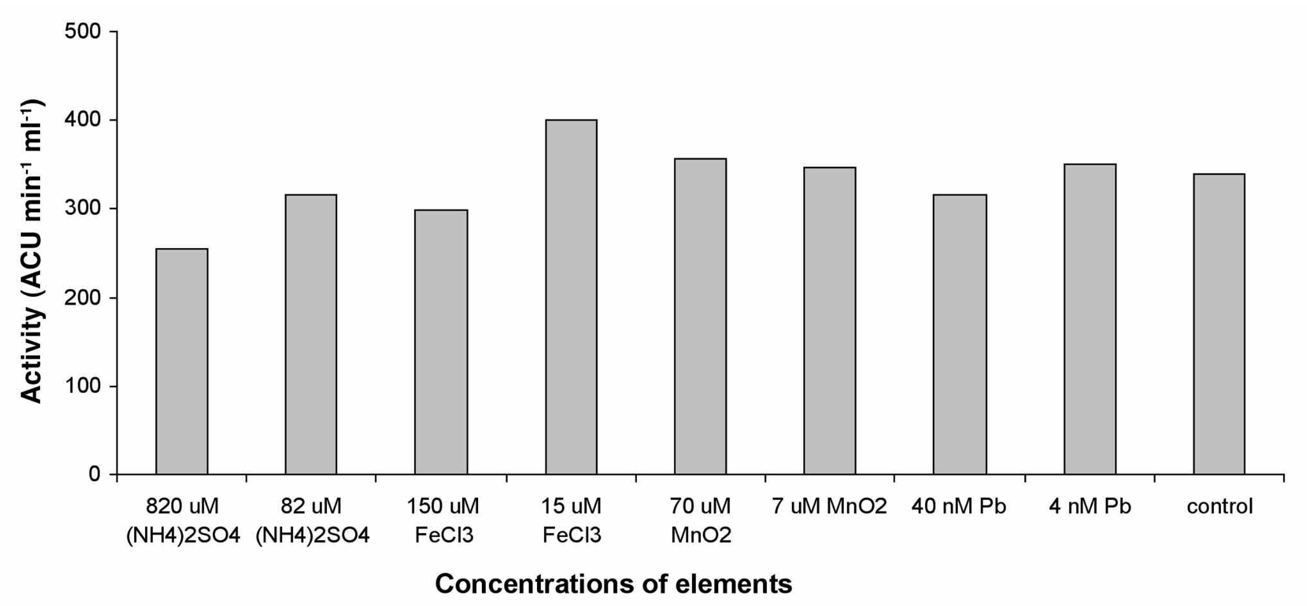


**Figure 2.** Effect of elements on protease production by the thraustochytrid isolate # 2a.
**Figure 2.** Effet des éléments traces sur l'activité protéase de l'isolat n° 2a de thraustochytridae.

*Isolation of micro organisms*

Bacteria and thraustochytrid protists from yellow and white zones, water samples and macroalgae were isolated using nutrient agar medium and pine pollen baiting method respectively (Raghukumar, 1992). The effect of various concentrations of S, Fe, Mn and Pb on growth and protease production on one of the isolates of the thraustochytrid protists was studied. The concentrations of elements used were 820 and 82 µM of $(NH_4)_2SO_4$, 150 and 15 µM of $FeCl_3$, 70 and 7 µM of $MnO_2$, 40 and 4 nM of Pb acetate. The elements were added to the modified Vishniac medium (MV) containing liver extract (0.001%), yeast extract (0.1%), glucose (0.4%) and peptone (0.15%). The growth was estimated as dry weight after 3 days. Protease activity was measured in the culture supernatant using Azocasein substrate (Hamamato et al., 1995). The effect of these elements on growth of 23 bacterial isolates grown in Zobell marine broth was compared by measuring the optical density at 400 nm. About nine isolates of thraustochytrid protists and 23 bacterial isolates were obtained from various algae, sediment from yellow zone and water samples. The thraustochytrid isolate # 2a showing very good growth was used for further studies regarding effect of metals on its growth and protease production. Two different concentrations of the elements were used for these studies and these were a) average concentration measured in the yellow and white zone b) concentrations ten times higher than the average values.

## Results and Discussion

We are reporting here for the first time the diversity of diatoms, trace metal analyses of dominant macroorganisms and the study of metal response of microorganisms in D. João de Castro shallow–water hydrothermal vent system.

*The benthic diatoms*

The sediment from the yellow zone contained the diatoms *Grammatophora marina* Kützing, *Thalassionema* sp., *Navicula* sp., sheathed *Navicula* sp., *Surirella* sp., *Amphora* sp., *Pleurosigma* sp., *Pseudonitzschia* sp. and *Pinnularia ambigua* Cleve. The sediment from the white zone contained rod, cocci and filamentous bacteria.

*Trace metals*

The trace metal concentrations of the dominant macroorganisms at the shallow water hydrothermal vent are presented in the Table 2. In general, organisms from the yellow zone contained higher concentrations of metals, with just a few exceptions. This was by large consistent with the fluid composition from the two zones (Table 1).

The accumulation ratios of the metals differ in the macroalgae and in the macroinvertebrates. Polychaetes accumulated the higher Cu and Zn concentrations than other organisms. While some species of algae accumulated the highest Mg and Iron, the sponge *Cliona viridis* Schmidt, showed the highest Al values. The same sponge species in Portugal mainland presented higher concentrations of Zn and lower concentration of Fe, and this is consistent with the variations present in the sediment of each area (Araújo et al., 2003).

The algae caught at the coasts of the S. Miguel and Santa Maria islands (*Zonaria tournefortii*), outside hydrothermal influence, also presented enriched values, higher than the ones published from Tyrrhenian coastal areas (Conti & Cecchetti, 2003).

As we used homogenate of the whole algae, special attention must be given in the future, since element concentration may be affected by the use of different parts of the algae (Riget et al., 1997). Nevertheless, further studies are needed in order to evaluate the metal accumulation, since there is a large inter and intra specific variability. It is very important to consider several taxa, since some of them take from water for their nutrition either the particular metal fraction such as the filter feeding sponges, or the dissolved metal such as algae (Conti, 2002). Further, it is important to evaluate the metal availability from the vent fluids. With this type of studies new insights on the biosorption capacity of the algae to remove heavy metals from the environment (Schiewer & Wong, 2000) can be achieved.

*Effect of some elements on the Microorganisms*

The growth of the thraustochytrid protist #2a was enhanced in the presence of all the elements tested (Fig. 1). Protease production was not inhibited in their presence (Fig. 2). Higher concentrations of elements did not favour the growth of this isolate as like the lower concentrations that have been tested (except for Pb). Here we provide preliminary evidence that thraustochytrid protist isolated from the brown alga *Sargassum vulgare* C. Agardh from shallow-water hydrothermal vent shows better growth in the presence of metals and its protease is not inhibited in the presence of S, Mn, Fe and Pb. The concentrations of Mn accumulated in the *Sargassum vulgare* collected from the shallow-water hydrothermal vents ranged between 25.1 ± 12.9 and 39.5 ± 25.1 µg. $g^{-1}$ (white and yellow zone respectivelly) and that of Fe between 6385 ± 7035 and 13855 ± 11368 µg. $g^{-1}$ (white and yewllow zone respectivelly), and therefore the ability of the thraustochytrid protist # 2a isolated from this species to tolerate high concentrations of these metals is highly significant. Association of thraustochytrid protists with living algae (as epibionts) has been shown earlier (Raghukumar et al., 1992).

All the 23 bacterial isolates attained maximum growth between 4-7 days in the medium without any elements. On the other hand 10, 2 and 15 isolates attained maximum growth within 2-4 days in media enriched with 82 µM of $(NH_4)_2SO_4$, 4 nM of Pb and 15 µM of $FeCl_3$ respectively indicating that these isolates have adapted to exist in metal-rich waters of the venting area. Hydrothermal vents are reported to be harbouring metal-tolerant bacteria (Vetriani et al., 2005) and flagellated protists (Atkins et al., 2002). As anoxic sulfur-rich vent fluids mix with oxygenated cold ambient sea water, a gradient in metal toxicity is created in the vent environment. It is expected that microbes inhabiting diffuse flows and plumes in the vent ecosystem show high metal–tolerance. However, the toxicity of a metal is not directly linked to its abundance but to its bioavailability which in turn depends on the chemical forms of the metal in the environment.

## Conclusion

This is a preliminary work on the influence of the shallow water hydrothermal activity on the marine biota. This study suggests presence of protists and bacteria adapted to metal rich waters of this ecosystem. Some macro-organisms, algae and animals, are also able to live in such environment. The heterogeneity of the vent fluids in the two contrasted areas is reflected by the differences found in the organisms collected and in accumulated metals in their tissues. Nevertheless, as differences have been also evidenced between species and the metals, more work needs to be carried out to understand the role of the different metal fractions in the environment and particularly in the food-web.

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