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Temporal trends and matrix-dependent behaviors of trace elements closed to a geothermal hot-spot source (Aeolian Archipelago, Italy)

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Abstract

Degassing activity from marine shallow-water hydrothermal vents represents a natural important source of trace elements of particular ecotoxicological concern, such as Hg, Cd, Pb, and As. In 2002, on November 3rd, a submarine area of the Aeolian Archipelago (Italy) was affected by an exceptional hydrothermal activity, presumably associated to a significant trace element release. This study developed in the highest impacted area, was focused on different environmental matrices (water, sediment, primary producers, and fishes) with the aim to evaluate on a statistical basis temporal dynamics involving trace elements closed to the emission source. Results evidenced that this event produced significant changes in water column, modifying the general assessment of the chemical descriptors selected (Na, K, Mg, Ca, Cl, Br, SO_4^{2-}, HCO_3^-). Furthermore, the degassing activity represents a significant input of Hg, Cd, Pb, and As for all of the sampled matrices, being it responsible of the higher levels observed in the study area compared to controls. Concentrations measured in sediments and biota (*Cystoseira compressa*, *Serranus cabrilla*) at temporal intervals of ten months distanced, supported the occurrence of different time scale dynamics which are both element and matrix-dependent. In particular, levels of Cd and As measured in liver tissues of *S. cabrilla* evidenced a significant trend to increase with the time, allowing to exclude a quick recovery of the study area. Measured values in edible fishes tissues are, also, notably higher than risk limits proposed by the 2001/22/CE Directive for human consumption, so, local-based caught have to be accurately regulated to avoid the occurrence of diet over-exposure.

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Keywords: Southern Tyrrhenian Sea, hydrothermal vents, trace elements, sediments, Cystoseira compressa, Serranus cabrilla

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1. Introduction

Potential harmful trace elements, such as mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As), do not achieve any biological functions and are naturally not soluble elements in not bio-available structures [1]. Nevertheless, natural phenomena could determine their leaching from rocks and sediments producing the increase in water of their soluble and bioavailable forms and favoring the transferring from the abiotic compartment towards the trophic web. Among natural processes able to affect trace element levels in marine ecosystems, phenomena related to submarine volcanic activities are of particular ecotoxicological interest. Literature evidences as venting could represent a local source of thermal gases coming from both subduction zones and carbonate decomposition of sediments [2], that are able to induce significant changes of major properties of the abiotic matrices [3,4]. Furthermore, geothermal emissions produce significant and measurable effects on biological communities principally related to the induced changes on pH [5], temperature, and major water chemicals such as carbonates and sulphides [2]. Vizzini et alii [6] evidenced as the increase of CO_2 emissions due to the hydrothermal vent activity, induces important biological effects on local primary producers. Even if recent researches well describe changes induced on physico-chemical characteristics of the water column, few data are available on dynamics involving potential harmful trace elements closed to these natural sources of emission. Several researches carried out in volcanoes have shown that trace elements are separated from magma during degassing and transported by rising gases as halides, native elements or sulphur compounds. Researches evidenced that Hg, Cd, Pb, As with others elements are often associated to fluids from marine shallow-water hydrothermal vents [7,8,9,10]. Even though these contributions seem to be of a little importance on a global scale [11], volcanic-derived emissions may become significant on a local base representing hot-spots for the occurrence of accumulation dynamics along the trophic web. In fact, if bioaccumulation occurs, local inputs of pollutants could became quickly exportable following migrations and dynamic of biota interesting wider geographical areas [12].

This paper focused its attention on trace element (Hg, Cd, Pb, As) dynamics occurring closed to a natural hydrothermal emission source sited in the Panarea volcanic complex and was articulated in two experimental phases characterized by different aims. The first one aimed to evaluate the significance and the order of magnitude of alterations occurred in water after a dramatic emission episode localizing the highest impacted site within a natural marine vent area. The second phase was sized only on the highest impacted area and aimed to evaluate matrix-dependent and temporal fluctuations of trace element levels in abiotic and biotic components.

2. Methods

2.1. Study areas

The Aeolian Archipelago arc (Italy, Southern Tyrrhenian sea) is a complex volcanic system constituted by seven islands: Vulcano, Lipari, Salina, Panarea Stromboli, Filicudi and Alicudi. The Panarea complex (fig. 1) is considered a quiescent volcano of the Aeolian Archipelago showing, spread within a 4 km^2 geothermal field [13], the most intense and diffuse shallow submarine hydrothermal activity known since historical times [2,14,15,16,17] due to the presence of numerous volcanoes, submarine canyons and hydrothermal sources [18,19]. The Ants of Panarea are formed by seven islets (Basiluzzo, Dattilo, Panarelli, Lisca Bianca, Lisca Nera, Bottaro, and Formiche) which are the emergent part of a subaqueous platform of the 20 km-wide crater of an ancient volcano [20,21]. In this area, exhalative vents release

about 106-107 Lday^{-1} [2] of hot thermal water (48-54 °C) characterized by low pH values ranged within 4.7-5.4 pH-units [22]. At the end of 2002, the southern portion of the Tyrrhenian Sea was affected by an earthquake (M = 5.8, epicenter 40 km NE of Palermo) linked to abrupt outgassing on the island of Panarea which occurred on November the 3rd. Stromboli was significantly active from December 2002 with a progressive reduction of vents intensity starting about from July 2003 [23].
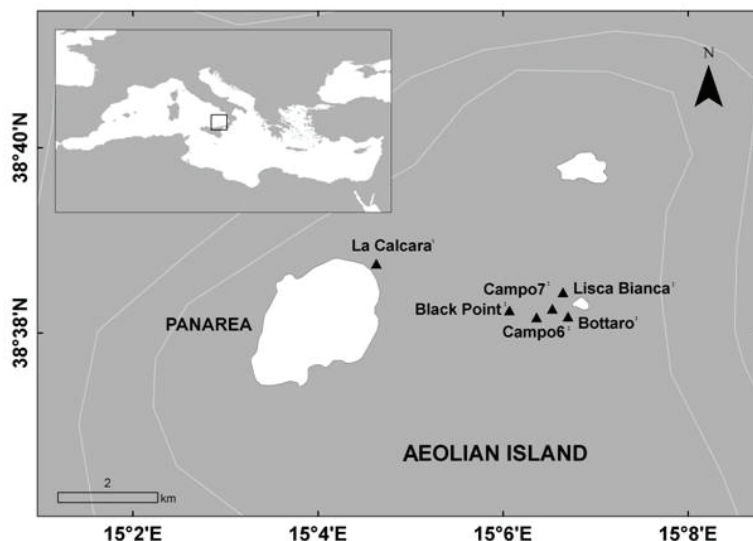

Fig. 1. The study area. The geographical localization of sampling stations is georeferenced in the reported scaled map of the Aeolian Archipelago. Panarea sampling station (La Calcara1) is located about 3 km far from the stronger paroxysmal activity, whereas Ants of Panarea islets sampling stations (Campo 7, Campo 6, Bottaro, Lisca Bianca, Black Point, indicated with the number 2) are located closer to the maximum exposed site (within 200 m).

Massive outgassing phenomena were located three kilometres East from the Bottaro and Lisca Bianca islets [24]. Further details related to the principal features of this event are reported by [6] and references therein. Literature documented very strong exhaling vents occurred at Point 21 and Bottaro North sites in which the individual gas flow rates of these fumaroles ranged between about 210 and 870 Lmin^{-1} [25] contributing respectively by 57 % and 78 % to the total gas output [26]. [26] observed that the Bottaro North, Fumaroles Field and Black Point sites evidenced almost an equal total gas output ranging within 2490-2740 Lmin^{-1} reporting an increase of more than twice of the emission rate determined in the mid 1980's by [21].

2.2. Rationale at the basis of this work

The experimental design was focused to evaluate, on a statistical basis, the significance of temporal fluctuations and matrix-dependent dynamics of potential harmful elements (Hg, Cd, Pb, As) in a shallow coastal marine ecosystem highly impacted by hydrothermal vent activity. To reach this target the experimental activity was organized in two different experimental phases. In both phases, samplings were designed to reduce Type I and Type II errors according to logic models [27,28,29] developed according to specific statistical targets a priori defined.

First experimental phase. Levels of eight different chemical descriptors of hydrothermal activity (Na, K, Mg, Ca, Cl, Br, SO_4^{2-}, HCO_3^-) were measured in marine water in October 2002 (before the event) and November 2002 (after the event). Field activities were performed in Panarea and Ants of Panarea islets; six sampling stations (La Calcara, Campo 7, Campo 6, Bottaro, Lisca Bianca, Black Point) were georeferenced and localized on a cartographic map as reported in figure 1. Sampling stations were selected with the aim to include maxima emission sources and controls. Samples (n = 36 records) were collected according to a logic model based on three factors: location (six levels, fixed), temporal replicates (two levels, fixed and orthogonal: BE versus AE, respectively before and after the critical event), and sampling replicates (three levels, random). Results were statistically analyzed in order to evaluate: i) the occurrence of a significant quantitative alteration of chemical water composition of selected descriptors; ii) the order of magnitude of observed alteration for each of the water descriptors; iii) the localization of the highest impacted site by hydrothermal activities within the six different levels selected for the factor location. This step represented the starting point for the develop of the second experimental phase.

Second experimental phase. On the basis of the results obtained from the previous phase, the highest impacted area (SA) was selected for the development of further samplings finalized to the evaluation of trace elements (Hg, Cd, Pb, As) levels in different environmental matrices: sediments, primary producers (*Cystoseira compressa*), and a carnivorous sedentary fish species (*Serranus cabrilla*). The choice to focus detailed researches only on the station which evidenced, during the first phase, the highest impact was related to the a priori decision to evaluate the maxima exposure effects both on abiotic and biotic matrices. This allowed to evaluate also the occurrence of temporal dynamics and to select the opportune experimental controls (C). The species *C. compressa* was selected as representative of dynamics interesting primary producers due to its abundance within both selected SA and controls. A sedentary benthonic fish species with longevity characteristics and widespread throughout the survey area was chosen in order to give a better description of investigated stations and on the occurrence of bioaccumulation phenomena. In particular, due to its ecological features and local commercial importance, the comber *S. cabrilla* was selected as study species. Sampling design was based on three factors: location (two levels, fixed: SA versus C), temporal replicates (two levels, fixed and orthogonal: T_0 versus T_1, respectively on November 2002 immediately after the event and on September 2003 ten months later), sampling replicates (six levels, random), collecting at all n = 24 records per matrix. Statistical analyses were performed on obtained results with the aim to evaluate the significance of: i) temporal differences among measured levels; ii) matrix-dependent dynamics involving selected trace elements.

2.3. Sampling procedures and laboratory analyses

2.3.1. First experimental phase: water

To collect samples of thermal waters, a system made of a Teflon probe and a syringe connected by a three-ways valve to a Pyrex bottle was used. The stopper of the bottle has two holes sealed by valves. The probe was put inside the emission point about 50 cm beneath the sea bottom and the whole sampling system was first filled by the CO_2- dominated gas to move out the seawater and keep seawater contamination at the lowest, possible, levels. Hence, by using the syringe, the thermal water is pumped into the glass bottle through one of the valves located on the stopper. Simultaneously, the same volume of gas is drawn out through the second valve and the procedure is repeated until the bottle was totally filled by the thermal water. The sample was brought to the surface keeping both of the stopper valves closed. On board the water sample was filtered through cellulose filters (0.45 μm) and acidified (100 μL HNO_3) for minor and trace elements determination. An Elan, mod. DRC 6100-c was used for trace elements analysis, reporting analytical results as μgL^{-1}.

2.3.2. Second experimental phase: sediments and biota

Superficial bottom sediments (0-5 cm) were sampled by scuba divers using an 8 cm diameter HDPE core tube, were extracted from the core, homogenized, and stored at +4 °C in pre-cleaned HDPE vials till to analyses. A methodological criteria was adopted to control grain size and mineralogical variability, responsible of the non homogeneity of the sediment, in order to ensure that variation in the observed data could be properly attributed to tested factors and not to grain-size differences among sediments. In fact, it is well-known by the literature that fine-grained particles tend to have relatively higher trace element contents than sandy ones [30,31]. A mineralogical check of collected sediments exclude significant mineralogical differences among stations. To ensure the comparability among sediment data, analyses were performed on <1 mm sediment fraction. Sediments were characterized by a sand dominance (higher than 98 %), nevertheless, grain-size variability was checked (unpublished data) excluding sediment samples which evidenced a silt content higher than 1 %. Biota samplings (both involving *C. compressa* and *S. cabrilla*) were performed contextually to the sediment ones. Tissues of *C. compressa* were washed using synthetic marine water (ISO 10253:1995 E) to remove small-diameter sediment particles and stored in pre-cleaned HDPE packets at +4 °C. *S. cabrilla* were caught using a fishing pole, details concerning the body size standardization are reported in [34]. This procedure represents an important phase in sampling activity, in fact, trace element levels in fishes tend to increase with the exposure time and are consequently directly related to the body length. *S. cabrilla* is a sedentary species living on rocky, sandy and muddy bottoms at depths ranging within 12-250 m. It feed mainly on fish and crustaceans (trophic level 3.35) with a maximum age of 7 years. In spite of its low commercial value, this species represents a food resource on a local-scale base. Ten specimens were sacrificed at each sampling stations, after that, excided portions of liver and edible flesh were wrapped in aluminum foil to reduce external contamination and stored at −20 °C until laboratory assays. Quantifications of trace elements (Hg, Cd, Pb, As) were performed on 0.5 g of dried (sediments and *C. compressa*) or wet (*S. cabrilla*) samples after the mineralization of solid matrices with a mixture H_2O_2–HNO_3 (4:1 v/v) in a microwave oven (Milestone, mod. ETHOS D Microwave Labstation), according to the US-EPA 3051A method. Extracts were filtered using a pre-cleaned cellulose fibre filters disk (Millipore) and opportunely diluted using ultrapure water. Determinations of Cd and Pb were performed by atomic absorption spectrometry with electrochemical atomization (GF-AAS, Perkin-Elmer, mod. AAnalyst 700). Hg and As were determined by atomic absorption via cold vapor generation (CV-AAS) according to the US-EPA 7473, and by atomic absorption after the hydride generation following the US-EPA 7011 method, respectively. Analytical results were expressed as $mgkg^{-1}$ dry weight (d.w.) for sediments and *C. compressa* and as $mgkg^{-1}$ wet weight (w.w.) for *S. cabrilla* tissues.

2.4. Quality assurance and Quality control (QA/QC)

Chemicals and reagents were analytical grade and glassware was carefully washed to avoid sample cross-over contamination. Concerning water samples, measurements were performed on 100 mL diluted, filtered and acidified water aliquots, spiked with a Y-Sc-Re internal standard solutions. Accuracy of the determinations (± 15 %) was checked by analyzing synthetic spiked seawaters. Concerning solid matrices, recoveries and reproducibility were checked both for sediments and organic matrices by analyzing procedural blanks and reference materials purchased from the National Institute of Standard and Technologies (NIST - Estuarine Sediment SRM1646a), the Department of Trade Industry as a part of the National Measurement System of the UK (SRM - Harbour sediment), the National Research Council, Canada (DORM-2 and DOLT-3). Analytical blanks were prepared, prior to test samples, using the same procedure. A solvent/matrices blank were checked every 15 samples to evaluate the response of detection instruments. Standard reference materials were analyzed in statistical replicates (n = 10) to calculate

averages and standard deviation of recoveries for all tested trace elements. Average percentages of recoveries were within 75.7 - 102.3 % range of variation for all measured variables and analytical concentrations were not recovery corrected. Limit of detection (LOD) was defined as the average blank (n=10) plus three standard deviation (SD); also the limit of quantification (LOQ) were calculated for the adopted procedures and were: concerning water variables 0.01 μgL^{-1} (Na, K, Mg, Ca, Cl, Br, $SO4^{2-}$, $HCO3^{-}$), while for variables measured in solid matrices 0.05 μgg^{-1} (Cd); 0.1 μgg^{-1} (Pb); 0.01 μgg^{-1} (Hg) 0.5 μgg^{-1} (As).

2.5. Statistical analyses

Concerning data collected during the first experimental phase, multivariate analyses were performed using Primer-E version 6.0 (Plymouth Marine Laboratory, UK), according to methods proposed by [32]. Principal Components Analysis (PCA) was applied to investigate correlations and similarities among environmental variables. The Euclidean distance resemblance matrix was calculated on weakly transformed ($\sqrt{}$) and normalized data. Non-metric multidimensional scaling (nMDS) is a technique characterized by a great flexibility in defining and converting dissimilarities to distances and its rationale is to preserve these relationships in low-dimensional ordination space [33]. The statistical significance of the three-dimensional nMDS projection obtained by application of Kruskal stress formula 1 and imposing minimum stress of 0.01, was verified by assessing the linearity of Shepard's diagram. The two-dimensional nMDS plot was superimposed on results obtained by the cluster analyses to evaluate the distance of observed segregations. The significance of observed ordinations related to different factors of interest was tested by the ANOSIM statistic R (one-way or two-way) test performed running 9,999 permutations. This procedure tests hypotheses for differences between groups of samples (according to an a priori defined factor), using permutation/randomization methods on a resemblance matrix and represents a well-sized strategy for multivariate experimental design characterized by few hierarchized factors [29]. The Pairwise routine test was, also, run to evaluate the significance of pairs of factors. Concerning the second experimental phase, univariate analyses were performed using the GraphPad Prism software version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). Average, maximum, minimum and standard deviation (SD) of each variable of interest were calculated. D'Agostino & Pearson omnibus normality test was performed to evaluate the best correlation matrix for each dataset, after that the opportune two-way ANOVA test was performed to evaluate significant differences linked to selected factors of interest.

3. Results

3.1. First experimental phase

Averages values (n = 3) for each chemical descriptor (Na, K, Mg, Ca, Cl, Br, $SO4^{2-}$, $HCO3^{-}$) grouped as levels measured in BE and AE are reported as logarithmic scale, in figure 2. Data are referred to values measured in sampling stations from the Ants of Panarea islets. Results of the statistical analyses confirmed the increase of the considered descriptors evidenced after the paroxysmic activity Actually, the ANOVA test performed to evaluate the significance of observed differences, evidences that the tested factor (BE versus AE) accounts for 33.31 % of the total variance with an associated F value of 8.83 (P = 0.0029), reporting a very significant effect related to the occurrence of the paroxysmic activity with a less than 0.29 % chance of randomly observing a bigger effect. Principal component analyses (fig. 3) performed on chemical descriptors evidenced that the first two axes (PC1 and PC2) accounted for the 95.2 % of the total variance (respectively 71.1 and 24.1 %). The first axis showed a significant negative

correlation to levels of Na, Cl, and Br, whereas the second axis was negatively correlated to K and Ca and positively related to SO_4^{2-}. The principal difference among data acquired at different sampling times (BE versus AE) is principally due to variables related to the first axis. In particular, the paroxysmic activity produces a significant increase of Na, Cl, and Br. Differences among AE stations are principally related to variables highly correlated to the second axis. In particular, some stations evidence a contextual increase of Mg and SO_4^{2-} and a decrease of K and Ca, while others evidence the opposite trend.
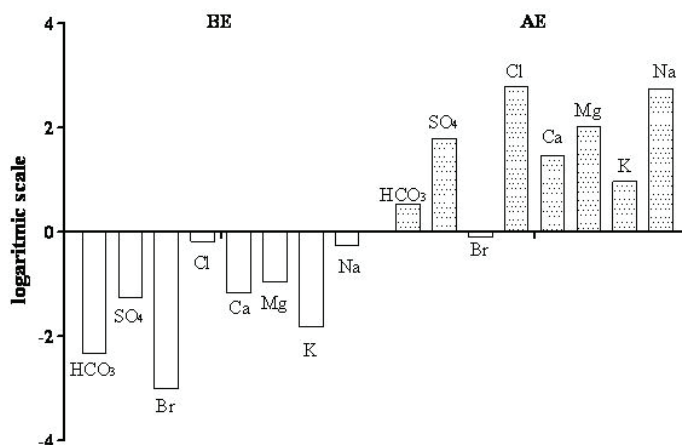

Fig. 2. Average levels of each chemical water descriptor in stations from the Ants of Panarea islets. Data are expressed as logarithmic transformation of measured concentration originally expressed as µgL^{-1}. Data are grouped as average before (BE, white lines) and averages immediately after (AE, grey lines) the paroxysmal activity. HCO^{3-} = carbonic acid, SO_4^{2-}=sulfate, Br = bromine, CL = chlorine, Ca = calcium, Mg = magnesium, K = potassium, Na = sodium.

Considering levels measured for each descriptor, averages changed as reported: Na (0.55→549.39 µgL^{-1}), K (0.02→9.25 µgL^{-1}), Mg (0.11→102.00 µgL^{-1}), Ca (0.07→28.30 µgL^{-1}), Cl (0.66→611.78 µgL^{-1}), Br (<0.01→0.78 µgL^{-1}), SO42- (0.05→60.72 µgL^{-1}), HCO3- (<0.01→3.35 µgL^{-1}). A difference related to the order of magnitude of increases after the event is evidenced. In particular, after the paroxysmic activity, Na, Mg, Ca, Cl, SO_4^{2-} increase of about three order of magnitude; K, HCO3$^-$, increase of about two order of magnitude, while Br increases of about one order of magnitude. Pearson's correlation matrix, calculated on averages acquired after the paroxysmic activity, evidences the occurrence of strong (p < 0.001) positive relationship between the following couples: Na-Cl (0.89), Na-K (0.84), Na-Ca (0.80), Na-Br (0.76), K-Ca (0.98), K-Cl (0.98), K-Br (0.91), Mg-SO_4^{2-} (0.92), Ca-Cl (0.97), Ca-Br (0.93), Cl-Br (0.90). On the contrary, significant (p<0.001) negative relationship are observed between the couples: Na-SO_4^{2-} (-0.51), K-Mg (-0.72), K-SO_4^{2-} (-0.83), Mg-Ca (-0.80), Mg-Cl (-0.67), Mg-Br (-0.72), Ca-SO_4^{2-} (-0.88), Cl-SO_4^{2-} (-0.79), and Br-SO_4^{2-} (-0.78).

Two-dimensional nMDS projection crossed with the results obtained from the cluster analyses is reported in figure 4. Minimum stress of this configuration occurred 22 times with a good linearity observed by the Shepard's diagram.
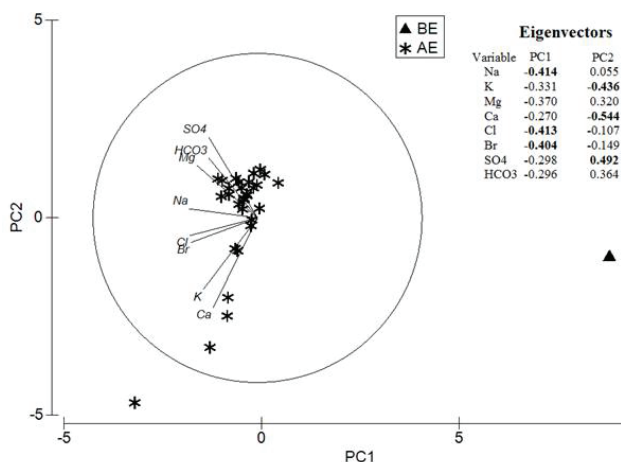
Fig. 3. Principal component analyses performed on chemical descriptors of water. Two dimensional projection of sampling station distribution both before (BE) and after (AE) the paroxysmic activity is reported according to the major related axes. Eigenvectors calculated for each variable are also evidenced. Higher significant relationships are highlighted using bold characters.
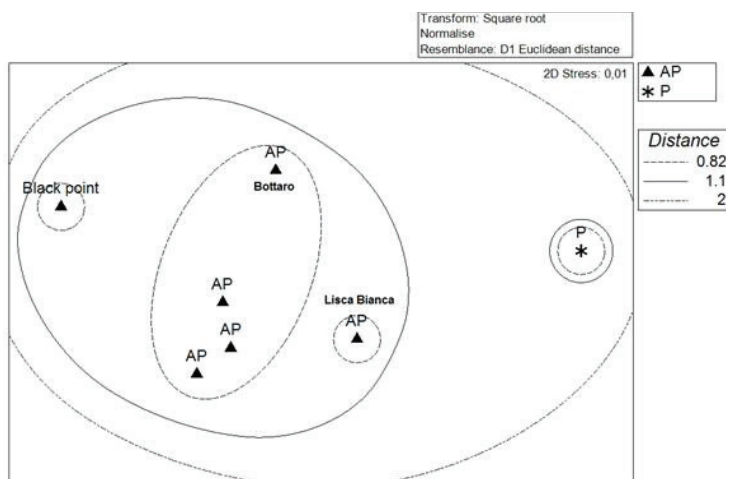
Fig. 4. nMDS performed on levels of chemical descriptors measured in water after the paroxysmal activity. nMDS (0.01 minimum stress) performed on Euclidean distance resemblance matrix calculated on transformed data is superimposed to results obtained by the single-linkage cluster analyses to evaluate the level of dissimilarity among groups. Black triangles indicate AP sampling stations while the black star indicates Panarea sampling station. AP = ants of Panarea islets (Campo 7, Campo 6, Bottaro, Lisca Bianca, Black point), P = Panarea sampling station (La Calcara). Distances from 0.82 to 2.00 are indicated by the black lines.

Results evidence that the assessment of considered chemical descriptors observed in sampling stations related to the Ants of Panarea islets differs significantly from those observed in Panarea sampling station (the cluster analysis evidences a distance higher than 1.1). In particular, as evidenced by the nMDS, even if the station Black Point located in the Ants of Panarea islets differs significantly from the others, all AP

sampling stations are within the 1.1 cluster analyses distance, evidencing a general similarity in chemical descriptors. Furthermore, even if P station differs significantly from the others, it is included within 2.0 cluster analyses distance. The ANOSIM test performed to estimate the significance of the segregation between the factors BE and AE on a multivariate basis, evidences the occurrence of a great significance (Global R = 0.229, significance level of sample statistic = 2.9 %, number of permuted statistics greater than or equal to Global R = 292).

3.2. Selection of study area and controls for the second experimental phase

On the basis of the results obtained by the first experimental phase (also see discussion par. 4.1), the Black Point station was selected as study area (SA) for the second experimental ones. Furthermore, controls were selected far from the Aeolian Archipelago due to the high similarity evidenced by the statistical analysis between AP and P stations concerning the assessment of chemical water descriptors after the paroxysmic activity. Controls, for the second experimental phase, were located in the Southern Tyrrhenian Sea along the Vibo Marina and Capo Vaticano coastal areas. These stations were selected as controls because never interested by volcanic and hydrothermal activities [34].

3.3. Second experimental phase

Measured levels of trace elements (Hg, Cd, Pb, As) in sediment samples collected near the emission source (SA) and controls (C) are reported as average (+ standard deviation) in figure 5, grouping data both related to the factor location (SA versus C), and the factor temporal replicates (T_0 versus T_1).

Fig. 5. Average trace element levels measured in sediments. Average (+ standard deviation) data are grouped both by the factor location (two levels, fixed: study area, SA versus controls, C), and time from the event (two levels, fixed and orthogonal: T_0 after the event, November 2002 versus T_1 ten months after the event, September 2003). Hg = Mercury, Cd = Cadmium, Pb = Lead, As = Arsenic, d.w. = dry weight
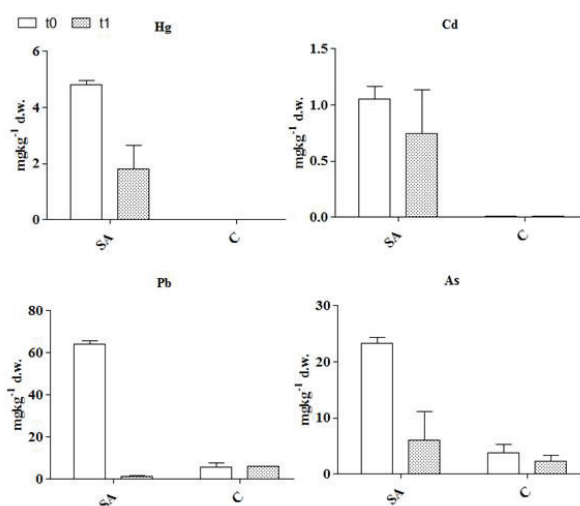
At T_0, average levels in SA are notably higher than values observed in controls for all considered trace elements. At T_1, levels measured in SA are similar to values reported in controls concerning Pb and As, whereas at the same time, Hg and Cd concentrations are notably higher in SA than in C. Furthermore, Hg levels in T_1 evidence a decrease compared to T_0, while Cd levels remain comparable after ten months from the first paroxysmic activity. Levels measured in C do not evidence any significant differences for all considered trace elements concerning the factor temporal replicates. Results obtained by the two-way ANOVA test performed on trace elements levels in sediments are summarized in table 1. Two factors of interest are evidenced: location (SA versus C), and temporal replicates (T_0 versus

T_1). The factor location resulted significant for all considered trace elements, whereas the factor temporal replicates evidences significance concerning Hg, Pb, and As, while not a significant difference is observed in T1 concerning Cd. Measured levels of trace elements (Hg, Cd, Pb, As) in *C. compressa* tissues collected near the emission source (SA) and controls (C) are reported as average (+ standard deviation) in figure 6, grouping data both related to the factor location (SA versus C), and the factor temporal replicates (T_0 versus T_1).

Fig. 6. Average trace element levels measured in *C. compressa*. Average (+ standard deviation) data are grouped both by the factor location (two levels, fixed: study area, SA versus controls, C), and time from the event (two levels, fixed and orthogonal: T_0 after the event, November 2002 versus T_1 ten months after the event, September 2003). Hg = Mercury, Cd = Cadmium, Pb = Lead, As = Arsenic, d.w. = dry weight

For all considered trace elements, levels measured in SA are notably higher than averages observed in controls. At T_1, averages in SA are similar to values reported in controls concerning As and Hg, whereas at the same time, Cd and Pb concentrations are higher in SA than in C. Even in this case, levels measured in C do not evidence any significant differences for all considered trace elements concerning the factor temporal replicates. Results obtained by the two-way ANOVA test performed on trace element levels measured in *C. compressa* tissues are summarized in table 2.

Table 1. Two-way ANOVA results obtained performing analyses on sediment database. X= effect not significant, *= significant, **= very significant, ***= extremely significant. Two factor of interest are considered: *location* (SA *versus* C), and the factor *temporal replicates* (T_0 *versus* T_1). Results obtained related to the total explained variance, F and P values and the level of significance of observed effect are also reported.

	Total explained variance (%)	F	P	Type I error prob. (%)	Effect
Mercury (Hg)					
f1 (location)	64.68	44.94	0.0026	0.26	**
f2 (temporal replicates)	13.37	18.59	0.0125	1.3	*
Cadmium (Cd)					
f1 (location)	67.76	17.01	0.0146	1.5	*
f2 (temporal replicates)	2.01	0.66	0.463	46	x
Lead (Pb)					
f1 (location)	26.79	512.85	<0.0001	0.01	***
f2 (temporal replicates)	36.21	650.57	<0.0001	0.01	***
Arsenic (As)					
f1 (location)	39.34	12.58	0.0239	2.4	*
f2 (temporal replicates)	25.26	20.73	0.0104	1	*

Table 2. Two-way ANOVA results obtained performing analyses on primary producers database. X= effect not significant, *= significant, **= very significant, ***= extremely significant. Two factor of interest are considered: *location* (SA *versus* C), and the factor *temporal replicates* (T_0 *versus* T_1). Results obtained related to the total explained variance, F and P values and the level of significance of observed effect are also reported.

	Total explained variance (%)	F	P	Type I error prob. (%)	Effect
Mercury (Hg)					
f1 (location)	37.55	5.63	0.0765	7.7	x
f2 (temporal replicates)	14.92	10.55	0.0314	3.1	*
Cadmium (Cd)					
f1 (location)	54.62	7.32	0.0538	5.4	x
f2 (temporal replicates)	9.5	225.72	0.0001	0.011	***
Lead (Pb)					
f1 (location)	73.39	19.4	0.0116	1.2	*
f2 (temporal replicates)	0.34	0.24	0.6474	65	x
Arsenic (As)					
f1 (location)	23.43	1.95	0.2354	24	x
f2 (temporal replicates)	8.9	5.56	0.0779	7.8	x

Even in this case, two factors of interest are evidenced: location (SA versus C), and temporal replicates (T_0 versus T_1). Even if measured levels in SA at T_0 are notably higher than related controls, the factor location represents a significant factor only for Pb. This apparently contradictory results is principally due to the fact that in SA, Hg, Cd, and As levels at T_1 tends to became similar to controls values. This is confirmed by the observed results for the factor temporal replicates: any significance is evidenced for Pb.

Measured levels of trace elements (Hg, Cd, Pb, As) in liver and muscles tissues of the species *S. cabrilla* collected near the emission source (SA) are reported as average (+ standard deviation) in figure 7,

grouping data by both the factor tissue (two levels, fixed: liver versus muscle), and temporal replicates (T_0 versus T_1).
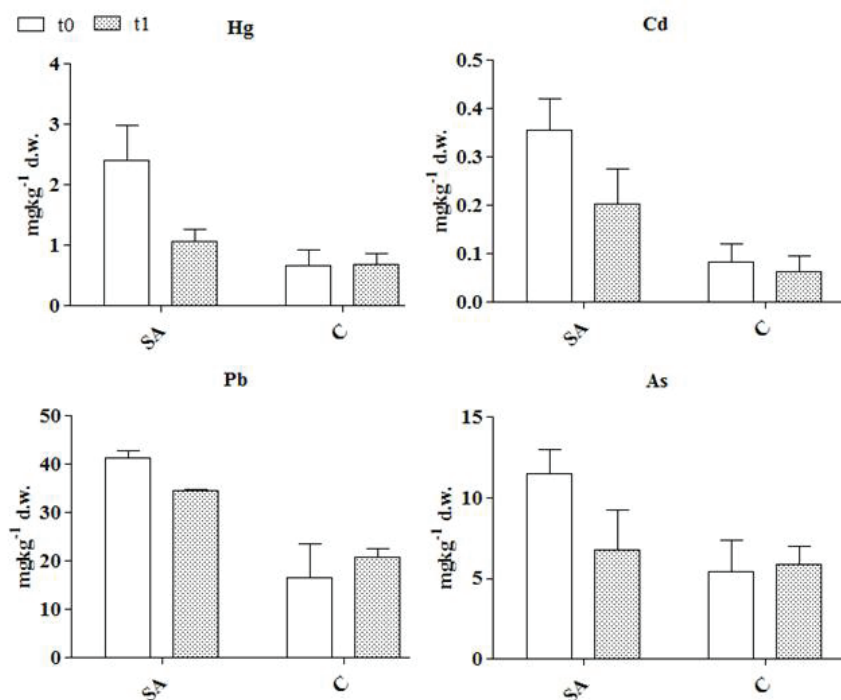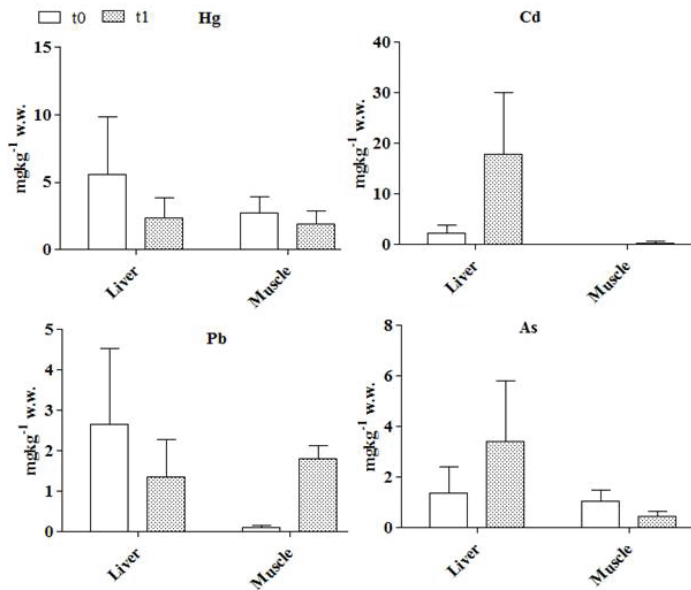

Fig. 7. Average trace element levels measured in *S. cabrilla*. Average (+ standard deviation) data (n = 24) are grouped by both the factor tissue (two levels, fixed: liver versus muscle), and time from the event (two levels, fixed and orthogonal: T_0 after the event, November 2002 versus T_1 ten months after the event, September 2003). Hg = Mercury, Cd = Cadmium, Pb = Lead, As = Arsenic, w.w. = wet weight.

Results obtained by the two-way ANOVA test are summarized in table 3. Both of the factors considered do not evidence significance. These results are due to the occurrence of different trace-element dependent dynamics that affect bioaccumulation processes. In fact, concerning Hg, maximum values are recorded at T_0 in liver, at the same time, muscle levels are significantly lower. After ten months from the paroxysmic activity, liver levels decrease (liver levels at T_1 are minor than T_0), becoming comparable to values measured in muscles. The same trend is observed for Pb even if a notably increase of muscle values after ten months is evidenced. Cd and As are characterized by a significant different behaviour compared to Hg and Pb ones. In fact, even if at T_0, Cd and As levels in livers are higher than in muscles (Cd evidences values quite closed to the LOQ), after ten months, liver values evidence a notably increase recording the absolute maximum, and muscles levels increase too.

4. Discussion

4.1. Dynamics involving water

Approximately 50 marine shallow-water hydrothermal sites are known worldwide, most of them actually poorly explored [35]. Hydrothermal circulation needs a large heat differential and it is controlled by geologic and tectonic conditions such as heat released from intruding magma bodies or by the generation of frictional heat along fractures [36].

Table 3. Two-way ANOVA results obtained performing analyses on fish tissues. X= effect not significant, *= significant, **= very significant, ***= extremely significant. Two factor of interest are considered: *tissue* (liver *versus* muscle), and the factor *temporal replicates* (T_0 *versus* T_1). Results obtained related to the total explained variance, F and P values and the level of significance of observed effect are also reported.

	Total explained variance (%)	F	P	Type I error prob. (%)	Effect
		Mercury (Hg)			
f1 (location)	4,82	0,27	0,6296	63	x
f2 (temporal replicates)	7,46	2,1	0,2208	22	x
		Cadmium (Cd)			
f1 (location)	18,86	2,08	0,2224	22	x
f2 (temporal replicates)	12,35	2,31	0,2028	20	x
		Lead (Pb)			
f1 (location)	9,01	0,56	0,4974	50	x
f2 (temporal replicates)	0,31	0,17	0,7044	70	x
		Arsenic (As)			
f1 (location)	14,03	0,88	0,4019	40	x
f2 (temporal replicates)	2,62	0,99	0,3759	38	x

For this reason, marine shallow-water hydrothermal systems are located near ocean island volcanoes (mantle hot spots), island arc volcanoes (subduction zones) and large active faults (transform faults) as evidenced by [35]. Geophysical characteristics of the hydrothermal sites could determine significant differences of chemical composition of observed gas emissions. [15], in their researches on the emissions observed in the Aeolian archipelago, evidenced that the low-temperature hydrothermal alteration of seawater is related to the chemistry of warm fluids which seep from volcanic sands. In this study, chemical descriptors selected to trace dynamics occurring in water column evidenced a clear, statistically supported, variation of their levels due to the paroxysmic activity. In particular, hydrothermal emissions are principally characterized by a notable increase of Na, Cl, and Br as evidenced by the PCA results. An early significant emission of K, Ca, and $SO4^{2-}$ was also associated to the event. [15] related the observed increase of dissolved K and other elements into surrounding seawater, to the occurrence of a chemical overprint of reactions resulting from the attack of igneous silicate phases by volcanic gases. On the contrary, observed Ca increases could be related to the occurrence of fluids seep from volcanic sand. [23] evidenced as compositional changes in gas emission of fumaroles are a complex function of time, chemistry, temperature and space. In particular, spatial variations across the fumaroles field were related to changes in redox conditions and components dominated by random variations. In particular, as observed by Sieland and colleagues [26] the fluid samples from Black Point were enriched with respect to seawater in Na, K, Ca, Cl, SiO_2 and Mn, but depleted in Mg and SO_4. In the same research, an enrichment of other minor trace elements such as Li, Br, B, Fe, Mn, Pb, and Zn up to 8 orders of magnitude in relation to normal seawater was observed. Sieland and colleagues (2009) also evidenced the presence of a significant difference among Black Point stations and other sampling sites, related to mineralization, pH (~ 3), redox potential (up to about +300 mV) as well as their minor and trace element enrichment [37]. This differences are supposed to be related to the occurrence of two distinct fluid types, in particular the Black Point fluid is supposed to be more influenced by the contribution of magmatic gases. The occurrence of partly reducing conditions might be caused by a higher input of magmatic gases such as

SO_2, HCl or HF [37]. These results support the clear multivariate-basis segregation among Black Point and other sampling stations observed in this paper.

4.2. Dynamics involving sediments

Sediments, due to their high percentages of silt and organic carbon and their mineralogical structure which tend to adsorb charged molecules and ions, represent the final reservoir of potential hazardous elements in aquatic ecosystems [30]. In marine shallow-water hydrothermal vents, typical surface ocean waters redox and pH conditions are altered by the emission of H_2S [15]. This occurrence also alters natural adsorption/desorption phenomena involving trace elements repartition equilibrium between sediments and water column [38]. Considering data collected in SA at T_0, a clear enrichment of Hg, Cd, Pb and As levels in sediments due to the hydrothermal activity was observed, confirming the importance of this matrix as reservoirs of trace elements. Hg values measured at T_0 are higher than levels recorded in highly human-due polluted sites such as the Gulf of Naples (0.18 mgkg^{-1}) [39]. Furthermore, values are also higher compared to cinnabar geological direct [40] or indirect (0.30-1.90 mgkg^{-1}) [41] enriched sites. Cd levels are notably higher than high human-due impacted harbours (Bari 0.40 mgkg^{-1}; Barletta 0.29 mgkg^{-1}; Gulf of Naples 0.27 mgkg^{-1}) [39] and back-ground Mediterranean levels (Aegean Sea 0.53 mgkg^{-1}; Southern Adriatic 0.20 mgkg^{-1}) [42,43]. Pb evidences values higher than or closed to those observed in highly human-due impacted areas (respectively Venice Treporti 10 mgkg^{-1} and Venice Porto Marghera 70 mgkg^{-1}) [44]. Concerning As, averages are similar to values observed in the Elba-Argentario coastal area (5.7-37.8 mgkg^{-1}) [41], characterized by a well-documented geomorphologic alteration due to its volcanic origin [45]. As reported by the literature, the source and therefore, initial chemical composition, of a hydrothermal fluid is an important factor controlling its final chemical composition [12]. [10] evidenced as marine shallow-water hydrothermal systems, which have abundant complexing ligands in the form of Cl and SO_4 ions, tend to transport high levels of Pb, and Cd metals while fluids from marine shallow-water hydrothermal systems, which circulate mainly water of meteoric origin generally transport trace elements, which form oxyanions, such as As [46]. This occurrence is probably related to the notably enrichment of Pb and Cd observed in SA compared to controls. Results evidenced as the occurrence of natural volcanic activity could represents a significant source of pollution for the surrounding marine ecosystem. Furthermore, the observed decrease of levels measured in sediments after ten months from the maximum emission, evidences the occurrence of specific equilibrium among the reservoir compartment (sediment) and the exchangeable ones (water-column). This result could be both related to changes in trace element emissions and redox potential values, factors which could equally affect trace element dynamics. In these systems, the oxidation rates of trace elements in seawater could proceed at different rates, as reported by [12] for the oxidation rates of the couples Fe(II)/Fe(III) and As(III)/As(V). Furthermore chemical balances in these systems are, also, influenced by temperature, and pH. These variables largely depend on water depth which affect the pressure curve, gas content, and salinity [47,48]. Furthermore, pH range is controlled by a combination of water-rock interaction, initial buffer capacity and near surface conditions [12]. Different behaviour of considered trace elements in SA at T_1 could be due to dynamics involving the coprecipitation with the hydrous ferric oxide fraction. In fact, while Pb levels in sediments after ten months tends to became similar to controls, Hg, Cd, and As levels remained higher; this is probably due to the higher stability produced by the hydrous ferric oxide fraction on these elements unless the physicochemical conditions surrounding the oxides change as observed by [12] occurring for As.

4.3. Dynamics involving biota

Trace elements measured in sediments could be present in a not-bioavailable chemical form, in particular the occurrence of possible hydrous ferric oxide precipitations could represent an important factor able to determine a reduction of the bioavailable fraction. Different physico-chemical dynamics affecting the water-column could determine significant release from sediments towards biota [49]. For these reasons, results obtained performing the analyses on biota could allow to evaluate bioaccumulation dynamics involving local species at different trophic level and to evaluate the possible risk related to human consumption of fish species.

Comparing results obtained in the present study to literature, values reported for other primary producers species (*Halimeda* spp. a green calcareous algae) collected in hydrothermally-influenced sites, evidenced As levels similar to those measured in the SA. In fact, [12] reported As levels ranging within 8.5-20.2 mgkg^{-1} d.w. closed to the hydrothermal area. Results obtained in this study evidenced in SA at T_0 an average enrichment factor of ~2.0 for Pb and As and ranging within 3.0-4.0 for Hg and Cd.

Even if trace element bioaccumulation in primary producers could not represent a significant problem involving plants survivor, concerning fishes, stocks decline could be related also to trace element pollution. A research performed by [50] on fish species of commercial interest in highly human stressed areas (Gulf of Naples) evidenced trace element values measured in *Pagellus erythrinus*, *Boops boops*, *Coris julis*, *Gobius paganellus*, *Scorpaena scrofa*, and *Trachinus araneus* values ranging within 0.005-0.340 mgkg^{-1} w.w. (Hg), 0.005-0.180 mgkg^{-1} w.w. (Pb), 0.004-0.110 mgkg^{-1} w.w. (Cd), and 0.005-0.150 mgkg^{-1} w.w. (As). Founding comparative bibliography to evaluate levels measured in this study for the *S. cabrilla* species was not an easy task. In fact, few data are available on this species due to the fact that it does not represent neither any global commercial interest nor toxicological ones. [51] observed in the Egypt area Pb values ranging within 0.92-2.53 mgkg^{-1} d.w. and Cd ones always < 0.006 mgkg^{-1} d.w. A research performed by [52] on a wide range of Mediterranean species clearly evidenced as hydrothermal vents produce trace element significant enrichments. In fact, Pb levels ranged within minimum of 0.006 mgkg^{-1} w.w. measured in Seabream (*Sparus aurata*) muscle tissues, and 0.386 mgkg^{-1} w.w. measured in Striped seabream (*Lithognathus mormyrus*) ones, while Cd values ranged within <LOQ measured in *S. cabrilla* and many other species and 0.015 mgkg^{-1} w.w. recorded in the Sharpsnout seabream (*Diplodus puntazzo*). Levels measured in muscles and liver tissues of *S. cabrilla* are notably higher than those reported by the literature for the European eels, a fish species considered a good bioaccumulator of pollutants [53,54,55,56,57].

Chemical pollution could represent a significant factor affecting the overall decline of fish species threaten energy storage, affecting lipid metabolism and leading, indirectly, to failed migration and/or the impairment of successful reproduction. Tissue levels are reliable to the occurrence of toxicological effects on biota which are variable in intensity and effects related to the species [58]. Nevertheless, some general information related to the possible effects could be generalized from the species to the *taxa*. Concerning ecotoxicological effects of trace element on fish species, [59] showed that Hg tends to accumulate in muscles rather than liver and other tissues, due to the high concentration in muscles of functional proteins having a high affinity with this element. In this study, after the maximum exposure observed at T_0 during which liver values were higher than muscles ones, muscle and liver tissues showed comparable levels. The liver is considered to be a good indicator of chronic exposure to trace elements as it is involved in metal metabolism [60]. Damages are evidenced at lower Hg concentration, in fact, values above 1 μM could cause blood damage [61]. The effects of Cd in tissues have been reported for blood and kidney (1-5 mgL^{-1}) [61], while in vitro exposure has shown the occurrence of effects on gills (3 days of exposure to 5 mgL^{-1}) [62] and on liver and lipid content in muscles (30 days of exposure to 0-5 μgL^{-1}) [63] . Pb levels higher than 300 μgL^{-1} cause blood alterations after 30 days of in vitro exposure [64].

Concerning the risk exposure related to human consumption, the European Commission (2001/22/CE) defined specific maximum levels for Hg (0.50 mgkg^{-1} w.w.), Pb (0.20 mgkg^{-1} w.w.), and Cd (0.05 mgkg^{-1}

w.w.) in muscle tissues of fish species. It should be pointed out that values for these elements measured in this study exceeded the maximum levels suggested for human consumption for Hg and Pb both at T_0 and T_1. Cd values does not exceeded those limits at T_0 but exceeded them at T_1 evidencing a notably increase of levels after ten months from the maximum exposure. Concerning livers, the values measured were highly above the risk levels at any sampling campaign for all of considered elements, and for this reason the human consumption of *S. cabrilla* livers should be avoided. It is to notice that, even if 2001/22/CE directive does not rules levels in liver, it evidences that defined values have to be assumed as reference even if fishes are consumed without evisceration. Furthermore, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) defines weekly limits for human consumption concerning trace elements [65]. On the basis of the analytical data obtained in this study in the worst case and considering as used for feeding purposes only the dorsal muscle portion, maximum weekly levels proposed by JECFA could be exceeded by the consumption of 100 g of *S. cabrilla* of: 96.6 times for Hg (0.005 mgweek^{-1}), 13.7 times for Cd (0.007 mgweek^{-1}), 9.4 times for Pb (0.025 mgweek^{-1}), and 3.7 times for As (0.05 mgweek^{-1}).

5. Conclusions

Results obtained in this study evidenced that the paroxysmic activity observed in correspondence of the Ants of Panarea islets produced a significant change in chemical descriptors of water column selected. Water descriptors changes segregates the Black Point station from each other concerning the typology of observed changes rather than magnitude allowing to select this area as study site for the evaluation of trace element dynamics. Furthermore, even if Panarea station (La Calcara) evidenced a significant difference from Ants of Panarea ones, it was closer to them on a statistical point of view excluding the possibility to consider this site as a reference control for trace element levels in a not impacted area from hydrotermalism. Trace element levels in sediments evidenced the occurrence of a significant input by degassing activity and the occurrence of element-dependant dynamics which are able to interfere to sediment releases. Levels observed in biota excluding a quick recovery of the area both concerning primary producers and fishes. Nevertheless levels measured in fish tissues evidenced a significant trend to increase with the time from the first event reaching values higher than risk limits for human consumption proposed by the 2001/22/CE directive.

References

[1] Stumm W, Morgan JJ. Aquatic chemistry: chemical equilibria and rates in natural waters, 3rd ed.; 1995.

[2] Italiano F, Nuccio PM. Geochemical investigations on submarine volcanic exhalations to the East of Panarea, Aeolian Islands, Italy. J Volc & Geoth Res 1991; 46: 125-141.

[3] Stuben D, Glasby GP. Geochemistry of shallow submarine hydrothermal fluids from Paleohori Bay, Milos, Aegean Sea. Exp Min Geol 1999; 8: 273–287.

[4] Hannington M, Herzig P, Stoffer P, Scholten J, Botz R, Garbe-Schonberg D, Jonasson IP, Roest W. The Shipboard Scientific Party, First observations of high-temperature submarine hydrothermal vents and massive anhydrite deposits off the north coast of Iceland. Mar Geol 2001; 177: 199–120.

[5] Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia MC. Volcanic carbon dioxide vents reveal ecosystem effects of ocean acidification. Nature 2008; 454, 96–99.

[6] Vizzini S, Tommasello A, Di Malda G, Pirrotta M, Mazzola A, Calvo S. Effect of explosive shallow hydrothermal vents on δ13C and growth performance in the seagrass Posidonia oceanica. J Ecol 2010; 98: 1284-1291.

[7] Dando PR, Stùben D, Varnavas SP. Hydrothermalism in the Mediterranean Sea. Prog Oceanogr 1999; 44: 333-367.

[8] Price RE, Pichler T. Distribution, speciation and bioavailability of arsenic in a shallow-water submarine hydrothermal system, Tutum Bay, Ambitle Island, PNG. Chem Geol 2005; 224: 122-135.

[9] Varnavas SP, Cronan DS (1988) Arsenic, antimony and bismuth in sediments and waters from the Santorini hydrothermal field, Greece. Chem Geol 1988; 67: 295-305.

[10] Vidal VMV, Vidal FV, Isaacs JD. Coastal submarine hydrothermal activity off northern Baja California. J Geoph Res 1978; 83(B4): 1757-1774.

[11] Lantzy RL, MacKenzie FT. Global cycles and assessment of man's impact. Geochim. Cosmochim. Acta 1979; 43: 511-515.

[12] Price R, Pichler T. Measuring toxic elements and toxicity in marine shallow-water hydrothermal systems. In: Merkel BJ, Schipek M, editors. Research in shallow marine and fresh water systems. 1st International Workshop Proceedings, FOG Freiberg Online Geology. 2009, ISSN 1434-7512. 22: 82-86.

[13] Gabbianelli G, Gillot PY, Lanzafame G, Romagnoli C, Rossi P L (1990) Tectonic and volcanic evolution of Panarea (Aeolian Islands, Italy). Mar. Geol. 92: 313-326.

[14] Kokelaar P, Romagnoli C (1995) Sector collapse, sedimentation and clast population evolution at an active island-arc volcano: Stromboli, Italy. Bull. Volc. 57: 240-262.

[15] Sedwick PN, Stuben D (1996) Chemistry of shallow submarine warm springs in an arc-volcanic setting: Volcano Island, Aeolian Archipelago, Italy. Mar. Chem. 53: 147-161.

[16] Inguaggiato S., Italiano F Proceeding of the 9th International Symposium on Water-Rock Interaction-WRI9, 30 March–3 April 1998, Taupo, New Zealand, Arehart andHulston Rotterdam, 1998, p. 727–730.

[17] Lucchi F. Late-Quaternary terraced marine deposits as tools for wide-scale correlation of unconformity-bounded units in the volcanic Aeolian archipelago (southern Italy). Sed. Geol. 2009; 216: 158–178.

[18] Italiano F. Gas exhalation from the sea floor. Invited lecture at 25th course: Observational database and mechanisms of climate, Erice, Sicily, 21-27 November, 1998.

[19] Chiocci FL, Bosman A, Romagnoli C, Tommasi P, De Alteris G. The December 2002 Sciara del Fuoco (Stromboli Island) submarine landslide: a first characterisation. EGS-AGU-EUG Joint Assembly, Nice, France, April 2003, Geophysical Research Abstracts. CDROM Version.Vol.5.

[20] Gamberi F, Marani PM, Savelli C. Tectonic, volcanic and hydrothermal features of a submarine portion of the Aeolian arc (Tyrrhenian Sea). Mar Geol 1997; 140: 167-181.

[21] Esposito A, Giordano G, Anzidei M. The 2002-2003 submarine gas eruption at Panarea volcano (Aeolian Islands, Italy): Volcanology of the seafloor and implications for the hazard scenario. Mar Geol 2006; 227: 119-134.

[22] Gugliandolo C, Italiano F, Maugeri T. The submarine hydrothermal system of Panarea (southern Italy): biogeochemical processes at the thermal fluids - sea bottom interface. Ann Geophys 2006; 49(2/3): 783-792.

[23] Buccianti A, Tassi F, Vaselli O. Compositional changes in a fumarolic field, Vulcano Island, Italy: a statistical case study. In: Buccianti A, Mateu-Figueras G, Pawlowsky-Glahn V (Eds) Compositional data analysis in the geoscience: from theory to practice. Geological Society, London, Special Publications. ISBN: 1-86239-205-6. http://www.geolsoc.org.uk 2006; 264: 67-77.

[24] Caliro S, Caracausi A, Chiodini G, Ditta M, Italiano F, Longo M, Minopoli C, Nuccio PM, Paonita A, Rizzo A. Evidence of a recent input of magmatic gases into the quiescent volcanic edifice of Panarea, Aeolian Islands, Italy. Geophysic Res Lett 2004; 31(7): L07619. doi: 10.1029/2003GL019359

[25] Bauer K, Bauer D, Barth G. Flow rate measurements at submarine volcanic gas emissions. In: Merkel BJ, Schipek M, editors. Research in shallow marine and fresh water systems. 1st International Workshop Proceedings, FOG Freiberg Online Geology. ISSN 1434-7512. 2009; 22: 112-117.

[26] Sieland R, Steinbrückner D, Hamel M, Merkel B, Schipek M. Geochemical investigations and gas quantification of submarine fluid discharges in the hydrothermal system of Panarea (Aeolian Islands, Italy) In: Merkel BJ, Schipek M (Eds) Research in shallow marine and fresh water systems. 1st International Workshop Proceedings, FOG Freiberg Online Geology. ISSN 1434-7512. 2009; 22: 87-93.

[27] Underwood AJ. On Beyond BACI: Sampling designs that might reliably detect environmental disturbances. Ecol Appl 1994; 4: 4-15.

[28] Underwood AJ, Chapman MG. Power, precaution, Type II error and sampling design in assessment of environmental impacts. J. Exp. Mar. Biol. Ecol. 2003; 296: 49-70.

[29] Benedetti-Cecchi L. Experimental design and hypothesis testing in ecology. Biol Mar Medit 2004; 11(1): 407-455.

[30] Sternbeck J, Östlund P. Metals in sediments from the Stockholm region: Geographical pollution patterns and time trends.Water Air Soil Pollut Focus 2001; 1: 151-165.

[31] Zhang L, Ye X, Feng H, Jing Y, Ouyang T, Yu X, Liang R, Gao C, Chen W. Heavy metal contamination in western Xiamen Bay sediments and its vicinity, China. Mar Poll Bull 2007; 54: 974-982.

[32] Clarke KR, Warwick RM. Change in marine communities: an approach to statistical analysis and interpretation. 2nd Edition. Primer-E: Plymouth 2001.

[33] Somerfield PJ, Clarke K.R. A comparison of some methods commonly used for the collection of sublittoral sediments and their associated fauna. Mar Environ Res 1995; 43: 143-156.

[34] Andaloro F, Romeo T, Renzi M, Guerranti C, Perra G, Consoli P, Perzia P, Focardi SE. Alteration of trace element levels in sediments and biota from the Aeolian Archipelago (Italy) following an episode of intense volcanic activity. Environ Monit Assessm 2011 (in press).

[35] Pichler T. Marine shallow-water hydrothermal systems as natural laboratories. In: Merkel BJ, Schipek M (Eds) Research in shallow marine and fresh water systems. 1st International Workshop Proceedings, FOG Freiberg Online Geology. ISSN 1434-7512. 2009; 22: 77-81.

[36] Fehn U, Cathles LM. The influence of plate movement on the evolution of hydrothermal convection cells in the oceanic crust. Tectonophysics 1986; 125: 289-312.

[37] Capaccioni B, Tassi F, Vaselli O, Tedesco D, Poreda R. Submarine gas burst at Panarea Island (southern Italy) on 3 November 2002: A magmatic versus hydrothermal episode. J Geophysic Res Solid Earth 2007; 112: 1-15.

[38] Prokop Z, Vangeheluwe ML, Van Sprang PA, Janssen CR, Holoubek I. Ecotox Environ Saf 2003; 34: 65.

[39] Romano E, Ausili A, Nadezhda Z, Magno MC, Pavoni B, Gabellini M. Marine sediment contamination of an industrial site at Porto of Bagnoli, Gulf of Naples, Southern Italy. Mar Poll Bull 2004; 49: 487-495.

[40] Barghigiani C, Ristori T, Lopez-Arenas J. Mercury in marine sediments from a contaminated area of the northern Tyrrhenian Sea: <20 _m grain-size fraction and total sample analysis. Sci Total Environ 1996; 192, 63–73.

[41] Borghini F. Gli elementi in tracce negli ecosistemi costieri dell'Argentario. At Soc Tos Sci Nat Mem 1988; 105(A): 9-16.

[42] Dalman Ö, Demirak A, Balci A. Determination of heavy metals (Cd, Pb) and trace elements (Cu, Zn) in sediments and fish of the Southeastern Aegean Sea (Turkey) by atomic absorption spectrometry. Food Chem 2005; 95(1): 157-162.

[43] Storelli MM, Storelli A, Marcotrigiano GO. Heavy metals in the aquatic environment of the Southern Adriatic Sea, Italy. Macroalgae, sediments and benthic species. Environ Int 2001; 26: 505-509.

[44] Volpi Ghirardini A, Birkemeyer T, Arzzi Novelli A, Delaney E, Pavoni B, Ghetti PF. An integrated approach to sediment quality assessment: the Venetian lagoon as a case study. Aquat Ecosyst Health Manage 1999; 2: 435-447.

[45] Leoni L, Sartori F, Damiani V, Ferretti O, Viel M. Trace element distributions in surficial sediments of the northern Tyrrhenian Sea: contribution to heavy-metal pollution assessment. Environ Geol Wat Sci 1991; 17: 103-116.

[46] Pichler T, Veizer J, Hall GEM.The chemical composition of shallow-water hydrothermal fluids in Tutum Bay, Ambitle Island, Papua New Guinea and their effect on ambient seawater. Mar Chem 1999; 64(3): 229-252.

[47] Henley RW, Ellis AJ. Geothermal systems ancient and modern: a geothermical review. Earth-Science Rev 1983; 19: 1-50.

[48] Nicholson K. Geothermal fluids. Springer-Verlag. 1992.

[49] Sadiq M. Solubility equilibria of major elements in seawater and marine sediments, Toxic metal chemistry in marine environments. Dekker, M. Inc., New York. 1992.

[50] Amodio-Cocchieri R, Amoroso S, Arnese A, Cirillo T, Montuori P, Triassi M. Pollution by Mercury, Arsenic, Lead, Chromium, Cadmium, and Polycyclic Aromatic Hydrocarbons of Fish and Mussels from the Gulf of Naples, Italy. Bull Environ Contam Toxicol 2003; 71: 551–560.

[51] Abdallah M. Trace element levels in some commercially valuable fish species from coastal waters of Mediterranean Sea, Egypt. J Mar Syst 2008; 73: 114–122.

[52] Celik U, kran Cakli S, Oehlenschl J. Determination of the lead and cadmium burden in some northeastern Atlantic and Mediterranean fish species by DPSAV. Eur Food Res Technol 2004; 218: 298–305.

[53] Pérez Cid B, Boia C, Pombo L, Rebelo E. Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electrothermal atomic absorption spectrometry. Food Chem 2001; 75: 93-100.

[54] Bordajandi LR, Gomez G, Fernandez MA, Abad E, Rivera J, Gonzales MJ. Study on PCBs, PCDD/Fs, organoclorinepesticides, heavy metals and arsenic content in freshwater fish species from the River Turia (Spain). Chemosp 2003; 53: 163-171.

[55] Usero J, Izquierdo C, Morillo J, Gracia I. Heavy metals in fish (Solea vulgaris, Anguilla anguilla and Liza aurata) from salt marshes on the southern Atlantic coast of Spain. Environ Int 2003; 29: 949-956.

[56] Ureña R, Peri S, del Ramo J, Torreblanca A. Metal and metallothionein content in tissues from wild and farmed Anguilla anguilla at commercial size. Environ Int 2007; 33: 532-539.

[57] Renzi M, Specchiulli A, Baroni D, Scirocco T, Cilenti L, Focardi S, Breber P, Focardi SE Trace elements in sediments and bioaccumulation in fish (Anguilla anguilla) in a Mediterranean lagoon (SE Italy) Int J Environ Anal Chem 2011 (in press).

[58] Geeraerts C, Belpaire C. The effects of contaminants in European eel: A review. Ecotoxicol 2010; 19: 239-266.

[59] Golovandova IL. Effects of heavy metals on the physiological and biochemical status of fishes and aquatic invertebrates. Inland Water Biol 2008; 1(1): 93-101.

[60] Miller PA, Munkittrick KR, Dixon DG. Relationship between concentrations of copper and zinc in water, sediment, benthic invertebrates, and tissues of white sucker (Catostomus commersoni) Can J Fish Aquat Sci 1992; 49: 978-984.

[61] Carlson E, Zelikoff JT. The immune system of fish: a target organ of toxicity. In: Di Giulio RT, Hinton DE (Eds). The toxicology of fishes. CRC Press, USA, 2008, p. 489-529.

[62] Gony S. Short note on the effects of cadmium on the gill of the glass eel (Anguilla anguilla). Int Rev Gen Hydrobiol 1990; 75(6): 835-836.

[63] Pierron F, Baudrimont M, Bossy A, Bourdinaud JP, Elie P, Massabuau JC. Impairment of lipid storage by cadmium in the European eel (Anguilla anguilla). Aquat Toxicol 2007; 81: 304-311.

[64] Santos MA, Hall A. Influence of inorganic lead on the biochemical blood composition of the eel, Anguilla anguilla L. Ecotoxicol Environ Saf 1990; 20:7-9.

[65] Food Standards Agency and the Department of Health (UK). Scientific Advisory Committee on Nutrition Metals and other elements in processed fish and fish products. Available online at http://www.food.gov.uk/science 2006.