

*Supplementary Materials*

## **Shallow-Water Hydrothermal Vents as Natural Accelerators of Bacterial Antibiotic Resistance in Marine Coastal Areas**

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**This file includes:**

Supplementary Tables S1–S2

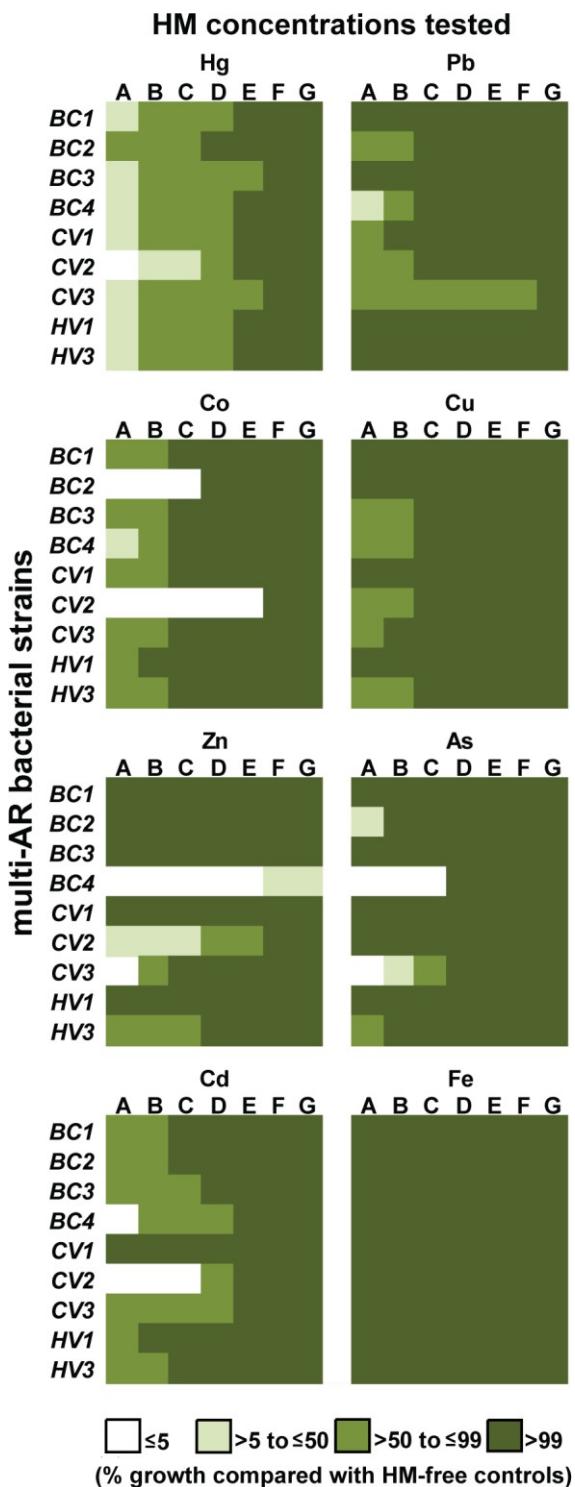
Supplementary Figure S1

**Table S1.** Concentrations of HMs in the sediments collected in this study. Reported are average values (in  $\mu\text{g g}^{-1}$ ) and SDs.

	"Hot" vent	"Cold" vent	Bottaro Crater
Al	10767 $\pm$ 1365	9675 $\pm$ 650	5333 $\pm$ 351
As	13.4 $\pm$ 1.5	15.3 $\pm$ 3.4	87.8 $\pm$ 64.0
Cd	0.03 $\pm$ 0.00	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01
Cr	5.6 $\pm$ 0.7	6.4 $\pm$ 0.5	17.8 $\pm$ 2.0
Fe	17333 $\pm$ 577	15250 $\pm$ 1258	18401 $\pm$ 11961
Mn	71.8 $\pm$ 8.9	81.5 $\pm$ 11.1	550 $\pm$ 564
Hg	0.06 $\pm$ 0.01	0.09 $\pm$ 0.05	0.12 $\pm$ 0.07
Ni	3.3 $\pm$ 0.6	3.5 $\pm$ 0.4	3.2 $\pm$ 1.7
Pb	7.7 $\pm$ 0.8	9.0 $\pm$ 1.2	27.7 $\pm$ 3.0
Cu	19.1 $\pm$ 3.4	18.5 $\pm$ 1.3	6.1 $\pm$ 4.3
V	61.4 $\pm$ 6.4	66.1 $\pm$ 6.0	118.4 $\pm$ 62.8
Zn	20.5 $\pm$ 5.0	20.1 $\pm$ 0.8	38.1 $\pm$ 13.0

**Table S2.** List of primers used in this study, with details of gene function, primer sequence, annealing temperature, amplicon size and detailed related references.

Name	Gene function	Primers sequences (5'-3')	Annealing temperatures (°C)	Amplicon size (bp)	References
<i>arsB</i>	Arsenite efflux ATP ase	GGTCTATGCGCTGGAGCAATTGAA TGCTGGCATTTGTCATTACCG	46	500	Bouskill et al., 2007
<i>czcA</i>	Cobalt/Zinc/Cadmium efflux protein	GTTCACCTGCTTCGCATGTT ACAGGTTGCGGATGAAGGAGATCA	63	320	Bouskill et al., 2007
<i>copA</i>	Copper translocating ATPase	CGGTCTCTA CGAATACCGCTTCAA GAAATAGCTCATGGCCGAGGCCTT	55	300	Bouskill et al., 2007
<i>merA</i>	Hg reductase	GAAGCGGGTGAAGTGATCC TCGTCAGGTAGGGAACAAAC	53	500	Larose et al., 2013
<i>pbrT</i>	Pb uptake protein	AGCGCGCCCAGGAGCGCAGCG TCTTGGCTCGAAGCCGTCGAGRTA	55	450	Roosa et al., 2014



**Figure S1. Results of the physiological tests conducted on the multi-AR bacterial strains identified in this study, to assess their tolerance/resistance to different heavy metals.** Letters form A to G indicate the different concentrations at which each HM was tested (A=10000 ppm, B=5000 ppm, C=1000 ppm, D=500 ppm, E=100 ppm, F=50 ppm, G=10 ppm). The color legend shows the different growth levels of each strain at each of the concentrations tested, compared with the respective HM-free controls.