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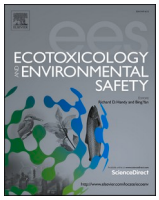

Relationship between wild-caught organisms for bioassays and sampling areas: Widespread serpulid early-development comparison between two distinct populations after trace element exposure

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ABSTRACT

Previous studies suggested the suitability of the brackish-water serpulid (*Ficopomatus enigmaticus*) to be used as model organism for both marine and brackish waters monitoring, by the performance of sperm toxicity and larval development assays. The present study focused on larval development after the exposure of two *F. enigmaticus* populations (Mediterranean and Atlantic, collected in Italy and Portugal, respectively) to different trace elements (copper, mercury, arsenic, cadmium, and lead) at different concentrations. Results of larval development assays were presented as the percentage of abnormal developed larvae. The effect, measured in terms of EC_{50} for all toxicants tested, showed that mercury was the most toxic metal for larvae of both populations. Specifically, the tested trace elements may be racked in the following order from the highest to the lowest toxicity: Mediterranean: mercury > copper > lead > arsenic > cadmium; Atlantic: mercury > copper > cadmium > arsenic > lead. Responses of both populations were similar for arsenic. Lead was the least toxic element for the Atlantic population, while cadmium showed the least toxicity for the Mediterranean population. These preliminary results demonstrate the sensitivity and suitability of the organisms to be used in ecotoxicological bioassays and monitoring protocols. Moreover, chemical analyses on soft tissues and calcareous tubes of collected test organisms and their sampling site water were performed, to identify and quantify the concentration of the tested trace elements in these 3 matrices. Populations exhibited less sensitivity to a certain element together with a relevantly higher concentration of the same element in soft tissues. This may indicate a certain resistance to particular contaminant toxic effects by organisms that tend to accumulate the same toxicant. This highlights the potential correlation between wild-caught test organisms' responses and a deep characterization of the sampling site to identify putative abnormalities or differences in model organism response during bioassay execution.

1. Introduction

Recently, embryotoxicity tests have gained considerable relevance along with other standard biological assays, being included in environmental monitoring and management programs (Schirling et al., 2006; Chiarelli; Roccheri, 2014). The efficiency of toxicity testing using living material relies on organism sensitivity and their ability to respond to low pollutant levels. In this context, several studies have demonstrated how early life stages of marine invertebrates' present higher sensitivity than adults of the same species in contaminated environments (Ross and Bidwell, 2001; Pineda et al., 2012). Besides high sensitivity of early developmental stages (Azad et al., 2009; Azad, 2013), they are also economically and ecologically relevant (His et al., 1999). Disturbances at this development level may result in future population imbalances and impaired ecosystem functioning (Caswell, 2000; Baurand et al., 2014).

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For ecologically relevant toxicology tests, suitable model organisms are represented by specimens that are both ready to use during most of the year (e.g. spawn of gametes), and geographically widespread (Richardson and Martin, 1994). Furthermore, to be considered as good models, organisms have to be abundant, easy to collect, and culture (Ross and Bidwell, 2001; Oliva et al., 2018). Despite such potential limitations, an increasing use of embryotoxicity tests has been observed, mainly using bivalves and sea urchins. As examples, Mai et al. (2012) performed embryotoxicity and genotoxicity tests to demonstrate that copper, Metolachlor, and Irgarol® (pesticides) can cause larval abnormalities in the oyster *Crassostrea gigas*. With the sea urchin (*Paracentrotus lividus*), Morroni et al. (2018) explored the reversibility of effects caused on its development by toxicants such as zinc, lead, copper, and cadmium, revealing developmental plasticity of contaminated embryos. Although polychaete embryotoxicity tests have increased popularity, there is still limited information available regarding the effects of contaminants in the larval stages of this class of organisms (Lewis and Watson, 2012). Furthermore, standardized bioassay protocols for marine and brackish waters monitoring are very limited (Oliva et al., 2018). Among polychaetes the species *Ficopomatus enigmaticus*, a sessile polychaete of the Family Serpulidae, has been already considered a good bioindicator for both marine and brackish water monitoring (Oliva et al., 2018, 2019). *Ficopomatus enigmaticus* is an abundant species with a wide spatial distribution, as well as being easy to collect, and reproductively active year-round (Oliva et al., 20182019). These organisms can endure a wide range of temperature and salinity conditions and are characterized by high growth rates and abundance, making this species a good candidate for ecotoxicity tests (Fornós et al., 1997).

Among the major sources of aquatic environment contaminations are the discharges of industrial effluents and the associated contamination by inorganic compounds, such as trace elements (Kaur et al., 2010). Ceramics, metallurgy, fertilizers, and plastics industries are important contributors, although the origin of these elements is also associated with acid mine drainage events and naturally occurring geochemical processes (Nordstrom, 2011). In marine and brackish ecosystems, these toxicants usually accumulate in the sediment which can already have natural background levels depending on the geological properties of the area (Acevedo-Figueroa et al., 2006; Ramachandra et al., 2018). In fact, cited ecosystem sediments may contain higher concentrations of these contaminants, if compared to the water column (Bryan and Langston, 1992; Fernandes et al., 2008). Furthermore, values in water may go up as a result of natural and man induced washout and sediment re-suspension episodes (Acevedo-Figueroa et al., 2006; Moreira et al., 2018), like bioturbation and dredging. Trace elements are highly toxic, persistent, and prone to bioaccumulate in invertebrates (Fernandes et al., 2008; Bhuyan et al., 2017). Previous works showed that polychaetes are severely affected by trace element bioaccumulation, resulting into oxidative stress and loss of redox homeostasis (Freitas et al., 2012; Coppola et al., 2016; Pires et al., 2017). Effects at a cellular level may impair organisms' embryogenesis, fertilization, larval settlement, and general physiological performance, including survival and growth (Gopalakrishnan et al., 2008; Hudspith et al., 2017).

Considering the above mentioned facts, namely widespread trace elements, their persistence in aquatic systems and impacts on wildlife, the present study aimed to: i) evaluate the effects of five trace elements (mercury, arsenic, copper, cadmium, and lead) on the larval development of the polychaete *F. enigmaticus*; ii) compare the responses to the aforementioned trace elements between two different populations (Mediterranean and Atlantic). These two goals aim to strengthen the ecological relevance of the *F. enigmaticus* larval development assay, focusing on the reproducibility of results between different populations collected from spatially distant areas. We tested the hypothesis that organisms from the same species would respond similarly to contaminants even when collected from different populations.

2. Material and methods

2.1. Ecological characteristics

The polychaete *F. enigmaticus* (Fauvel, 1923) is a filter-feeding serpulid tubeworm throughout the temperate waters of the Northern and Southern hemispheres. Colony structures of *F. enigmaticus* are characterized by massive calcareous reefs (Schwindt et al., 2004) as a consequence of their high fecundity (Dittmann et al., 2009) and larval dispersal behaviour (Dittmann et al., 2009). Considering its habitat, brackish water with vertical hard structures such as concrete walls, pylons and ship hulls represent the selective substrate of the species.

2.2. Organisms, collection, and maintenance

Adult organisms of *F. enigmaticus* were collected in S. Rossore-Migliarino Regional Park – Fiume Morto (Pisa, Italy) and in the Ria de Aveiro coastal lagoon (Cais da Fonte Nova Channel, Western Portugal), both for genetic analyses and to perform the larval development assay. Polychaetes from the two populations were collected during late spring period (end of May-beginning of June) and transferred to the laboratory together with water from the sampling sites. The correct identification of the individuals used in ecotoxicological tests was performed by a genetic analysis following the protocols described in Oliva et al. (2020). All specimens used in this study were correctly identified and attributed to the same taxonomic *F. enigmaticus* group.

Aquaria salinity was the same as in the sampling area: 25 at the Ria de Aveiro and 15 at S. Rossore-Migliarino Regional Park. Salinity was increased daily up to a maximum of 5 points, until 30, as this value is within the optimum salinity range for larval development (Oliva et al., 2018). Organisms were fed daily with an *Isochrysis galbana* suspension (3×10^5 cells/ml). At the end of the acclimation period (1 day for organisms from the Ria de Aveiro and 3 days for the organisms from S. Rossore-Migliarino Regional Park) aquaria conditions were: temperature 22 ± 1 °C, oxygen saturation >90%, salinity 30 and pH 8.1 ± 0.1. Aquaria were maintained under a photoperiod of 10 h light: 14 h darkness.

2.3. Contaminants

A set of trace element ions was chosen for the exposure assay, in the following nominal concentration: Cu^{2+} - 11.25-22.5-45-90 μg/L; Hg^{2+} - 0.45–0.9-4.5–9 μg/L; As^{3+} - 90-180-360-720 μg/L; Cd^{2+} - 90-450-900-1800 μg/L; Pb^{2+} - 45-225-450-1800 μg/L. These concentration ranges were based on those reported by Gopalakrishnan et al. (2008), who compared the toxicity of mercury, cadmium, nickel, lead, and zinc in early life stages of the serpulid polychaete *Hydroides elegans*.

Dilutions of all assessed contaminants were prepared by dissolving the respective salt (copper chloride, mercury chloride, sodium arsenate, cadmium chloride and lead nitrate) in artificial seawater (ASW, salinity 30), prepared following the ASTM (2013) standard formulation. Copper sulphate pentahydrate was used as reference toxicant (Manfra et al., 2016) at concentrations of 11.25–22.5-45-90-180 μg/L of Cu^{2+}.

All reported exposure concentrations represent 90% of each prepared contaminant dilution. The reduction in concentration was due to the fact that 1 mL of fertilized egg suspension (in clean filtered seawater) was added to 9 mL of each concentration replicate, as reported in section below (2.4.3). All chemicals were purchased from Merck/Sigma-Aldrich (Milan, Italy).

2.4. Experimental setup

2.4.1. Gametes emission and collection

The embryotoxicity tests followed the protocol reported in Oliva et al. (2019). Gamete release was induced by de-tubing serpulids (Hadfield et al., 1994). Each polychaete was collected individually,

quickly rinsed in tap water and then placed in 1 mL of 30 ASW. After gametes emission (5–10 min), single female eggs were selected with a fertilization pre-test using an inverted microscope (Leica DMIL) for division synchronization check. Eggs of selected females were transferred together in a 300 mL beaker filled with ASW.

2.4.2. Gametes preparation and fertilization

The egg suspension was rinsed three times with fresh ASW to remove immature and damaged eggs (supernatant). Then the suspension was diluted to reach a concentration of 300 cells/mL. Sperm from 4 to 5 males was freshly collected, mixed and pipetted in the rinsed egg suspension at a concentration of about 3×10^5 cells/mL. The obtained suspension was gently stirred and then placed at 22 ± 1 °C for 40 min to allow fertilization.

2.5. Larval development exposure assay

One mL of fertilized homogeneous egg suspension was pipetted into each dilution replicate, making up to 10 mL of final volume (9 mL of testing substance). Three replicates were set for each treatment and control (ASW). Then, all replicates were incubated at 20 ± 2 °C for 48 h, with a photoperiod of 10 h light: 14 h darkness. After the incubation the assay was stopped by adding few drops of buffered 37% formaldehyde. The number of correctly developed and abnormal larvae was counted for each condition and a percentage of poorly-developed larvae was calculated as in Oliva et al. (2020). A 20% of poorly-developed larvae in controls was set as the threshold for the acceptability of the assay.

2.6. Trace element quantification

Trace element analyses were performed on the three different relevant matrices: water, whole organism tissue and calcareous tubes of *F. enigmaticus*, all collected at the same sampling site mentioned in section 2.2. Salinity, pH, and oxygen saturation of water samples were measured. Samples were then stabilized with 2% concentrated, ultrapure, HNO_3 for further analyses. Regarding organisms, they were collected and transported as reported in section 2.2. In laboratory, worms were gently removed from their tubes and about 5 g of fresh whole-body tissue and 5 g of calcareous tubes were collected, weighed, and then dried at 40 °C for 48 h. The dried samples were directly analysed for evaluating the concentration of mercury by using a DMA-80 Tri Cell (Milestone, FKV) according to the EPA 7473 (2007) method. As regards other trace element analyses, both dried tubes and tissues of *F. enigmaticus* were digested, following EPA, 2007A (2007), by using an Ethos 1 Microwave Digestion System (Milestone, FKV). Copper analysis was performed with an ICP Varian 720-ES (Agilent), according to EPA 6010D (2014), while arsenic, cadmium, and lead were quantified with an AAS Varian SpectrAA240Z following EPA 7010 (2007). Limits of Quantification (LOQ) for each analysis are reported in Tables 3 and 4 (for water samples and for tissues/calcareous tubes, respectively). Mercury and copper standard, as well as a multi-element standard with arsenic, cadmium, and lead, were 2% HNO_3 solution of certified reference material quality. All reference solutions were purchased from Titolchimica, Italia.

2.7. Data analysis

After 48 h of exposure, samples were evaluated as the percentage of poorly-developed larvae in comparison to controls. EC_{50} calculations were normalized to the control mean percentage of success using Abbott's formula (Volpi-Ghirardini and Arizzi-Novelli, 2001):

$$P = \left(\frac{P_e \times P_c}{100 - P_c}\right) \times 100$$

where Pc and Pe are control and experimental percentage response, respectively. EC_{50} and their 95% confidence intervals were calculated according to PROBIT analysis (Finney, 1971). Each experiment was run in triplicate and a mean EC_{50} with relative 95% confidence limits was calculated. Moreover, both population responses were compared for each element at each concentration via a Student's t-test, to underline statistically significant differences in terms of concentration-response relationship.

3. Results

3.1. Ecotoxicology evaluation

Concentration-response curves for each trace element used in the present study showed a percentage of well-developed larvae decrease along the toxicant concentration increase (Fig. 1). Copper and arsenic concentration-response curves showed similar behaviour. The curves for each trace element in both populations (Mediterranean vs Atlantic) decreased in a linear and gradual way. With respect to mercury and cadmium good larval development was seen in the Mediterranean population as it decreased more gradually, while the Atlantic population showed an abrupt drop of values. An opposite pattern was reported for lead. The proportion of well-developed larvae in the Mediterranean population showed a sharp decrease, against the proportion of the Atlantic one, which reached a very low percentage, though in a more progressive way.

The percentages of effect in terms of poorly-developed larvae for each concentration of each assessed element were used to calculate different Effect Concentration (EC_{10}-EC_{50}). In particular, the value for EC_{50} was adopted as the main term of comparison between the two populations. All the EC_{50} values, together with 95% confidence limits, are reported in Table 1. The highest similarities between Mediterranean and Atlantic populations were obtained for arsenic, with an EC_{50} value of 460.26 μg/L for the Mediterranean population and 411.40 μg/L for the Atlantic one. Also, with copper, the results obtained revealed values of EC_{50} of 110.02 μg/L and 67.91 μg/L for Mediterranean and Atlantic population, respectively. Looking at mercury and cadmium EC_{50} values, it was possible to observe that the Mediterranean population was less sensitive, for both elements, with values of 3.97 μg/L (mercury) and 1563.28 μg/L (cadmium). The same elements assessed with the Atlantic population, showed EC_{50} values of 0.27 μg/L and 249.75 μg/L for mercury and cadmium, respectively. On the contrary, lead resulted in an EC_{50} value for the Atlantic population more than the double that of the Mediterranean one.

Table 2 reports results in terms of EC_{10} of all assessed elements for both populations. Atlantic organisms were more sensitive to cadmium (62.65 μg/L) in comparison to those from the Mediterranean region (393.57 μg/L). The opposite behaviour was observed for lead: 66.65 μg/L and 252.61 μg/L of EC_{10} for the Mediterranean and Atlantic population, respectively. For arsenic and copper, the Atlantic population was more tolerant given that the EC_{10} values were higher than those listed for the Mediterranean one. The Mediterranean population showed values of 4.08 μg/L for copper and 41.61 μg/L for arsenic, while these were of 30.46 μg/L copper and 123.39 μg/L arsenic for the Atlantic organisms. Lastly, regarding mercury, the Mediterranean population displayed higher tolerance, with an EC_{10} of 0.72 μg/L, while this value was of 0.019 μg/L for the Atlantic population.

3.2. Trace elements determination

Pollutant quantifications were performed on water, calcareous tubes, and organism whole tissues (Tables 3 and 4). Concentrations of selected elements, measured in water samples, were close to the instrumental limit of quantification (LOQ) and comparable between the two sampling sites, Fiume Morto (Pisa, Italy) and Ria de Aveiro (Aveiro, Portugal) (Table 3). Similar results were obtained for quantified element concentrations in calcareous tubes (Table 4). Furthermore, concentrations

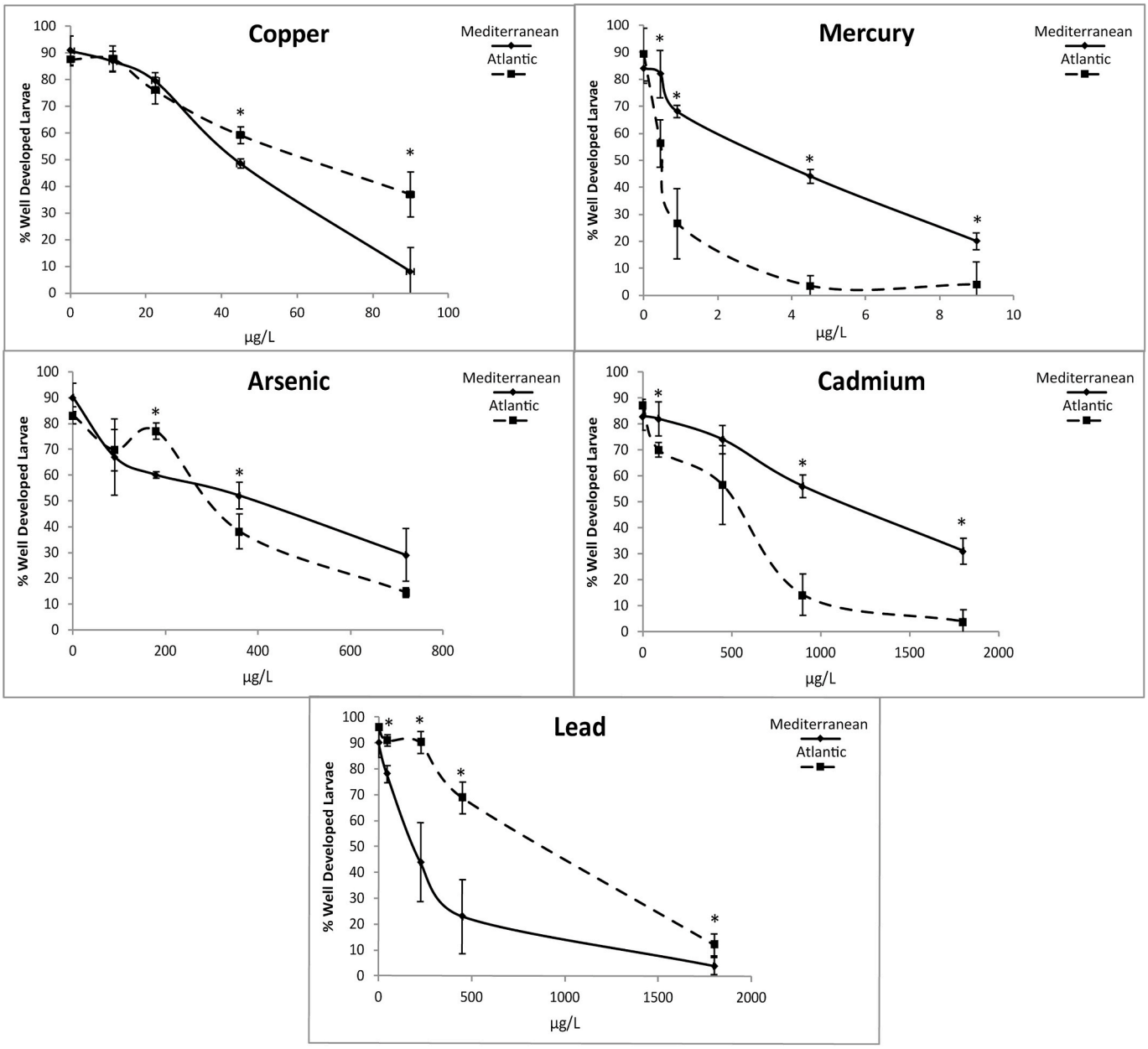

Fig. 1. Concentration-effect curves, calculated for all assessed elements, after 48 h exposure of *F. enigmaticus* embryos. Each graph reports the curves for both assessed populations. For each concentration the mean percentage of well-developed larvae ± standard deviation was reported, n = 3. Differences between populations at each concentration were compared via Student's *t*-test, * = statistically significant differences, $p < 0.05$.

Table 1
Values of EC50 (with relative 95% confidence limits) calculated for each trace element and both Mediterranean (Italy) and Atlantic (Portugal) populations. Results were obtained with a PROBIT model for the estimation of median effect concentration.

Ion	Italy		Portugal	
	EC50 (µg/L)	C.L. 95%	EC50 (µg/L)	C.L. 95%
Hg^{2+}	3.97	2.64–5.33	0.27	0.08–0.57
Cd^{2+}	1563.28	1206.47–2082.25	249.75	145.52–360.63
Cu^{2+}	110.02	63.51–309.57	67.91	n.c.
As^{3+}	460.26	328.27–681.13	411.40	169.99–648.02
Pb^{2+}	255.82	185.89–320.59	566.85	525.77–676.64

Table 2
Values of EC10 (with relative 95% confidence limits) calculated for each trace element and both Mediterranean (Italy) and Atlantic (Portugal) populations. Results were obtained with a PROBIT model for the estimation of the effect concentration.

Ion	Italy		Portugal	
	EC10 (µg/L)	C.L. 95% (µg/L)	EC10 (µg/L)	C.L. 95% (µg/L)
Hg^{2+}	0.72	0.22–1.31	0.019	0.001–0.067
Cd^{2+}	393.57	163.05–600.22	62.65	16.91–115.45
Cu^{2+}	4.08	0.60–9.28	30.46	n.c.
As^{3+}	41.61	10.07–81.84	123.39	9.73–247.69
Pb^{2+}	66.65	31.87–104.28	252.61	188.21–309.69

Table 3
Trace element concentration in water samples collected in both sampling areas: Fiume Morto (Pisa, Italy) and Ria de Aveiro (Aveiro, Portugal). For each element it was reported the relative instrumental limit of quantification (LOQ).

Element	Limit of Quantification (LOQ) (μg/L)	Sample Concentration (μg/L)	
		Fiume Morto	Ria de Aveiro
Hg	0.05	< LOQ	< LOQ
Cd	0.1	0.22	0.21
Cu	2.5	5.17	3.40
As	2.5	< LOQ	< LOQ
Pb	2.5	4.65	<2.5

Table 4
Trace element concentration in organisms' tissue and calcareous tubes samples collected in both sampling areas: Fiume Morto (Pisa, Italy – Mediterranean population) and Ria de Aveiro (Aveiro, Portugal – Atlantic population). For each element it was reported the relative instrumental limit of quantification (LOQ). Measure unit for each quantified element was mg/Kg dry weight.

Element	Limit of Quantification (LOQ)	Whole Tissue		Calcareous Tubes	
		Fiume Morto	Ria de Aveiro	Fiume Morto	Ria de Aveiro
Hg	0.005	0.56	0.07	0.06	0.01
Cd	0.2	1.57	0.72	0.06	0.05
Cu	0.6	25.36	35.52	5.71	3.79
As	0.6	8.30	9.65	0.84	0.95
Pb	0.3	1.07	8.79	2.76	2.66

were comparable between the two populations and deposition of the measured contaminants into tube composition appeared to be minimal. Element concentrations in serpulid whole tissues were similar between the two sampling sites for copper and arsenic (Table 4). In particular, tissues from the Mediterranean organisms had values of 25.36 mg/kg dry weight (d.w.) for copper and 8.30 mg/kg d. w. for arsenic, compared to 35.52 mg/kg d. w. copper and 9.65 mg/kg d. w. arsenic in tissues from those from the Atlantic population. Values for mercury were eight times higher in the Mediterranean population than the Atlantic populations (0.56 mg/kg d. w. vs 0.07 mg/kg d. w.), and cadmium was almost double that of the Atlantic populations (1.57 mg/kg d. w. vs 0.72 mg/kg d. w.). Lead concentration in the Mediterranean population tissues was eight times lower than the one obtained for Atlantic organisms (1.07 mg/kg d. w. compared to 8.79 mg/kg d. w.).

4. Discussion

4.1. Embryotoxicity tests: Comparison between F. enigmaticus data with other serpulid species results

The effect, in terms of EC_{50}, of the larval exposure to environmentally relevant trace element pollutants, was evaluated with *F. enigmaticus* trochophores, comparing an Atlantic and a Mediterranean populations of the same species. Among the few embryotoxicity studies using serpulids, Gopalakrishnan et al. (2008) compared the toxicity of mercury, cadmium, nickel, lead, and zinc in the early life stages of *H. elegans*. Authors observed that the EC_{50} values, based on the percentage of poorly-developed larvae, were dose-dependent, confirming the sensitivity of this species to the used trace elements. Comparing these results with those obtained in the current study, the Mediterranean population was more tolerant to cadmium in comparison to *H. elegans* (EC_{50}: 86.66 μg/L), but similar sensitivity to this trace element was detected for the Atlantic population. Considering lead, while the Atlantic population of *F. enigmaticus* was more sensitive to this element if compared to *H. elegans* (EC_{50}: 260.64 μg/L), the Mediterranean population displayed a comparable response with this serpulid species (Gopalakrishnan et al., 2008). On the contrary, both populations of *F. enigmaticus* showed higher sensitivity to mercury when compared with *H. elegans* (EC_{50}: 9.33 μg/L). Regarding copper, Atlantic *F. enigmaticus* showed higher sensitivity if compared with *H. elegans* (exposed to 100 μg/L of Cu) (Gopalakrishnan et al., 2007). In particular, *H. elegans* had 40% well-developed larvae, while the percentage of *F. enigmaticus* from the Atlantic population, was only approximately 10% at the same concentration. However, the Mediterranean specimens, under the same conditions, showed a percentage of well-developed larvae similar to *H. elegans,* confirmed by comparable EC_{50} values (122 μg/L). Effects of copper were also compared with another study conducted by Ross and Bidwell (2001) that used another serpulid species, *Galeolaria caespitosa*. The authors tested an endpoint similar to that of the present work, and had an EC_{50} value lower (between 16 and 40 μg/L) than the one obtained for *F. enigmaticus*.

Overall, for the Mediterranean population, trace element toxicity varied from mercury to cadmium in a decreasing order, according to the EC_{50} values: mercury (3.97 μg/L) > copper (110.02 μg/L) > lead (255.82 μg/L) > arsenic (460.26 μg/L) > cadmium (1563.28 μg/L). For the Atlantic population, contaminants were ranked in the following order, also of decreasing toxicity, again in terms of EC_{50}: mercury (0.27 μg/L) > copper (67.91 μg/L) > cadmium (249.75 μg/L) > arsenic (411.40 μg/L) > lead (566.85 μg/L). Comparing the two populations, lead switched with cadmium in the toxicity order for the lowest toxic element, while mercury in both populations, was the most toxic element for early development.

4.2. Embryotoxicity tests: Comparison between F. enigmaticus data and other model species

Due to the lack of information concerning serpulids in ecotoxicological bioassays, the findings of the present study were compared with more consistent literature relative to other widely used model species in ecotoxicology: the echinoid *Paracentrotus lividus* and the oyster *Crassostrea gigas.* Relative to *P. lividus*, Arizzi-Novelli et al. (2003) found EC_{50} values for cadmium and copper (of 230 μg/L and 62 μg/L, respectively), which are both similar to those calculated for the Atlantic *F. enigmaticus*. Mai et al. (2012) working with *C. gigas*, reported an EC_{50} of 212.30 μg/L for cadmium, which was similar to the response of the Atlantic population. For the same species, Moreira et al. (2018) obtained 215.20 μg/L as EC_{50} for arsenic, which was two times lower than the one of the Atlantic populations (411.40 μg/L).

4.3. Chemical analyses on tissues, calcareous tubes and water

Considering all the obtained results, the detected differences between the assessed populations of *F. enigmaticus* could be related to genetic inference (Harding et al., 2019) and/or other different environmental issues. This assumption has already been demonstrated to be one of the most diffused mechanisms of resistance in free spawning organisms (Galletly et al., 2007; Pease et al., 2010). Indeed, sensitivity could be related to diverse population with varying exposure histories to different trace element, which is site specific and can lead to different bioaccumulation levels (Rainbow et al., 2009).

It was expected that the trace element concentrations in the environment would also be found in tissues and organisms' calcareous tubes. The element quantification in polychaete tubes was based on the idea that they could act as a stocking matrix for xenobiotics and toxic concentrations of micronutrient. Due to the lack of this type of information in the literature, a similar hypothesis was assumed with peculiar behaviour of some aquatic arthropods that accumulate toxic compounds in their exoskeleton and discard them as they molt. For example Auffan et al. (2013) observed that on *Daphnia pulex* the shedding of the chitinous exoskeleton (ecdysis) was a pivotal mechanism for the release of CeO_2 nanoparticles (ingested or adsorbed). Bergami et al. (2016) observed that larvae of *Artemia franciscana* exposed to toxic PS-NH_2 nanoparticles underwent multiple molts, hypothesizing that this occurrence was a physiological mechanism for toxicants release from

the body. However, contrarily to what was expected, results of the present study showed trace element concentrations in calcareous tubes close to instrumental detection limits for both populations, indicating that these structures may not prevent metal accumulation in polychaete soft tissues. Also, the water collected at both sampling areas did not present relevant trace element concentrations. On the other hand, analysis of the whole tissues revealed significant concentrations of the trace elements of interest, and important differences between populations, pointing out bioaccumulation could be controlled by biotic factors such as gender, maturity, and size rather than by environmental contamination (Ray, 1984). In fact, trace elements are reported to be very prone to accumulate in invertebrate organism tissues (Radomyski et al., 2018).

Considering and interpreting the obtained results, and given that quantifications were performed once, this bioaccumulation may be due to other matrices not evaluated in the present study, such as suspended organic matter and suspended inorganic particles. This hypothesis could be related to the poorly selective filter-feeding activity of serpulids. Moreover, to better establish the link between adults and larvae responsiveness to contaminants, some studies tested for inherited resistance to trace elements using marine oligo- and polychaetes (Langdon et al., 2003). For example, Grant et al. (1989) observed in *N. diversicolor* that the tolerance to copper and zinc was inherited, having seen that the offspring from the contaminated site was more resistant to both contaminants when compared to the one from the clean site. This genetic event might also happen for *F. enigmaticus,* likely explaining part of the sensitivity displayed by the larvae. Therefore, polychaete eggs when in contact with high concentrations of contaminants may produce low quality abnormal embryos (Gopalakrishnan et al., 2008).

4.4. Relevance in ecotoxicological monitoring

In the present study EC_{10} values representing no-effect concentrations, instead of NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration), agreed with Warm and van Dam (2008). The Italian (Lgs. D. 173, 2016) and Portuguese (Portaria 1450, 2007) laws on Materials Classification, relative to threshold contamination levels for marine sediments, report concentrations much higher than the contaminants assessed in the present study, if compared to obtained EC_{10} values in this study. Moreover, considering that standardized tests for whole sediment assessment are lacking, there is a mandatory need to work with manipulated matrices such as elutriates (Haring et al., 2010), which usually hold only a portion of all hydrophilic contaminants present in whole sediment samples. For this reason, it is advantageous to work with a species able to show sensitivity to those lower concentrations, as it is the case of the polychaete *F. enigmaticus*.

5. Conclusion

Genetic analysis confirmed that all individuals from both tested populations belong to the species *Ficopomatus enigmaticus*. Experimental data from the present work showed the toxic effects of selected trace elements on the larval development of this species, further confirming the sensitivity of *F. enigmaticus* early life stages to trace elements exposure. Demonstrated sensitivity is comparable between the two populations tested, thus strengthening the ecological relevance of this *F. enigmaticus* larval development assay. However, it is necessary to underline how specific sensitivity of bioassay endpoints with wild-caught organisms to specific contaminants can be affected by the presence of the contaminants in the organisms' sampling site, coupled with the bioaccumulation ability of the test organism. Overall, our results contribute to the scarce understanding of the effects of contaminants on larval stages of polychaete species and might aid in the standardization of bioassay protocols for marine and brackish waters monitoring. Considering the initial hypothesis, our results showed that test organisms collected in different areas can be used in wide-range monitoring plans. However, it is necessary to well characterize sampling areas in order to select organisms coming from environments with similar chemical status to avoid misinterpretations and over- or under-estimation of effects. For that, next-generation tests with embryos, adults, and multigenerational experiments on different species could be a possible the next research step.

Author contribution statements

Matilde Vieira Sanches, Matteo Oliva, Rosa Freitas and Carlo Pretticonceived and planned the experiments.Carlo Pretti supervised the project.Matteo Oliva, Carlo Pretti and Adília Pires conceived of the presented idea.Matilde Vieira Sanches, Matteo Oliva, Lucia De Marchi, Adília Pires, Alessia Cuccaro and Mariella Baratti carried out the experiment.Matilde Vieira Sanches, Matteo Oliva, Lucia De Marchi, Adília Pires, Alessia Cuccaro, Rosa Freitas, Mariella Baratti and Carlo Pretti contributed to the interpretation of the results.Matteo Oliva, Rosa Freitas and Carlo Pretti developed the theory and performed the computations.Matilde Vieira Sanches wrote the manuscript with support from Matteo Oliva, Lucia De Marchi, Adília Pires, Alessia Cuccaro, Rosa Freitas, Mariella Baratti and Carlo Pretti.Matilde Vieira Sanches, Matteo Oliva and Carlo Pretti verified the analytical methods.All authors discussed the results and contributed to the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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