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A preliminary study of 5 sediment samples collected in the lagoon of Venice highlighted that the larval development rate (LDR) and the early-life stages (ELS) mortality endpoints with A. tonsa are more sensitive than the standard amphipod mortality test; moreover LDR resulted in a more reliable endpoint than ELS mortality, due to the interference of the sediment with the recovery of unhatched eggs and dead larvae. The LDR data collected in a definitive study of 48 sediment samples from the Venice Lagoon has been analysed together with the preliminary data to evaluate the statistical performances of the bioassay (among replicate variance and minimum significant difference between samples and control) and to investigate the possible correlation with sediment chemistry and physical properties.
The results showed that statistical performances of the LDR test with A. tonsa correspond with the outcomes of other tests applied to the sediment-water interface (Strongylocentrotus purpuratus embryotoxicity test), sediments (Neanthes arenaceodentata survival and growth test) and porewater (S. purpuratus); the LDR endpoint did, however, show a slightly higher variance as compared with other tests used in the Lagoon of Venice, such as 10-d amphipod lethality test and larval development with sea urchin and bivalves embryos. Sediment toxicity data highlighted the high sensitivity and the clear ability of the larval development to discriminate among sediments characterized by different levels of contamination. The data of the definitive study evidenced that inhibition of the larval development was not affected by grain-size and the organic carbon content of the sediment; in contrast, a strong correlation between inhibition of the larval development and the sediment concentrations of some metals (Cu, Hg, Pb, Zn), acid-volatile sulphides (AVS),

polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) was found. No correlation was found with DDTs, hexachlorobenzene and organotin compounds.

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# TESTING LAGOONAL SEDIMENTS WITH EARLY LIFE STAGES OF THE COPEPOD *ACARTIA TONSA* (DANA): AN APPROACH TO ASSESS SEDIMENT TOXICITY IN THE VENICE LAGOON

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## Abstract

The early-life stages of development of the calanoid copepod *Acartia tonsa* from egg to copepodite I is proposed as an endpoint for assessing sediment toxicity by exposing newly released eggs directly onto the sediment-water interface.

A preliminary study of 5 sediment samples collected in the lagoon of Venice highlighted that the larval development rate (LDR) and the early-life stages (ELS) mortality endpoints with *A. tonsa* are more sensitive than the standard amphipod mortality test; moreover LDR resulted in a more reliable endpoint than ELS mortality, due to the interference of the sediment with the recovery of unhatched eggs and dead larvae. The LDR data collected in a definitive study of 48 sediment samples from the Venice Lagoon has been analysed together with the preliminary data to evaluate the statistical performances of the bioassay (among replicate variance and minimum significant difference between samples and control) and to investigate the possible correlation with sediment chemistry and physical properties.

The results showed that statistical performances of the LDR test with *A. tonsa* correspond with the outcomes of other tests applied to the sediment-water interface (*Strongylocentrotus purpuratus* embryotoxicity test), sediments (*Neanthes arenaceodentata* survival and growth test) and porewater (*S. purpuratus*); the LDR endpoint did, however, show a slightly higher variance as compared with other tests used in the Lagoon of Venice, such as 10-d amphipod lethality test and larval development with sea urchin and bivalves embryos. Sediment toxicity data highlighted the high sensitivity and the clear ability of the larval development to discriminate among sediments characterized by different levels of contamination. The data of the definitive study evidenced that inhibition of the larval development was not affected by grain-size and the organic carbon content of the sediment; in contrast, a strong correlation between inhibition of the larval development and the sediment concentrations of some metals (Cu, Hg, Pb, Zn), acid-volatile sulphides (AVS), polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) was found. No correlation was found with DDTs, hexachlorobenzene and organotin compounds.



Keywords – Ecotoxicology, sediment-water interface, planktonic larvae, larval development

# Introduction

Planktonic copepods represent the major component of the marine ecosystem. They feed on phytoplankton and protozoans and serve as food reservoir for the planktonic larvae of fishes (Turner, 2004). As such they play a key role in the food webs of the marine and oceanic environments. A number of factors make the copepods a useful bioindicator for the assessment of the adverse effects of chemicals and effluents in surface waters after short-term exposure, including their worldwide distribution, ecological relevance, short generation times and easy culturing (US EPA, 1978). Indeed copepods have been used for this purpose since the late seventies (USEPA, 1978; Bengtsson, 1978); in fact, copepod mortality is a methodologically easy endpoint to evaluate. An international standard is also available for the acute lethality test with the calanoid *Acartia tonsa* Dana, the harpacticoids *Nitocra spinipes* Boeck and *Tisbe battagliai* Volkmann-Rocco (ISO, 1999).

Nevertheless, several studies performed over recent years have evidenced the ability of endpoints such as egg-production, hatching success and early life stage development to indicate significant impairments at concentration levels several times lower than those affecting survival (Hutchinson et al., 1994; Kusk and Petersen, 1997; Lotufo, 1997; Andersen et al., 1999; Breitholtz and Bengtsson, 2001; Christoffersen et al., 2003; Gorbi et al., 2012; Zhou et al., 2016). In addition, the endocrine regulation of critical processes including molting, sexual differentiation and growth, makes the copepods particularly well suited for detecting the effects of chemicals that affect or interfere with neuro-endocrine signalling (the synthesis of hormones, binding to receptors or simulating hormone functions). The suitability of the copepods and the growing interest for endocrine disrupters testing has led to the recent development of several methods for detecting sub-lethal and chronic effects towards the calanoid *A. tonsa* (Andersen et al., 2001) and the harpacticoids *N. spinipes* (Breitholtz et al., 2003), *T. battagliai* (Hutchinson et al., 1999a,b), *Tigriopus japonicus* Mori (Marcial et al., 2003; Kyun-Woo et al., 2013), *Amphiascus tenuiremis* Brady and Robertson (ASTM, 2004a) and *Eurytemora affinis* Pope (Forget-Lerayet al., 2005; Lesueur et al., 2013). An OECD guideline for detecting reproductive and developmental effects of endocrine disrupters with *A. tonsa*, *N. spinipes*, *T. battagliai* and *A. tenuiremis* has been under validation (Kusk and Wollenberger, 2007; OECD 2007) and a ISO Standard Method (ISO 16778) for assessing water quality with the early life stages of *A. tonsa* has been recently published (ISO 2015).

Although several studies have been dedicated to testing chemicals, the use of sub-lethal endpoints and chronic tests for assessing environmental matrices is not yet widespread; in fact, only very few references are available in the literature on elutriate (Williams, 1992) and sediment testing (Kovatchet al., 1999; Wollenberger and Kusk, 2006). This is surprising given that a number of

91 factors make copepods particularly useful for elutriate, pore-water and sediment testing including
92 their sensitivity to toxic substances, easy laboratory cultivation, the widespread need for small
93 volume samples and the availability of several sub-lethal endpoints.

94 Sediment toxicity is most often assessed using adult amphipods (or less frequently with
95 polychaetes) following their acute exposure to the whole sediment (Kennedy et al., 2009);
96 nevertheless, although these methods have proved to be a valuable tool for the detection of acute
97 effects in hot-spots of contamination, their ability to highlight impairments in sediment with low to
98 moderate contamination is uncertain (USEPA, 2001; Picone et al., 2016). Sub-lethal and chronic
99 effects are seldom investigated in whole-sediment testing, since the procedures are often expensive
100 and time-consuming, both for amphipods and polychaetes, requiring greater scientific and
101 technological effort as compared to acute tests (Costa et al., 2005). In contrast, early-life stages of
102 benthic and planktonic invertebrates offer the possibility of assessing the sub-lethal effects exerted
103 by sediment-bound contaminants on the sediment-water interface (SWI) (Anderson et al., 1996,
104 2001). SWI testing with early-life stages is ecologically relevant for a number of reasons: firstly,
105 early-life stages are more sensitive than adult stages (Ringwood, 1992; Hutchinson et al., 1998) and
106 the sub-lethal effects that can be measured on early-life stages may have greater ecological
107 relevance than lethality to identify possible impairments due to the exposure to the contaminants.

108 Secondly, the SWI plays a crucial role in the marine environment since gametes and larval stages of
109 many marine species are negatively buoyant and spend most of their life span at this interface,
110 where mineralization of detritus, deposition from water columns and release from the sediments
111 may contribute to the accumulation of toxicants (Anderson et al., 2001, and citation herein).

112 The larval development test (LDR test) proposed by Wollenberger and Kusk (2006) allows the
113 determination of the effects due to the fraction of soluble contaminants released from the sediment
114 into the overlying water during the test. The method is based on the exposure of the copepods at
115 early stages of development to the sediments, starting with the egg-stage. The test assesses the ratio
116 of surviving animals that passed through the nauplii stages and reached a copepodite stage after 5–6
117 days of exposure (Andersen et al., 2001). Within this period about the 50% of control animals
118 should have metamorphosed into at least copepodite stage I. The 50% ratio was selected as it
119 represents the optimal development point to enable the observation of potential inhibitory or
120 stimulatory effects (Kusk and Wollenberger, 2007). Furthermore, nauplii and copepodites are
121 morphologically clearly distinct, so that the transition from the sixth naupliar stage to the first
122 copepodite stage is quite easy to observe under a dissecting microscope. Moreover, the
123 establishment of a laboratory culture of *A. tonsa* enables the recruitment of organisms of good

quality and sensitivity throughout the year, avoiding the disadvantages (limited in-field availability and seasonal changes of health and sensitivity) related to the use of wild populations of other crustaceans, e.g. amphipods.

Taking the Lagoon of Venice as the selected area of investigation, the present study focuses on the evaluation of the sensitivity of the larval development test with *A. tonsa* as an applicable method for the routine testing of sediment toxicity. A first, preliminary application of the LDR test on a restricted number of frozen samples (n = 5) has been performed to compare the sensitivity of the early life stages toxicity test with *A. tonsa* with the standard 10-d lethality test with the amphipod *Corophium orientale* (both performed on whole sediment, but *C. orientale* on freshly collected, not frozen sediment). The promising results obtained in the preliminary study suggested the need to perform further assessment on LDR test performance in a definitive study, by testing a larger number of freshly collected sediments, characterized by different grain size, total organic carbon (TOC) content and occurrence of contaminants which may cause risk or adverse effects to the biota (Contaminants of Potential Concern - COPC). The definitive study was then performed on 48 sediment samples collected in the framework of the HICSED. This whole-lagoon scale project is promoted and funded by the Ministero delle Infrastrutture e dei Trasporti – Magistrato alle Acque di Venezia through its concessionaire Consorzio Venezia Nuova, with the aim of quantifying the hazard due to sediment contamination, using ecotoxicological criteria.

The data collected during both the preliminary and definitive study have been analysed with the following specific goals: 1) the assessment of the statistical performances of the bioassay (especially as concern the LDR endpoint), through analysis of the variance and subsequent calculation of a toxicity threshold (TT) using the minimum significance difference criterion (Thursby *et al.*, 1997); 2) the evaluation of the potential role of grain-size, organic carbon and acid volatile sulphides as confounding factors; 3) a verification of the ability of the test to discriminate among sediments of different degrees of contamination; 4) an identification of possible correlations between LDR and contaminant's concentrations, also taking into account their bioavailability and mobility, i.e. Simultaneously Extracted Metals (SEM) and Acid Volatile Sulphides (AVS).

# 1 Materials and methods

## *1.1 Study area*

The Venice lagoon is a coastal wetland with a surface of about 549 $km^2$ located in North-Eastern Italy, with an average mean depth referred to the mean sea level of about 1 m (Sfriso and Marcomini, 1996). The lagoon is connected with the Adriatic Sea by 3 sea inlets that allow for the exchange of about 60% of the water in any 12-h cycle (Sfriso and Marcomini, 1996). It includes

estuarine and marine environments, pristine salt marshes and shallows, as well as reconstructed marshes, reclaimed land and human environments such as the city of Venice and the industrial district. Point and nonpoint sources of pollution flowing into the lagoon include: industrial waste from the area of Porto Marghera, treated and untreated municipal wastewaters from the cities of Venice and Mestre, streams, agricultural runoff, boat traffic and atmospheric deposition (Volpi Ghirardini et al., 2005).

### *1.2 Sediment sampling*

The 5 sampling sites for the preliminary study (Figure 1, black triangles) have been selected from shallows already investigated during prior surveys undertaken in the Lagoon of Venice (Picone et al., 2008, 2016). Site 22B, which is characterized by clayey-silt sediments and low contamination levels, has been selected as a candidate reference site. Site 24B has been chosen as representative of the sandy/silty-sand low contamination areas located close to the sea inlets, whilst site 32B is representative of the contaminated sediments of the industrial area of Porto Marghera. Sites 2B and 16B have been selected as low to medium-low contaminated sites typical of the shallows of the northern (2B) and southern (16B) basin of the Lagoon (Picone et al., 2008, 2016).

The 48 sampling sites of the definitive study are also reported in Figure 1 (47 sites marked with a black dot plus site 32B, sampled also for the definitive study). The sites were divided into 3 groups (α, β and γ) on the basis of the expected contamination level (MAG.ACQUE, 1999), according to the Venice Sediment Management Criteria (Ministero Ambiente, 1993): α-samples, located near the sea inlets and far from industrial and urban areas, were expected to be characterized by the lowest level of contamination (Class A sediments, according to 1993 Venice Sediment Management Criteria); β-samples expected to be affected by medium-low contamination (Class B sediments); γ-samples (including 32B), located mainly in shallows and canals between the city of Venice and the industrial area of Porto Marghera, were expected to be moderately to highly contaminated (Class C sediments).

Sediments were collected on two separate occasions; in September 2005 as part of the preliminary study and from February to April 2008 in the definitive study. In shallows, sampling was performed following the integrated design and Quality Assurance/Quality Control (QA/QC) procedures reported in Volpi Ghirardini et al. (2005) using a 10-cm diameter Plexiglas® corer. Canal sediments were gathered by scuba divers, who collected cores of sediment along 2 perpendicular axis within a circular area of approximately 10m-diameter. Only the first 20cm of sediment were used for chemistry and toxicity testing.

The samples were stored in 2-L glass containers until their arrival in the laboratory, where the sediments were immediately homogenized and divided into different aliquots for chemistry, grain-size analysis and toxicity testing. Sediments were kept at 4°C under darkness until toxicity testing, which was performed within 1 month from sampling (ASTM, 2014). Frozen aliquots (-20°C) of sediment were only used in the *A. tonsa* LDR-test performed in the preliminary study.

### *1.3 Acartia tonsa culturing*

*A. tonsa* eggs were obtained from the Technical University of Denmark (DTU), Lyngby, Denmark. The presence of *A. tonsa* has been identified in the Lagoon of Venice (Camatti et al., 2000) and often represents the dominant component of the zooplankton (Bianchi et al., 2003). The use of the Danish laboratory-cultured population, isolated in 1981 (Andersen et al., 2001), was, however, preferred in order to avoid the use of a wild population that was potentially tolerant to a wide range of contaminants.

Laboratory cultures were started by adding 600–800 newly released eggs to 1.8-L of culturing medium in a 2-L glass bottle. The culture medium was obtained by diluting the ASTM standard water (ASTM, 2004b) with Milli-Q® purified water (Millipore, Bedford, MA, USA) up to a salinity of 25 psu and then adding trace elements and nutrients as described in Kusk and Wollenberger (1999). The cultures were kept at 20 ± 1°C, under a 16-h light and 8-h dark photoperiod and continuous aeration; under these conditions *A. tonsa* reaches the adult stage after 10–11 days and starts to reproduce. Newly released eggs were removed daily from the culture by siphoning out the medium from the bottom of the culture and then filtering this through two nets with mesh sizes of approximately 170-µm and 50-µm respectively. This procedure made it possible to separate the eggs (passing through the 170-µm net but retained by the 50-µm net) from detritus, faecal pellets and dead specimens. Each culture was then kept for a period of up to 6–7 weeks. The copepods were fed with the cryptophycean *Rhodomonas salina* (Hill and Wetherbee 1989), at a daily rate of about 6 x $10^4$ cell/mL; the algae were added three times per day using a timer-controlled peristaltic pump. Algal cultures were maintained following the method described by Kusk and Wollenberger (1999).

### *1.4 Toxicity testing*

The test was performed under static conditions, in 100-mL glass beakers filled with 3.5 g of wet sediment and 80-mL of culture medium. Test vessels were maintained at the same conditions as the cultures (T = 20 ± 1°C; 16-h light, 8-h dark photoperiod; without aeration). The experiment started by adding a known number of eggs between 50 and 80 to each beaker; six and nine replicates were used for the samples and the control sediment respectively. Sample 22B (frozen) was used as a

control in the preliminary study, whilst freshly collected sediment from site 24B has been used as the control in the definitive study (sampled during the 2008 campaign). Only newly released eggs or eggs stored in refrigerators at 4°C for a period not exceeding 48-h were used for testing. Larvae were fed on day 0 and day 2 with 6 x $10^4$ cell/mL of *R. salina*. Algal density was measured using a Beckman Multisizer™ 3 Coulter counter. To determine the time at which 50% of the animals reached the copepodite stage, one control replicate was fixed and stained on day 6 by adding 0.8-mL of Lugol's solution to the medium. The developmental stage was then examined by filtering the water phase through a mixed cellulose ester filter (diameter 47-mm, porosity 0.45-µm, Advantech, Japan) with gridlines; before filtering was carried out, the content of the beaker was gently shaken to allow for the resuspension of the stained early stages whilst taking care to minimize the resuspension of the sediment. After filtering, the sediment surface was carefully rinsed with the culture medium to recover the early stages that could have remained on the sediment surface. All unhatched eggs, nauplii and copepodites recovered on the filter were counted under a dissecting microscope and the ratio of copepodites was calculated.

Control results were deemed acceptable if an average copepodites percentage in the range 30–70% was found at the end of the exposure.

According to the intra-laboratory QA/QC procedure, the sensitivity of each of the cultures used for testing the sediments was verified by testing the effects of 3,5-Dichlorophenol (3,5-DCP) on the larval development of *A. tonsa* after a 120 h exposure, following the methods described in Andersen et al. (2001). Preliminary tests were performed at DTU in October 2005; tests on the 48 freshly collected sediment samples were performed in Venice from February through May 2008.

The 10-d lethality test with *C. orientale* in the preliminary study was performed as reported in Picone et al. (2008). Briefly, the tests were performed by exposing juvenile amphipods (passing through a 1000 µm-mesh sieve, but retained by a 500-µm mesh sieve) to 200-mL wet sediment and 750-mL of overlying water (natural filtered seawater) in 1l wide-mouth glass beakers. Four replicates per sediment were tested, using 25 amphipods for each replicate. The beakers were kept in a thermostatic chamber at T = 16 ± 2°C, S = 35 psu and under constant illumination (> 100 lux).

### *1.5 Physico-chemical analyses*

Sediment grain-size was determined following a gravimetric procedure (ICRAM, 2001) and was subsequently classified according to Shepard (1954). TOC analyses were performed using a CHNS-O analyser (mod. EA1110, CE-Instruments, ThermoElectron, Milan, Italy), on aliquots of 10–20 mg of dry sediment acidified with 20-µL of 1N HCl solution and dried at 105°C for 15 minutes.

Dry-weight total-metal concentrations were measured using inductively coupled plasma-atomic emission spectrometry (ICP-AES) for Cu, Cr, Ni, Pb, V and Zn (EPA method 6010B), atomic absorption-furnace technique for As and Cd, (EPA methods 7060 and 7131respectively) and atomic absorption spectrophotometry for Hg (EPA method 7473). Prior to analysis, samples were digested through microwave assisted acid digestion with aqua regia (EPA method 3051A mod.). SEM and AVS were determined following the procedure by Allen *et al*. (1993). Polynuclear aromatic hydrocarbons (PAHs) were analysed using a reverse phase HPLC (EPA method 8310 mod.) after extraction through sonication (EPA method 3550 mod.). Polychlorobiphehyls (PCBs) and organochlorine pesticides (OCPs) were measured by gas chromatography (EPA method 8082 and 8081, respectively), after pressurized fluid extraction (PFE, EPA method 3545) cleanup for sulphur removal (EPA method 3660) and sulphuric acid/potassium permanganate cleanup (EPA method 3665). Organotin compounds (tributyltin, dibutyltin and monobutyltin) were determined on only half of the sediment samples using HGA-AS spectrophotometry following the procedure reported in ICRAM (2001).

### *1.6 Data analyses*

The results of the test were expressed as Larval Development Rate (LDR) and Early Life Stage (ELS) mortality. LDR is reported as the number of copepodites divided by the total number of early stages (nauplii plus copepodites) recovered at the end of the test:

$$LDR = \frac{copepodites}{nauplii + copepodites}$$

LDR was then normalized with respect to the average control LDR in order to calculate the percentage of inhibition (In-%). Negative values of inhibition indicate stimulatory effect. ELS mortality (measured only in the preliminary study) is reported as follows:

$$ELS_{mortality} = \frac{initial\ eggs - (unhatched\ eggs + nauplii + copepodites)}{initial\ eggs - unhatched\ eggs}$$

Effective concentration 50 ($EC_{50}$) for the reference toxicant was calculated using a statistical program for continuous response data and log-normal distribution of the observed effects at the tested concentrations, developed at the Technical University of Denmark (Christensen et al. 2009).

One-way analysis of variance (ANOVA) and Fisher *post-hoc* test on log-transformed LDR data were used to highlight significant differences between samples and controls. Data normality and variance homogeneity were checked using Kolmogorov-Smirnov's and Bartlett's test respectively. Spearman's non-parametric correlation was used to check for significant correlations between the inhibition of development and grain-size, TOC, AVS and contaminants. The toxicity threshold for

the larval development test was calculated using the Minimum Significant Difference (MSD) criterion (Thursby et al.,1997; Phillips et al., 2001) applied on the whole dataset available for the sediment test with *A. tonsa* larval stages (n = 65). MSDs were calculated according to the following equation:

$$MSD = t_{(\alpha,n+m-2)} \cdot \sqrt{\frac{s_n^2}{n} + \frac{s_m^2}{m}}$$

where t is the tabulated t-value derived from standard tables, α the significance level (fixed at 0.05), n and m the number of replicates for control and sample respectively, $s_n^2$ and $s_m^2$ the variance observed for control and sample, respectively.

Samples were considered toxic only if the MSD-derived toxicity threshold was exceeded. Stimulatory and inhibitory effects were classified as follows: absence of effects from 0% up to ± 24%, possible effects from ± 25% up to ± 39% and significant effects for values > ± 40%. Rationale for these intervals is reported in the Discussion section. All statistical analyses were performed using StatSoft Statistica 7.

# 2 RESULTS

## *2.1 Preliminary study*

*Toxicity testing*

A single *A. tonsa* culture was used for the preliminary study; the 3,5-Dichlorophenol (DCP) $EC_{50}$ (162 μg $l^{-1}$) is consistent with the data reported by Andersen et al. (2001) (130 - 210 μg $l^{-1}$). LDR in the control sediment (22B) was 0.44 ± 0.04 according to QA/QC criteria (LDR = 0.50 ± 0.20), whilst ELS mortality was 0.44 ± 0.05, above the 0.30 criterion stated by Kusk and Wollenberger (2005) for water-only exposure (no criteria are available for ELS mortality for sediment testing).

The $LC_{50}$ for the reference toxicant test (Cd) with *C. orientale* was 1.64 mg $l^{-1}$, consistent with the range 3.3 ± 2.5 mg $l^{-1}$ reported in Picone et al. (2008). Amphipod survival in the control (native sediment) was always > 90%.

The toxicity data of *A. tonsa* and p-values are reported in Table 1, together with chemical data and the main physical characteristics of the samples (grain size as percentage of sand and TOC). The results of the comparative study on the effects between copepod larval development and amphipod survival are reported in Figure 2. Here the data are reported as the % of amphipod and ELS survival (instead of mortality), and as the % of the *A. tonsa* LDR, in order to allow for a graphical comparison. The amphipod test did not discriminate among the samples (survival ranging from 87 ±

6% in 32B up to 97 ± 2% in 24B), whilst both LDR and ELS survival showed a clear gradient of effects with LDR ranging from 60 ± 6% (24B, sandy-silt reference) to 15± 5% (16B); this latter was also the sample with the lowest survival of ELS (55 ± 10%), whilst the highest survival was measured in 24B (82 ± 7%). The LDR calculated in the silty-sand sample 24B was significantly higher than the LDR measured in the other samples, which also included the clayey-silt reference sample 22B; the other three sites (2B, 16B and 32B) were significantly different both from 22B and 24B (one-way ANOVA, $p < 0.05$), but negligible differences among the shallows (2B and 16B) and the industrial area (32B) have been observed. The toxicity gradient highlighted by the LDR endpoint is as follows: 32B = 16B = 2B > 22B > 24B (one-way ANOVA and *post-hoc* Fisher's test).

*Sediment chemistry and physico-chemical features*

Sediment contamination differed very slightly among sites 2B,16B, 22B and 24B. The mean Effect Range Median quotients (mERMq), a normalized chemical summary calculated by normalizing each chemical concentration to its respective Effect Range Median (ERM) (Long et al., 2006), ranged from 0.05 (24B) and 0.13 (2B); ERM values (i.e. concentrations above which acute effects would frequently occur) have been exceeded only for Hg (0.71 mg $kg^{-1}$dw, Long et al., 1995) in site 2B (0.94 mg $kg^{-1}$dw). PAHs, OCPs and PCBs were always below Effect Range Low (ERL, i.e. values below which effects would be rarely observed); OCPs were also always below detection limits (< 0.1 µg $kg^{-1}$dw) (Picone, 2006).

Industrial site 32B showed a clearly higher metal contamination with sediment concentration of Hg (3.28 mg $kg^{-1}$dw) and Ni (115 mg $kg^{-1}$dw) above ERM values; no data were available for organic contaminants in site 32B.

TOC was below 1% in samples 22B (0.9%) and 24B (0.5%), whilst a higher value was measured in the peaty substrate of site 16B (5.0%). $\Sigma$SEM-AVS$f_{OC}^{-1}$(molar difference between SEM and AVS, normalized in respect to the fraction of organic carbon in the sediment) was lower than the critical value of 130 µMol $g^{-1}$ of OC in all samples. Metal bioavailability is therefore expected to be improbable (USEPA, 2005) under the static conditions in which the test was performed.

The Spearman's correlation calculated for larval development inhibition and the physico-chemical variables highlighted a significant correlation ($p < 0.05$) with TOC,As, Cd, Cr, Cu, Ni, Pb and Zn, although the $\Sigma$SEM-AVS $f_{OC}^{-1}$ index indicated an improbable bioavailability of the metals. No correlation has been observed for ELS-mortality. The Spearman's correlations for the preliminary study are summarized in Table 2.

## 2.2 Definitive study

*Toxicity testing*

Three different *A. tonsa* cultures were used during the experimental period (February–May 2008); the $EC_{50}$ ranged from 33μg $l^{-1}$ up to 204 μg $l^{-1}$ of 3.5-DCP. These values fell within the intralaboratory control chart (31–250 μg $l^{-1}$) and are in accordance with the data obtained from specimens of the same Danish population cultured at lower salinity (Andersen et al., 2001).

The results of the 48 sediment LDR tests are summarized in Table 3 together with the main physico-chemical characteristics of the samples (grain size as percentage of sand, grain size classification according to Shepard diagram, TOC), the p-values, and the classification of the effects. The analysis of the MSD's dataset and the calculation of its $90^{th}$ percentile, as reported in Thursby et al. (1997), made it possible to identify a toxicity threshold corresponding to 60% of development relative to the control; as a consequence, only the samples showing an inhibitory or stimulatory effect higher than 40% were considered to be really effective. Most of the γ-samples (11 out of 13) and β-samples (14 out of 20) showed a percentage of inhibition of the larval development higher than the threshold; in particular, the 62% of the γ-samples and the 20% of the β-samples were characterized by an inhibition > 80%. On the contrary, only 2 α-samples out of 15 exceeded the calculated toxicity threshold. Stimulatory effects were measured in 7 sites; however, stimulation reached noteworthy levels only in a few sites (α-06, α-14, β-18 and γ-05) and only in γ-05 was it above the 40% threshold.

*Sediment chemistry and physico-chemical features*

Sediment samples were characterized by a wide range of TOC content (0.1–13.3%); both the highest (β-12) and the lowest (γ-05) values were found in the shallows of the central basin of the Lagoon. However, β-12 is clearly an outlier, since all the other measured concentrations are significantly lower (in the range 0.1–7.7%). The α-samples showed the higher mean content of TOC (4.1%), followed by β-samples (3.3%, excluding β-12) and γ-samples (1.9%).

With respect to the grain-size, most of the sediments were classified as sandy-silts (60%) and silty-sands (19%); sandy sediments were distributed only in the areas closest to the southern sea inlet. Clay content was generally negligible in all sites except β-04, a confined mudflat of the northern Lagoon that receives water from the Dese River, where clay comprises up 36% of the sediments.

The results of the chemical analyses are summarized in Supplementary Material Table 1SI and Table 2SI. Sediment chemistry showed that the differences between α- and β-samples are generally negligible; the COPC in these sediments are mainly Hg and As, as evidenced by the recurring value

above the ERL for both these metals (8.2 and 0.15 mg $kg^{-1}$dw respectively for As and Hg) and the limited data readings above the ERM for Hg (Long et al., 1995; 2006). With respect to the other contaminants, only infrequent values above the ERL for Cr, Cu, Ni and Zn were measured. The mean ERM quotient (mERMq) was in the range 0.07–0.25 and 0.07–0.26 for α- and β-samples, respectively.

As expected, the γ-samples were significantly more contaminated, especially with regard to the presence of metals; in fact, most of the metals occurred at concentrations above the ERL values and several exceeding ERM for Hg and Zn have also been registered. The mERMq values are clearly higher than in α- and β-samples, ranging from 0.17 (γ-05) to 1.78 (32B).With respect to the organics, the differences among the three typologies of sediments are less marked; PAHs concentrations were found above the ERL only in samples γ-04 and 32B, while PCBs exceeded the ERM only in sample 32B; this latter sediment resulted in the most contaminated among the examined samples. DDTs were found at concentrations above ERL at seven sites (β-07, β-20, γ-02, γ-04, γ-08, γ-09, 32B); these were all located close to the mainland. Chlordane, aldrin, dieldrin and hexacyclohexane were below the detection limits (0.1 $ngg^{-1}$dw) in all the analysed samples.

The highest concentrations of acid volatile sulphides (AVS) were measured in the shallows, especially in the mudflats near the industrial area of Porto Marghera (γ-04, γ-08, γ-09, γ-10, 32B) and in some of the mudflats characterized by higher residence time (γ-02, β-04, β-05 and β-06) (Cucco and Umgiesser, 2005). Only in samples β-11, γ-03 and γ-06 did the SEM occur at concentrations higher than the AVS, underlining the potential bioavailability of the divalent metals. Since the value of the index ΣSEM-AVS $f_{OC}^{-1}$in all these samples is lower than the critical value of 130 μMol $g^{-1}$ of OC reported by USEPA (2005), the occurrence of metal toxicity is also unlikely. With respect to the organotins, minimal differences were noted among the sites, with values in the range 2–35 μg Sn $kg^{-1}$dw. In most of the analysed samples TBT concentrations were above the Interim Sediment Quality Guidelines-low trigger value (ISQG-low) of 5 μg $kg^{-1}$ dw adopted in Australia (ANZECC and ARMCANZ, 2000; McCready et al., 2006). The maximum concentration was found in sample β-07, but higher concentrations were more widespread in the γ-samples.

*Statistical analysis*

Correlation data for the definitive study are summarized in Table 4. The non-parametric Spearman's correlation evidenced the lack of significant relations between larval development inhibition and both TOC (Spearman's R = 0.05; p = 0.72) and grain-size (Spearman's R= -0.27; p = 0.06). To avoid any possible misleading of the relations due to the simultaneous occurrence of inhibitory and stimulatory effects in the dataset, the analysis was repeated excluding the samples eliciting

stimulation; the results confirmed the absence of a correlation between In-% and TOC (Spearman's R = -0.02; p = 0.90) and grain-size (Spearman's R = -0.30; p = 0.06). In contrast, a significant correlation was found between larval development and AVS in the sediments (p = 0.009).

The inhibition of the larval development showed a significant correlation (p < 0.001) with most of the contaminants that were analysed, as well as with the mERMq (Spearman's R = 0.58) that summarizes the overall contamination state of the sediments.

With respect to the metals, as in the preliminary study, a significant correlation was found with Pb (R = 0.65), Zn (R = 0.61), Cu (R = 0.59) and Hg (R = 0.55). A significant but clearly weaker correlation was found with sediment concentrations of As (R = 0.43), V (R = 0.35) and Cd (R = 0.33) (p < 0.05). No correlations were found with Cr and Ni. A correlation with organic contaminants was evaluated after normalization of the sediment concentrations to TOC content; as a result, significant correlations were found with PCBs (Spearman's R = 0.35, p = 0.01) and PAHs (Spearman's R = 0.33, p = 0.02), while no relationship between larval development inhibition and DDTs (p > 0.05), HCB (p = 0.07) and organotin compounds (p = 0.22, p = 0.42 and p = 0.16 for TBT, DBT and MBT, respectively) was evidenced.

# 3 DISCUSSION

## *3.1 Preliminary study*

ELS mortality was quite high in all samples except 24B (18%); the observed mortality is clearly higher than that usually observed in water-only exposures with *A. tonsa* (Kusk and Petersen, 1997), but comparable with other experiences on sediment testing (Wollenberger and Kusk, 2006). The higher ELS mortality measured in the sediment test can be attributed to a number of possible factors including unhatched eggs remaining 'trapped' in the sediment that stayed in the beaker together with the sediment phase after the test chamber was mixed; some larvae may have been buried in the sediment after this was fixed with Lugol's solution and mixed. In both cases, the buried eggs/larvae would not have been counted, leading to a biased estimation of ELS mortality.

The larval development test with *A. tonsa* was much more sensitive to sediment contamination than the standardized, acute lethality test with amphipods, as already noted by Wollenberger and Kusk (2006). Even if exposure conditions are different (sediment bound toxicants and/or dissolved in the pore water as concern amphipods; contaminants released at the sediment-water interface for *A. tonsa*), the use of the larval development test with copepods can greatly improve the

characterization of sediment toxicity, by providing complementary information on the possible effects of exposure to low concentrations of soluble toxicant in the sediments.

The different storage conditions of the sediments (frozen storage at -20°C for *A. tonsa*; freshly collected sediments kept at 4°C until testing for amphipods) could have introduced some changes in the contaminant's availability as a consequence of freezing and thawing. Beiras et al. (1998) observed an increase of toxicity, towards larvae of *Crassostrea gigas*, of the elutriates extracted by frozen sediment samples; in contrast, in a spiking experiment with DDTs and endrin, Schuytema et al. (1989) reported that frozen sediment samples showed reduced toxicity toward the amphipod *Hyalella azteca* in respect to freshly collected sediments. It seems unlikely that the higher toxic response observed with the copepods as compared with amphipods could be exclusively related to changes in the contaminant's availability; in any case, to avoid any disturbance due to freezing and thawing, for the definitive study the decision was made to operate only with freshly collected sediment, stored at 4°C for less than 4 weeks (ASTM, 2014).

LDR seemed a more consistent and reliable endpoint than ELS mortality for the sediment testing with *A. tonsa*; for this reason, the definitive study has focused only on LDR and on the possible factors affecting it.

### *3.2 Definitive study*

*Statistical performance assessment*

The $90^{th}$ percentile of the MSDs normalized to control for the larval development test with *A. tonsa* (0.40) yielded a higher result than the values obtained for other species used in the Lagoon of Venice (Volpi Ghirardini et al., 2005; Picone et al., 2008; Losso and Volpi Ghirardini, 2010) (data summarized in Supplementary Material, Table 3SI) and for the Atlantic and Pacific amphipods and bivalves summarized by Phillips et al. (2001). This higher threshold could be easily explained by analysing the plot reported in Supplementary Material (Figure 1SI), showing the cumulative distributions of the variances for the main species and tests used for assessing sediment toxicity in the Lagoon of Venice. The larval development test with *A. tonsa* is characterized by a larger among-replicate variance as compared with the other tests taken into consideration. Since the MSDs are a function of both sample and control variance, this led automatically to an increase of all the MSD values, including the $90^{th}$ percentile.

The variance observed for the larval development test is at least in part associated with its higher sensitivity as compared with most of the tests mentioned in Table 3SI, which emerged also from the preliminary study (i.e. amphipod lethality test). The higher threshold in any case does not affect the discriminating ability of the test and is not an exception in the literature; it is similar or even lower

than the thresholds summarized by Phillips et al. (2001) for the survival (64%) and growth (44%) of the polychaetous annelid *Neanthes arenaceodentata*, the development of the sea urchin *Strongylocentrotus purpuratus* exposed to pore-water (55%), the development test with the abalone *Haliotis refuscens* (64%) and the development test with *S.purpuratus* at sediment-water interface (59%). This latter data is particularly relevant, since it evidences that the statistical performances of the sediment-water interface test with *A. tonsa* match the performances of the methods proposed by Anderson et al. (1996) for testing sediment toxicity with early-life stages.

The ability of the toxicity threshold to distinguish between "toxic" and "non-toxic" samples has been also confirmed by the output of the one-way ANOVA (see data on Table 3). Only for one sample (β-01) ANOVA and toxicity threshold provided contradictory results, since the inhibition of the larval development (48%) resulted above the toxicity threshold but no statistical difference between sample and control has been detected by ANOVA, at a confidence level of 5% ($p = 0.06$). For all the other samples, ANOVA confirmed the response obtained with the toxicity threshold.

In any case, care should be taken when considering not-effective samples with an effect higher than 20–25% relative to control, as pointed out by Thursby et al. (1997); data characterized by relatively high variance could partially mask the potential toxic effects of the sediments, increasing the risk of 'false negatives' (type II or *beta* errors). For these reasons, we propose the following classification of the effects, based on the precautionary criterion, both for stimulatory and inhibitory effects: absence of effects from 0% up to ± 24%, possible effects from ± 25% up to ± 39% and significant effects for values > ± 40% (see Table 3).

*Influence of the non-contaminant factors*

The non-toxic samples were characterized by wide ranges both of grain-size (sand ranging from 5% to 88%) and TOC (0.2% - 13.3%), suggesting that these physical features of the sediments had little influence on the larval development of *A. tonsa*. This hypothesis was supported also by the correlation analyses performed on the dataset. The possible interference of grain-size (suspension of the finest particles and trapping-effect of the sediment) and TOC (release of organic acids) on the larval development of *A. tonsa* thus does not appear to be of concern.

The significant correlation observed between larval development and AVS in the sediments suggests that amorphous sulphides and probably other sulphur compounds (i.e. elemental sulphur) should be carefully taken into consideration to avoid possible 'false positive' responses. In the Lagoon, the areas where sediments are commonly rich in sulphur compounds are generally restricted to mudflats and canals characterized by silty sediments (as γ-02, β-04 and β-05), where significantly toxic effects were measured despite a low to moderate contamination level.

*Sediment testing*

The samples collected in the areas close to the sea inlets and some of those located far from industrial and urbanized area are generally characterized by negligible effects on larval development; toxic effects were mainly elicited by the samples retrieved from areas located landward and in the proximity of the industrial area. Surprisingly some α samples collected close to the barrier islands (the area less affected by chemical contamination) significantly inhibited the larval development of *A. tonsa* (α-05, α-09, α-11 and α-12); As and Hg were the only chemicals exceeding the ERL in these sites and are probably the toxicants in need of monitoring and of most potential concern in that area. The toxic response provided by sample β-07, collected in a confined mudflat of the southern basin of the Lagoon, is quite interesting: in this sample organotin compounds and ∑DDTs were detected at levels comparable with those of the industrial area, highlighting a possible contribution of agricultural runoff and small shipyards to the observed toxicity. ∑DDTs together with metals are also the possible causes of the impairments observed in sample β-20.

With the exception of the above-mentioned samples, the observed spatial distribution of the toxicity is in agreement with the results expected on the basis of sediment chemistry and with previous studies (Picone, 2006; Picone et al.,2009, 2016).

Despite the occurrence of significant correlations between exposure and effect, as well as the lack of correlation with some key chemicals (i.e. ∑DDTs and organotin compounds), it is not possible to identify with a good level of confidence the toxicants effectively involved in the inhibition of the development. Dissolved organotin compounds are strong inhibitors of the larval development of *A. tonsa* at levels as low as 1 ng $l^{-1}$ of TBT (Kusk and Petersen, 1997); nevertheless, in the tested sediment no significant correlation has been found between larval development and organotin compounds, although sediment concentrations were above the values proposed by ANZECC as interim guideline. The lack of correlation is most probably due to high hydrophobicity of TBT that favours its absorption in sediment organic carbon, including the black carbon fraction (Brändli et al., 2009); the fate of the sediment-bound TBT is thus to be accumulated in the surface sediment, where it can be taken up by ingestion by deposit feeder organisms, while its release in the overlying water could be strongly limited. As a consequence, the occurrence of TBT toxicity toward *A. tonsa* in the sediment test is less likely, but cannot be excluded, especially for the samples characterized by the concentrations above detection limits.

Furthermore, Spearman's correlation pointed out that the divalent metals Cd, Cu, Pb and Zn are positively correlated with the inhibition of larval development, but the estimation of metal

539 bioavailability through the ΣSEM-AVS $f_{OC}^{-1}$ index suggested that most of these metals are bound to
540 ligands, as amorphous sulphides and organic matter, restricting their mobility in a static exposure.
541 In this context, the positive correlation could have been generated by the co-occurrence of metals
542 and other, "effective" pollutants whose bioavailability is not limited. This hypothesis is supported
543 by previous studies carried out on the Venice Lagoon, that evidenced a clear superimposed gradient
544 of increasing concentrations from the sea inlets towards the industrial area for metals and organic
545 micro-pollutants, as well as consistent contamination near the urban centres (Picone et al., 2016). It
546 cannot be, however, excluded that this significant correlation could have been generated by the
547 oxidation of amorphous sulphides and the subsequent release of free metal ions in the overlaying
548 water during the exposure (Wang and Chapman, 1999; Vandegehuchte et al., 2007; Sei et al.,
549 2016).

550 The inhibition of the larval development in crustaceans is often associated with the occurrence of
551 metals (Cd) and various classes of organochlorine compounds as PCBs, DDTs and other pesticides
552 that may affect molting and growth of the larvae by interfering with the endocrine regulation of
553 these processes (Rodriguez Moreno et al., 2003; Bondgaard and Bjerregaard, 2005; Kusk and
554 Wollenberger, 2007; Rodriguez et al., 2007). The correlation found between PCBs sediment
555 concentrations and larval development might suggest that at least part of the toxicity measured
556 towards *A. tonsa* could be due to endocrine disruption exerted by the PCBs, especially the *ortho*-
557 chlorinated congeners, that Zou and Fingerman (1997) already reported as effective disrupting
558 agents towards the water flea *Daphnia magna* Straus.

559 Nevertheless, as stressed by Barata et al. (2004) and remarked by Rodriguez et al. (2007), a wide
560 range of contaminants can exert toxic effects on small crustaceans as cladocerans and copepods by
561 reducing energy intake, both inhibiting food intake and increasing the energy demand as response to
562 the environmental stressor. Other substances may also slow down the molting of the crustaceans by
563 inhibiting the activity of enzymes involved in digestion of the chitinous exoskeleton (Zou and
564 Fingerman, 1999). As a consequence, care should be taken before ascribing the observed
565 impairments in the larval development or molting cycle to endocrine disruption, especially if
566 biochemical analyses to evaluate possible alteration of the endocrine metabolism or *in vivo* testing
567 for endocrine disruption, as the ecdysteroid agonist- and antagonist-sensitive $B_{11}$-cell line assay
568 (Wollenberger et al., 2005), have been not performed coincidentally with the toxicity tests.

569 Of particular interest is the stimulation observed in sample γ-05; in fact, *in vivo* studies performed
570 on pure chemicals evidenced that molting stimulation in copepods as well as other crustaceans is by
571 far less frequent than inhibition, both in adults and larval stages (Rodriguez et al., 2007, and citation

572 therein). Dahl et al. (2006), in a study on *N. spinipes*, observed stimulatory effects of the drug
573 Simvastatin at low concentrations (up to 1.6 μg $l^{-1}$) and related this outcome to alterations in
574 endocrine control. Andersen et al. (2001) hypothesized that the shortening of the intermolt period
575 elicited by hormone mimics with ecdysone agonist potential, could be due to the ability of these
576 compounds to bind to the ecdysone receptors site, causing a decrease of the threshold concentration
577 of ecdysone needed to trigger the shedding of the exoskeleton.

578 Due to the incomplete knowledge of the endocrine regulation in crustaceans and the lack of
579 chemical data concerning the possible occurrence of hormone mimics in the sediments other than
580 DDTs and PCBs, it is impossible to formulate any hypothesis concerning causality in site γ-05.
581 Toxicity data underline that further and deeper investigation concerning organic micropollutants
582 with possible endocrine disrupting potential that are not routinely investigated (i.e. polybrominated
583 diphenylethers, alkylphenol ethoxylates, synthetic hormones, personal care products and the related
584 degradation products) are needed, also in consideration of the recent detection of fragrance
585 materials (FM) in the surface waters of the Lagoon of Venice (Vecchiato et al., 2016). The
586 occurrence of FM, in the nearness of the urban centres as well as in shallows receiving waters from
587 the drainage basin (Vecchiato et al., 2016), provides a further line of evidence concerning the need
588 to perform deeper investigations in the areas expected to receive untreated or partially treated
589 sewages from the urban centres of the Lagoon (as site γ-05).

590 **CONCLUSION**

591 The larval development test with *A. tonsa* proved to be a valuable tool for assessing the
592 impairments elicited by sediment contamination when the LDR endpoint is used.

593 The LDR test showed excellent discriminating ability among samples characterized by different
594 contamination levels and good tolerance to wide ranges of potential confounding factors as
595 sediment grain-size and organic carbon content.

596 Despite the substantial among-replicate variance observed, the statistical performances of the test
597 were consistent with the data reported for other bioindicators used for sediment and sediment-water
598 interface testing, and the threshold calculated through the MSD criterion allows for a quick, reliable
599 and easy identification of the significantly toxic and/or stimulatory samples. To minimize the
600 possible 'false negative' responses and at the same time improve the statistical performance of
601 testing, it could be appropriate to use a larger number of replicates both for samples and controls, in
602 order to reduce both the among-replicate variance and the MSDs.

603 Since crustaceans are more vulnerable to the effects elicited by some classes of organic pollutants
604 (pesticides, PCBs, human pharmaceuticals, natural and xenobiotic hormone mimics) than other

organisms usually employed in short-term toxicity testing, e.g. bivalve embryos and larvae (His *et al*.,1999), the development test with *A. tonsa* can provide critical data for a comprehensive site-specific Ecological Risk Assessment. In this context, the LDR endpoint could be used in a tiered approach to screening levels, to identify sites characterized by major hazard and in need of further investigation (Tier 1), or at a deeper level of research, to provide a comprehensive characterization of the toxicity (Tier 2).

In any case, the *A. tonsa* test on sediment-water interface should be considered a complementary approach to the classic whole-sediment testing with infaunal species of amphipods and annelids, which has continued to be the most relevant laboratory approach to estimate the toxicity of bulk sediment.

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839

**Table 1**

Table 1. Summary of the physico-chemical analyses and toxicity test results for the preliminary study. In-% and p-value refers to the comparison with the reference site 22B.

| Site | **Total metals** (mg kg$^{-1}$ dw) | | | | | | | | | **Organics** (µg kg$^{-1}$ dw) | | | | | | | | | | | | TOC (%) | Sand (%) | Silt (%) | Clay (%) | LDR (± se) | p-value | In-% (± se) | ELS mortality (± se) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | As | Cd | Cr | Cu | Hg | Ni | Pb | Zn | N | AY | AE | F | Pn | An | FL | PY | BaA | Cn | BaP | DA | ∑PCB | | | | | | | | |
| **2B** | 7.9 | 0.40 | 50 | 23 | 0.94 | 25 | 31 | 79 | 30 | 1 | 1 | 5 | 49 | 7 | 58 | 50 | 22 | 24 | 18 | 5 | 1.0 | 1.5 | 3.7 | 61.6 | 34.7 | 0.20 ± 0.04 | < 0.001 | 0.54 ± 0.09 | 0.36 ± 0.06 |
| **16B** | 15.9 | 0.80 | 64 | 24 | 0.27 | 38 | 34 | 120 | 4 | 1 | 0.3 | 3 | 19 | 5 | 53 | 37 | 12 | 14 | 9 | 2 | 0.9 | 5.0 | 20.2 | 39.3 | 40.5 | 0.15 ± 0.05 | < 0.001 | 0.67 ± 0.10 | 0.45 ± 0.10 |
| **22B** | 6.9 | 0.21 | 38 | 15 | 0.93 | 19 | 24.8 | 70 | 33 | 0.6 | 2.3 | 9 | 10 | 3 | 6 | 14 | 22 | 16 | 12 | 3 | 0.2 | 0.9 | 4.5 | 73.5 | 22.0 | 0.44 ± 0.04 | - | - | 0.44 ± 0.06 |
| **24B** | 5.4 | 0.20 | 28 | 13 | 0.20 | 14 | 12 | 38 | 7 | 0.2 | 0.2 | 2 | 17 | 2 | 9 | 10 | 4 | 7 | 5 | 1 | 0.2 | 0.5 | 60.4 | 29.9 | 9.7 | 0.60 ± 0.06 | < 0.001 | -0.37 ± 0.07 | 0.18 ± 0.07 |
| **32B** | 13.9 | 1.87 | 144 | 97 | 3.28 | 115 | 64.9 | 375 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 2.4 | 45.5 | 25.1 | 29.4 | 0.18 ± 0.07 | < 0.001 | 0.58 ± 0.16 | 0.36 ± 0.09 |

AY = acenaphthylene; AE = acenaphtene; F = fluorene; Pn = phenanthrene; An = anthracene; FL = fluoranthene; PY = pyrene; BaA = benzo[a]anthracene; Cn = chrysene; BaP = benzo[a]pirene; DA = dibenz[a.h]anthracene

**Table 2**

Table 2. Spearman correlations between inhibition of larval development, ELS mortality and physical and chemical parameters for the preliminary study. In bold are reported the significant correlations. Correlations with organic chemicals (PAH, PCBs) have been calculated for data normalized to organic carbon content.

| Parameter | N | In-% | | ELS-mortality | |
|---|---|---|---|---|---|
| | | Spearman R | p-value | Spearman R | p-value |
| Sand (%) | 5 | -0.20 | 0.74 | -0.46 | 0.22 |
| TOC | 5 | **1.00** | **< 0.001** | 0.67 | 0.22 |
| As | 5 | **1.00** | **< 0.001** | 0.67 | 0.22 |
| Cd | 5 | **0.90** | **0.04** | 0.41 | 0.49 |
| Cr | 5 | **0.90** | **0.04** | 0.41 | 0.49 |
| Cu | 5 | **0.90** | **0.04** | 0.41 | 0.49 |
| Hg | 5 | 0.40 | 0.50 | 0.05 | 0.93 |
| Ni | 5 | 0.90 | **0.04** | 0.41 | 0.49 |
| Pb | 5 | **0.90** | **0.04** | 0.41 | 0.49 |
| Zn | 5 | **0.90** | **0.04** | 0.41 | 0.49 |
| PAHs | 4 | 0.80 | 0.20 | 0.40 | 0.60 |
| PCBs | 4 | 0.74 | 0.26 | 0.21 | 0.79 |

**Table 3**

Table 3. Summary of the larval development rate (LDR) toxicity test results for the 48 samples of the definitive study. Negative values of the percentage of inhibition (In-%) indicate stimulatory effect. P-values highlighted in italic and bold indicate significant difference from control sample (ANOVA and *post hoc* test).
Effect classification was performed as follows: stimulatory effect if In-% < -40, possible stimulation if -39 < In-% < -25, no effect in -24 < In-% < 24, possible toxicity if 25 < In-% < 39, significant toxicity if In-% > 40%.

| Site | Typology | LDR (± se) | Control LDR | p-value | In-% (± se) | TOC (%) | sand (%) | Class | Effect |
|---|---|---|---|---|---|---|---|---|---|
| α–01 | Shallow | 0.57 ± 0.06 | 0.55 ± 0.06 | 0.727 | -5 ± 10 | 5.2 | 23 | Sandy-silt | Absent |
| α–02 | Shallow | 0.50 ± 0.10 | 0.55 ± 0.06 | 0.522 | 8 ± 19 | 3.4 | 5 | Silt | Absent |
| α–03 | Shallow | 0.53 ± 0.05 | 0.55 ± 0.06 | 0.846 | 3 ± 9 | 3.5 | 54 | Silty-sand | Absent |
| α–04 | Shallow | 0.51 ± 0.09 | 0.62 ± 0.03 | 0.436 | 18 ± 15 | 0.9 | 67 | Silty-sand | Absent |
| α–05 | Shallow | 0.09 ± 0.04 | 0.36 ± 0.06 | ***0.012*** | 75 ± 13 | 4.1 | 18 | Silt | Toxic |
| α–06 | Shallow | 0.75 ± 0.05 | 0.68 ± 0.03 | 0.461 | -11 ± 7 | 3.3 | 45 | Sandy-silt | Absent |
| α–07 | Shallow | 0.47 ± 0.13 | 0.62 ± 0.03 | 0.215 | 24 ± 21 | 7.7 | 51 | Silty-sand | Absent |
| α–08 | Shallow | 0.55 ± 0.06 | 0.55 ± 0.07 | 0.987 | 0 ± 0 | 4.0 | 32 | Sandy-silt | Absent |
| α–09 | Shallow | 0.35 ± 0.04 | 0.55 ± 0.07 | 0.100 | 35 ± 7 | 3.5 | 59 | Silty-sand | Possibly toxic |
| α–10 | Shallow | 0.56 ± 0.05 | 0.55 ± 0.06 | 0.846 | -3 ± 9 | 1.5 | 34 | Sandy-silt | Absent |
| α–11 | Channel | 0.26 ± 0.11 | 0.37 ± 0.09 | 0.174 | 31 ± 30 | 2.9 | 26 | Sandy-silt | Possibly toxic |
| α–12 | Channel | 0.04 ± 0.01 | 0.55 ± 0.06 | ***< 0.001*** | 92 ± 2 | 5.3 | 22 | Sandy-silt | Toxic |
| α–13 | Shallow | 0.44 ± 0.06 | 0.55 ± 0.07 | 0.333 | 19 ± 12 | 4.8 | 17 | Sandy-silt | Absent |
| α–14 | Shallow | 0.69 ± 0.07 | 0.55 ± 0.07 | 0.248 | -26 ± 13 | 4.1 | 58 | Silty-sand | Stimulatory |
| α–15 | Shallow | 0.46 ± 0.08 | 0.46 ± 0.09 | 0.953 | 0 ± 5 | 7.6 | 88 | Sand | Absent |
| β–01 | Shallow | 0.29 ± 0.06 | 0.54 ± 0.08 | 0.060 | 48 ± 11 | 2.6 | 25 | Sandy-silt | Toxic |
| β–02 | Shallow | 0.14 ± 0.06 | 0.36 ± 0.06 | ***0.033*** | 62 ± 17 | 5.8 | 13 | Sandy-silt | Toxic |
| β–03 | Shallow | 0.28 ± 0.09 | 0.54 ± 0.08 | ***0.019*** | 49 ± 17 | 5.6 | 15 | Sandy-silt | Toxic |
| β–04 | Shallow | 0.26 ± 0.07 | 0.55 ± 0.07 | ***0.008*** | 53 ± 12 | 3.4 | 4 | Clayey-silt | Toxic |
| β–05 | Shallow | 0.21 ± 0.09 | 0.55 ± 0.07 | ***0.003*** | 62 ± 16 | 5.1 | 48 | Silty-sand | Toxic |
| β–06 | Shallow | 0.51 ± 0.03 | 0.68 ± 0.03 | 0.107 | 24 ± 4 | 0.3 | 49 | Silty-sand | Absent |
| β–07 | Shallow | 0.06 ± 0.05 | 0.46 ± 0.09 | ***< 0.001*** | 87 ± 12 | 2.7 | 10 | Silt | Toxic |
| β–08 | Shallow | 0 ± 0 | 0.46 ± 0.09 | ***< 0.001*** | 100 ± 0 | 1.0 | 19 | Sandy-silt | Toxic |
| β–09 | Shallow | 0.02 ± 0.01 | 0.46 ± 0.09 | ***< 0.001*** | 97 ± 3 | 7.2 | 16 | Sandy-silt | Toxic |
| β–10 | Shallow | 0.13 ± 0.02 | 0.42 ± 0.05 | ***< 0.001*** | 69 ± 5 | 4.5 | 39 | Sandy-silt | Toxic |
| β–11 | Channel | 0.08 ± 0.03 | 0.37 ± 0.09 | ***< 0.001*** | 79 ± 9 | 2.4 | 37 | Sandy-silt | Toxic |
| β–12 | Shallow | 0.49 ± 0.11 | 0.62 ± 0.03 | 0.324 | 20 ± 18 | 13.3 | 24 | Sandy-silt | Absent |
| β–13 | Shallow | 0.44 ± 0.05 | 0.46 ± 0.09 | 0.879 | 4 ± 10 | 0.2 | 76 | Sand | Absent |
| β–14 | Shallow | 0.12 ± 0.05 | 0.46 ± 0.09 | ***< 0.001*** | 73 ± 10 | 6.9 | 75 | Sand | Toxic |
| β–15 | Shallow | 0.58 ± 0.07 | 0.55 ± 0.06 | 0.683 | -6 ± 12 | 1.6 | 11 | Silt | Absent |
| β–16 | Shallow | 0.37 ± 0.10 | 0.68 ± 0.03 | ***0.001*** | 45 ± 14 | 3.4 | 25 | Sandy-silt | Toxic |
| β–17 | Channel | 0.03 ± 0.03 | 0.55 ± 0.06 | ***< 0.001*** | 95 ± 5 | 3.4 | 21 | Sandy-silt | Toxic |
| β–18 | Shallow | 0.81 ± 0.06 | 0.68 ± 0.03 | 0.196 | -20 ± 9 | 3.2 | 47 | Sandy-silt | Absent |
| β–19 | Shallow | 0.38 ± 0.17 | 0.54 ± 0.08 | 0.119 | 31 ± 31 | 1.5 | 14 | Clayey-silt | Possibly toxic |
| β–20 | Shallow | 0.26 ± 0.06 | 0.54 ± 0.08 | ***0.036*** | 53 ± 10 | 1.6 | 58 | Silty-sand | Toxic |
| γ–01 | Channel | 0.06 ± 0.03 | 0.37 ± 0.09 | ***< 0.001*** | 83 ± 9 | 2.3 | 39 | Sandy-silt | Toxic |
| γ–02 | Shallow | 0.27 ± 0.14 | 0.55 ± 0.07 | ***0.010*** | 52 ± 26 | 1.9 | 27 | Sandy-silt | Toxic |
| γ–03 | Channel | 0.06 ± 0.03 | 0.37 ± 0.09 | ***0.002*** | 84 ± 6 | 1.8 | 10 | Silt | Toxic |
| γ–04 | Channel | 0.02 ± 0.01 | 0.37 ± 0.09 | ***< 0.001*** | 94 ± 4 | 3.2 | 74 | Silty-sand | Toxic |
| γ–05 | Shallow | 0.70 ± 0.09 | 0.42 ± 0.05 | ***< 0.001*** | -87 ± 11 | 0.1 | 24 | Sandy-silt | Stimulatory |
| γ–06 | Channel | 0.02 ± 0.01 | 0.42 ± 0.05 | ***< 0.001*** | 96 ± 2 | 4.4 | 23 | Sandy-silt | Toxic |
| γ–07 | Shallow | 0.02 ± 0.01 | 0.54 ± 0.08 | ***< 0.001*** | 96 ± 2 | 4.0 | 31 | Sandy-silt | Toxic |
| γ–08 | Shallow | 0.04 ± 0.04 | 0.42 ± 0.05 | ***< 0.001*** | 91 ± 9 | 1.3 | 23 | Sandy-silt | Toxic |
| γ–09 | Shallow | 0.13 ± 0.05 | 0.68 ± 0.03 | ***< 0.001*** | 81 ± 8 | 2.2 | 31 | Sandy-silt | Toxic |
| γ–10 | Shallow | 0.04 ± 0.03 | 0.42 ± 0.05 | ***< 0.001*** | 91 ± 6 | 1.8 | 20 | Sandy-silt | Toxic |
| γ–11 | Shallow | 0.54 ± 0.08 | 0.62 ± 0.03 | 0.510 | 13 ± 13 | 0.2 | 26 | Sandy-silt | Absent |
| γ–12 | Channel | 0.16 ± 0.05 | 0.62 ± 0.03 | ***< 0.001*** | 75 ± 9 | 1.0 | 26 | Sandy-silt | Toxic |
| γ–13 | Channel | 0.11 ± 0.03 | 0.37 ± 0.09 | ***0.005*** | 71 ± 9 | 1.4 | 36 | Sandy-silt | Toxic |

**Table 4**

Table 4. Spearman correlations between inhibition of larval development and physical and chemical parameters for the definitive study. In bold are reported the significant correlations. Correlations with organic chemicals (PAH, PCBs, DDTs, HCB and organotin compounds) have been calculated for data normalized to organic carbon content.

| Parameter | n | Spearman R | p-value |
|---|---|---|---|
| Sand (%) | 48 | -0.27 | 0.06 |
| TOC | 48 | 0.05 | 0.72 |
| As | 48 | **0.43** | < **0.001** |
| Cd | 48 | **0.33** | **0.02** |
| Cr | 48 | 0.18 | 0.23 |
| Cu | 48 | **0.59** | < **0.001** |
| Hg | 48 | **0.55** | < **0.001** |
| Ni | 48 | 0.09 | 0.53 |
| Pb | 48 | **0.65** | < **0.001** |
| Zn | 48 | **0.61** | < **0.001** |
| Cr (VI) | 48 | 0.08 | 0.60 |
| V | 48 | **0.35** | **0.02** |
| AVS | 48 | **0.36** | **0.01** |
| mERMq | 48 | **0.58** | < **0.001** |
| PAHs | 48 | **0.33** | **0.02** |
| PCBs | 48 | **0.35** | **0.01** |
| o.p'-DDT | 48 | 0.11 | 0.45 |
| p.p'-DDT | 48 | 0.14 | 0.33 |
| o.p'-DDD | 48 | 0.22 | 0.14 |
| p.p'-DDD | 48 | 0.14 | 0.35 |
| o.p'-DDE | 48 | 0.07 | 0.62 |
| p.p'-DDE | 48 | 0.29 | 0.06 |
| ∑DDTs | 48 | 0.26 | 0.07 |
| HCB | 48 | 0.27 | 0.07 |
| TBT | 24 | 0.30 | 0.22 |
| DBT | 24 | 0.20 | 0.42 |
| MBT | 24 | 0.34 | 0.16 |

**Figure 1**

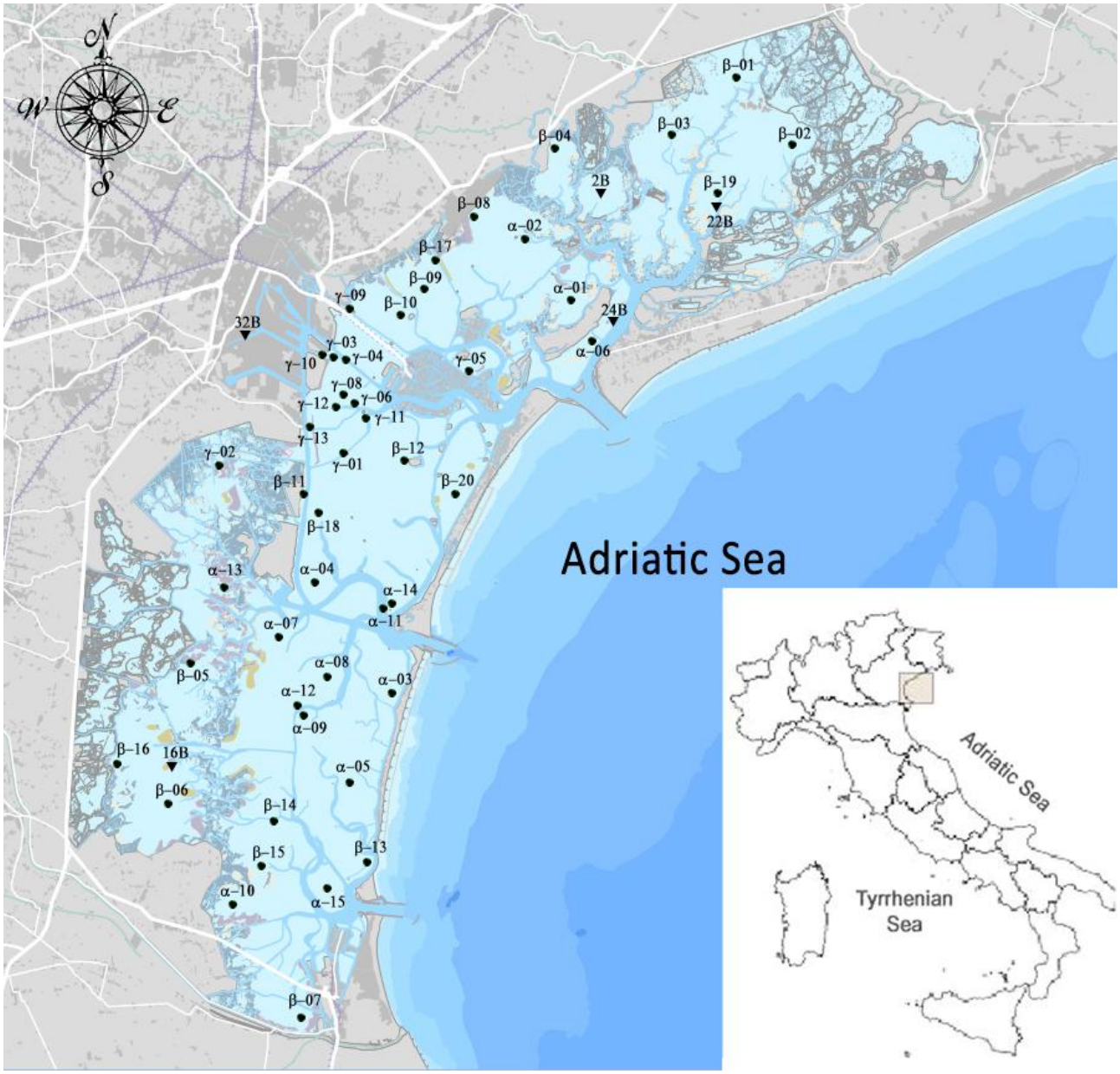


Figure 1. Location of the sampling sites in the Lagoon of Venice. ▼= sites investigated in the preliminary study; ● = sites investigated in the definitive study. Site 32B has been investigated both in the preliminary and definitive study. Site 24B has been used as control sample in the definitive study. Labels α, β and γ indicate expected low, medium and high contaminated samples, according to the dataset of the project " Mappatura dell'inquinamento dei fondali lagunari" (MAG.ACQUE, 1999), based on 1993 Venice Sediment Management Criteria (Ministero Ambiente, 1993).

**Figure 2**

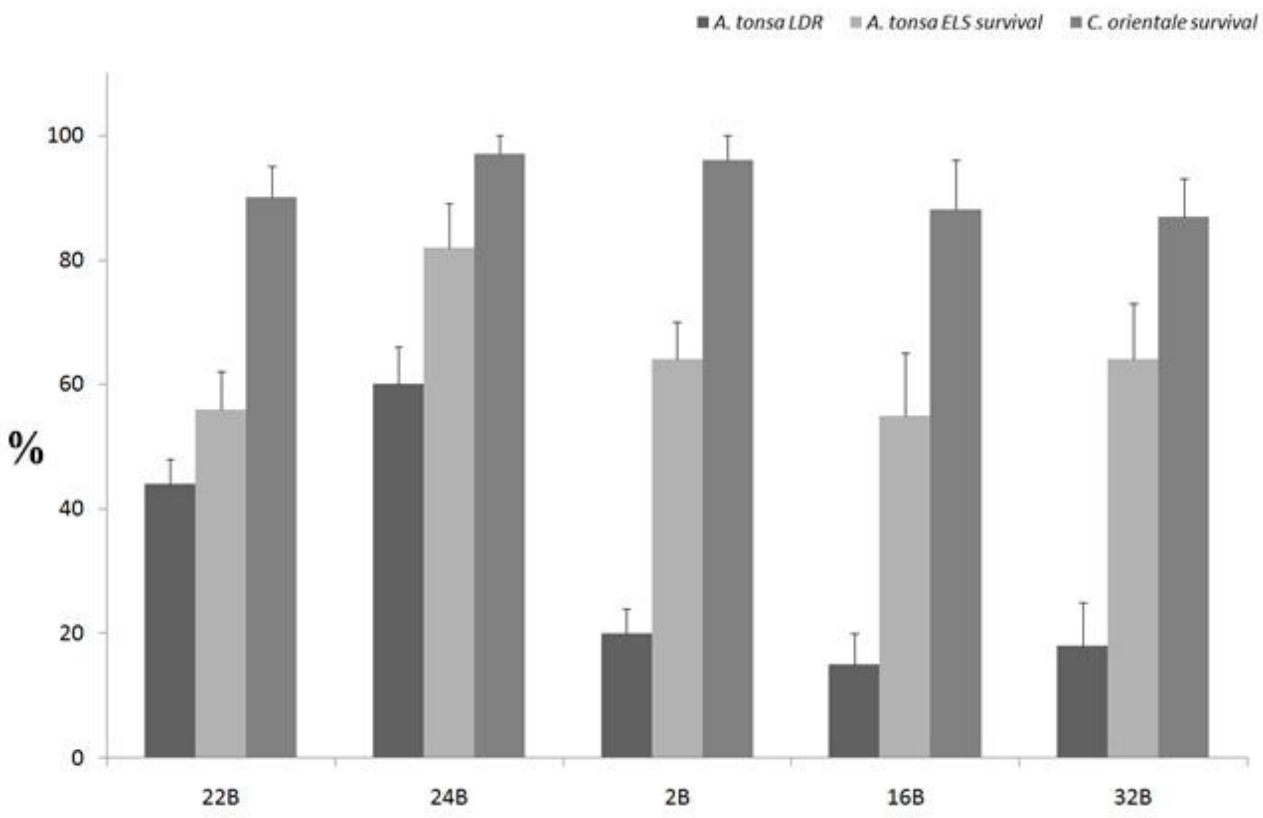


Figure 2. Comparison among the larval development test with *A. tonsa* and the 10-d lethality test with the amphipod *C. orientale*. Data are reported as % of *A. tonsa* larval development rate (LDR) and % of *C. orientale* and *A. tonsa* ELS survival. Error bars correspond to standard deviation.

**Supplementary Material Figure 1SI**

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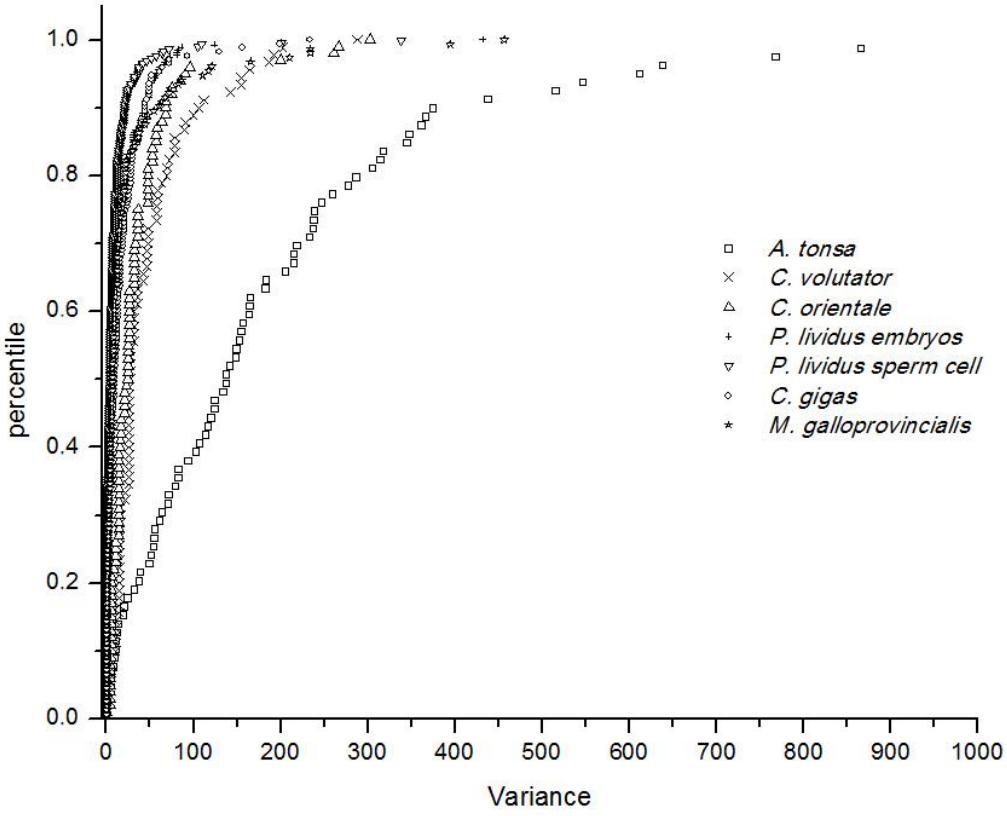


Figure 1SI. Percentile rank plot of the obtained variances for both controls and samples with the toxicity tests used in the Lagoon of Venice. Only 40 percent of samples tested with *A. tonsa* showed a variance < 100, whilst for all the other bioassays more than 90% of the samples were characterized by a variance < 100.

**Supplementary Material Table 1SI**

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Table 1SI. Summary of the chemical analyses performed on sediment samples: total metals, organics and organotins. * indicates ERL exceedings; ** indicates ERM exceedings (Long et al., 1995). No ERL/ERM are available for V, TBT, DBT and MBT.

| Site | Total metals (mg/kg) | | | | | | | | | Organics (µg/kg) | | | Organotins (µg Sn/kg) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | As | Cd | Cr | Cu | Hg | Ni | Pb | V | Zn | ΣPAHs | ΣPCBs | ΣDDTs | TBT | DBT | MBT |
| α–01 | *13* * | 0.07 | 34 | 14 | *0.75* ** | 14 | 14 | 42 | 52 | 137 | 0.7 | 0.4 | | | |
| α–02 | *17* * | 0.06 | 61 | 16 | *0.27* * | 19 | 18 | 51 | 40 | 209 | 2.2 | 0.6 | < 2 | < 4 | < 4 |
| α–03 | 6 | 0.10 | 32 | 12 | *0.26* * | 17 | 7 | 36 | 57 | 220 | 1.1 | 0.4 | 2 | < 4 | < 4 |
| α–04 | *16* * | 0.12 | 46 | 16 | *0.27* * | *21* * | 15 | 44 | 85 | 80 | 1.8 | 0.5 | 6 | < 4 | < 4 |
| α–05 | *38* * | 0.08 | 19 | 3 | 0.06 | 11 | 5 | 18 | 26 | 134 | 0.1 | 0.3 | | | |
| α–06 | *10* * | 0.05 | 25 | 9 | *0.24* * | 9 | 8 | 33 | 36 | 75 | 0.7 | 0.4 | < 2 | < 4 | < 4 |
| α–07 | *11* * | 0.10 | 24 | 12 | *0.17* * | 13 | 10 | 32 | 61 | 299 | 0.7 | 0.3 | | | |
| α–08 | *16* * | 0.10 | 45 | 13 | *0.27* * | 20 | 15 | 52 | 27 | 100 | 0.7 | 0.4 | 2 | < 4 | < 4 |
| α–09 | 8 | 0.01 | 53 | 21 | *1.03* ** | *26* * | 18 | 56 | 79 | 137 | 1.6 | 0.7 | | | |
| α–10 | *13* * | 0.05 | 32 | 16 | 0.13 | 19 | 14 | 35 | 66 | 170 | 0.8 | 0.4 | | | |
| α–11 | *11* * | 0.06 | 20 | 6 | *0.17* * | 10 | 8 | 27 | 40 | 130 | 1.0 | 0.4 | | | |
| α–12 | *16* * | 0.10 | 36 | 18 | *0.47* * | 19 | 19 | 49 | 78 | 163 | 1.9 | 0.8 | | | |
| α–13 | *26* * | 0.16 | *84* * | 33 | *0.55* * | *39* * | 38 | 85 | 102 | 717 | 3.7 | 1.2 | | | |
| α–14 | *9* * | 0.06 | 19 | 5 | *0.19* * | 8 | 6 | 27 | 65 | 33 | 0.3 | 0.3 | < 2 | < 4 | < 4 |
| α–15 | *9* * | 0.05 | 30 | 19 | 0.05 | 11 | 12 | 29 | 55 | 17 | 0.2 | 0.3 | < 2 | < 4 | < 4 |
| β–01 | *11* * | 0.08 | 49 | 20 | *0.95* ** | 20 | 23 | 58 | 47 | 428 | 1.0 | 0.7 | | | |
| β–02 | *14* * | 0.08 | 36 | 11 | 0.11 | 17 | 10 | 47 | 33 | 113 | 0.2 | 0.3 | < 2 | < 4 | < 4 |
| β–03 | *11* * | 0.10 | 39 | 19 | *1.03* ** | *22* * | 18 | 52 | 56 | 141 | 0.2 | 0.4 | < 2 | < 4 | < 4 |
| β–04 | *16* * | 0.10 | 61 | *36* * | *0.57* * | *29* * | 38 | 61 | 115 | 257 | 3.6 | 0.8 | | | |
| β–05 | *19* * | 0.10 | 52 | 27 | *0.21* * | *30* * | 26 | 50 | 99 | 214 | 1.7 | 0.9 | | | |
| β–06 | *24* * | 0.04 | 64 | 25 | *0.33* * | *30* * | 28 | 61 | 130 | 134 | 1.8 | 1.4 | | | |
| β–07 | *20* * | 0.03 | 65 | *34* * | *0.31* * | 15 | 37 | 61 | 146 | 72 | 6.8 | *2.1* * | 10 | 5 | 8 |
| β–08 | *25* * | 0.06 | 49 | 28 | *0.95* ** | 19 | 33 | 63 | *153* * | 342 | 3.5 | 0.9 | < 2 | < 4 | < 4 |
| β–09 | *9* * | 0.06 | 39 | 21 | *0.58* * | 18 | 29 | 54 | 115 | 175 | 2.7 | 0.6 | 2 | < 4 | < 4 |
| β–10 | *38* * | 0.03 | 37 | 24 | *0.76* ** | 20 | 32 | 51 | 93 | 169 | 2.0 | 0.4 | | | |
| β–11 | *15* * | 0.07 | 15 | 5 | 0.09 | 9 | 8 | 19 | 42 | 40 | 0.5 | 0.7 | | | |
| β–12 | 7 | 0.11 | 37 | 16 | *0.41* * | 15 | 17 | 42 | 57 | 1,075 | 0.8 | 0.4 | | | |
| β–13 | *14* * | 0.06 | 21 | 15 | 0.1 | 10 | 18 | 31 | 111 | 68 | 0.2 | 0.3 | | | |
| β–14 | *18* * | 0.02 | 51 | 17 | *0.28* * | *25* * | 18 | 52 | 83 | 221 | 1.1 | 0.4 | 5 | < 4 | < 4 |
| β–15 | *16* * | 0.04 | 65 | 18 | *0.37* * | *29* * | 17 | 60 | 42 | 87 | 1.2 | 0.7 | | | |
| β–16 | *20* * | 0.11 | 62 | 28 | *0.3* * | *31* * | 34 | 70 | 122 | 375 | 2.0 | 1.0 | 4 | < 4 | < 4 |
| β–17 | *16* * | 0.05 | 30 | 21 | *0.45* * | 16 | 22 | 36 | 103 | 476 | 3.3 | 1.1 | | | |
| β–18 | *13* * | 0.04 | 23 | 22 | 0.1 | 19 | 17 | 28 | 78 | 38 | 0.5 | 0.3 | | | |
| β–19 | *11* * | 0.08 | 34 | 15 | *0.56* * | 17 | 15 | 44 | 50 | 118 | 0.6 | 0.4 | < 2 | < 4 | < 4 |
| β–20 | *11* * | 0.13 | 34 | 21 | *1.04* ** | 17 | 21 | 38 | *206* * | 429 | 4.4 | *2.0* * | | | |
| γ–01 | *17* * | *1.84* * | 41 | *44* * | *0.97* ** | *22* * | 39 | 48 | *258* * | 1,099 | 6.1 | 0.9 | 6 | 4 | 11 |
| γ–02 | *30* * | *2.00* * | *84* * | *39* * | *0.40* * | *35* * | 44 | 89 | *275* * | 947 | 5.1 | *1.9* * | 4 | < 4 | 5 |
| γ–03 | *14* * | *1.93* * | 22 | 25 | *0.59* * | 11 | 44 | 27 | *208* * | 535 | 8.2 | 1 | 7 | 8 | 20 |
| γ–04 | *22* * | *2.55* * | 48 | *47* * | *1.5* ** | *23* * | 47 | 56 | *328* * | *6,622* * | 12.7 | *2.1* * | | | |
| γ–05 | *13* * | 0.25 | 58 | 8 | *0.34* * | *38* * | 8 | 29 | 45 | 1,010 | 6.8 | 0.9 | | | |
| γ–06 | *16* * | *4.28* * | 50 | *85* * | *2.42* ** | 21 | *62* * | 55 | *1,359* ** | 1,308 | 5.8 | 1.3 | 2 | < 4 | 7 |
| 32B | *23* * | *2.40* * | *81* * | *110* * | *7.3* ** | *27* * | *89* * | 55 | *315* * | *26,341* * | *888* ** | *41.9* * | | | |
| γ–08 | *33* * | *7.57* * | 37 | *86* * | *2.7* ** | 17 | *62* * | 57 | *1,228* ** | 1,074 | 11.0 | *3.0* * | 3 | < 4 | 9 |
| γ–09 | *23* * | *4.28* * | 41 | *49* * | *1.05* ** | 17 | *49* * | 46 | *679* ** | 1,491 | 15.8 | *2.1* * | 9 | 6 | 20 |
| γ–10 | *33* * | *4.28* * | 50 | *98* * | *1.98* ** | 20 | *90* * | 53 | *1,921* ** | 2,040 | 5.6 | 0.8 | 3 | < 4 | 4 |
| γ–11 | *14* * | 1.11 | 26 | 22 | *0.77* ** | 12 | 24 | 37 | 120 | 2,266 | 3.1 | 0.8 | 2 | < 4 | < 4 |
| γ–12 | *22* * | *6.00* * | 36 | *57* * | *1.10* ** | 16 | *68* * | 48 | *987* ** | 1,522 | 4.9 | 0.8 | | | |
| γ–13 | *16* * | *2.00* * | 35 | 30 | *1.00* ** | 19 | 30 | 39 | *215* * | 569 | 7.6 | 1.2 | 7 | 4 | 10 |

**Supplementary Material Table 2SI**

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Table 2SI. Summary of the chemical analyses performed on sediment samples: SEM, AVS, mERMq

| Site | Simultaneously Extracted Metals (µMol/kg dw) | | | | | | | | | AVS (µMol/kg) | ΣSEM-AVS/$f_{OC}$ | mERMq |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | As | Cd | Cr | Cu | Hg | Ni | Pb | Zn | ΣSEM | | | |
| α–01 | 1.2 | 0.22 | 43 | < 0.8 | < 0.02 | 23 | 7 | 43 | 75 | 1198 | -22 | 0.17 |
| α–02 | 0.6 | 0.24 | 16 | < 0.8 | < 0.02 | 7 | 6 | 350 | 363 | 2708 | -69 | 0.13 |
| α–03 | 2.4 | 0.25 | 26 | < 0.8 | < 0.02 | 15 | 5 | 57 | 77 | 2627 | -73 | 0.10 |
| α–04 | 3.3 | 0.72 | 45 | < 0.8 | < 0.02 | 34 | 9 | 167 | 211 | 558 | -39 | 0.14 |
| α–05 | 3.5 | 0.29 | 32 | < 0.8 | < 0.02 | 15 | 4 | 52 | 72 | 659 | -14 | 0.09 |
| α–06 | 2.2 | 0.14 | 49 | 1.87 | < 0.02 | 28 | 5 | 34 | 69 | 1357 | -39 | 0.08 |
| α–07 | 5.1 | 0.62 | 70 | 5.83 | < 0.02 | 44 | 10 | 187 | 247 | 2107 | -24 | 0.09 |
| α–08 | 1.4 | 0.18 | 27 | < 0.8 | < 0.02 | 12 | 6 | 32 | 49 | 1133 | -27 | 0.12 |
| α–09 | 3.8 | 0.57 | 2 | < 0.8 | < 0.02 | 15 | 15 | 120 | 151 | 1484 | -38 | 0.24 |
| α–10 | 1.6 | 0.25 | 18 | < 0.8 | < 0.02 | 10 | 8 | 60 | 78 | 398 | -21 | 0.10 |
| α–11 | 11.5 | 0.45 | 130 | 10.33 | < 0.02 | 58 | 15 | 102 | 185 | 1820 | -56 | 0.07 |
| α–12 | 2.0 | 0.41 | 46 | < 0.8 | < 0.02 | 19 | 11 | 89 | 120 | 2693 | -49 | 0.16 |
| α–13 | 5.2 | 3.06 | 272 | < 0.8 | < 0.02 | 173 | 33 | 671 | 881 | 4700 | -80 | 0.25 |
| α–14 | 2.3 | 0.11 | 11 | < 0.8 | < 0.02 | 4 | 3 | 20 | 28 | 751 | -18 | 0.07 |
| α–15 | 1.8 | 0.10 | 6 | < 0.8 | < 0.02 | 3 | 3 | 19 | 25 | 275 | -3 | 0.07 |
| β–01 | 0.1 | 0.17 | 28 | < 0.8 | < 0.02 | 12 | 4 | 35 | 51 | 2324 | -87 | 0.21 |
| β–02 | 0.4 | 0.16 | 63 | < 0.8 | < 0.02 | 30 | 3 | 11 | 44 | 557 | -9 | 0.09 |
| β–03 | 1.0 | 0.14 | 23 | < 0.8 | < 0.02 | 15 | 4 | 35 | 53 | 244 | -3 | 0.22 |
| β–04 | 30.9 | 1.41 | 129 | 163.78 | < 0.02 | 65 | 85 | 455 | 771 | 7477 | -197 | 0.22 |
| β–05 | 2.7 | 1.04 | 11 | < 0.8 | < 0.02 | 10 | 16 | 219 | 247 | 14148 | -273 | 0.16 |
| β–06 | 0.6 | 1.37 | 76 | < 0.8 | < 0.02 | 41 | 11 | 248 | 301 | 12983 | -4227 | 0.19 |
| β–07 | 2.0 | 0.76 | 49 | 2.69 | < 0.02 | 31 | 11 | 176 | 221 | 2362 | -79 | 0.18 |
| β–08 | 0.8 | 0.82 | 41 | 6.37 | < 0.02 | 30 | 15 | 198 | 250 | 6495 | -624 | 0.26 |
| β–09 | 2.6 | 0.67 | 28 | 11.12 | < 0.02 | 18 | 12 | 136 | 178 | 2472 | -32 | 0.18 |
| β–10 | 2.7 | 0.53 | 13 | 4.05 | < 0.02 | 9 | 8 | 152 | 174 | 3586 | -76 | 0.24 |
| β–11 | 2.5 | 0.26 | 6 | < 0.8 | < 0.02 | 3 | 5 | 352 | 360 | 301 | 2 | 0.07 |
| β–12 | 4.6 | 0.57 | 20 | < 0.8 | < 0.02 | 23 | 10 | 229 | 262 | 529 | -2 | 0.13 |
| β–13 | 5.4 | 0.22 | 18 | 2.96 | < 0.02 | 18 | 6 | 80 | 108 | 195 | -44 | 0.09 |
| β–14 | 2.1 | 0.63 | 47 | < 0.8 | < 0.02 | 22 | 14 | 104 | 140 | 2981 | -41 | 0.15 |
| β–15 | 1.8 | 0.41 | 9 | < 0.8 | < 0.02 | 6 | 6 | 64 | 77 | 430 | -22 | 0.16 |
| β–16 | 1.4 | 1.45 | 26 | < 0.8 | < 0.02 | 17 | 17 | 280 | 315 | 1300 | -29 | 0.19 |
| β–17 | 6.8 | 1.67 | 150 | < 0.8 | < 0.02 | 57 | 41 | 407 | 507 | 3522 | -89 | 0.16 |
| β–18 | 2.1 | 0.30 | 42 | < 0.8 | < 0.02 | 23 | 8 | 84 | 115 | 3939 | -120 | 0.10 |
| β–19 | 0.7 | 0.07 | 7 | < 0.8 | < 0.02 | 4 | 2 | 21 | 27 | 1484 | -97 | 0.15 |
| β–20 | 1.8 | 1.93 | 134 | < 0.8 | < 0.02 | 56 | 14 | 381 | 453 | 1987 | -96 | 0.26 |
| γ–01 | 1.0 | 1.37 | 96 | < 0.8 | < 0.02 | 50 | 20 | 712 | 783 | 4120 | -145 | 0.31 |
| γ–02 | 3.8 | 2.60 | 222 | 3.83 | < 0.02 | 108 | 30 | 644 | 789 | 22327 | -1134 | 0.29 |
| γ–03 | 1.5 | 2.11 | 33 | < 0.8 | < 0.02 | 14 | 17 | 499 | 531 | 220 | 17 | 0.21 |
| γ–04 | 1.4 | 2.90 | 29 | < 0.8 | < 0.02 | 10 | 37 | 942 | 991 | 11843 | -339 | 0.43 |
| γ–05 | 0.6 | 0.39 | 24 | < 0.8 | < 0.02 | 9 | 6 | 157 | 172 | 4372 | -4200 | 0.17 |
| γ–06 | 2.1 | 6.87 | 28 | < 0.8 | < 0.02 | 18 | 25 | 1699 | 1749 | 973 | 18 | 0.78 |
| 32B | 19.2 | 4.48 | 313 | 465.16 | < 0.02 | 155 | 133 | 1296 | 2053 | 6212 | -104 | 1.78 |
| γ–08 | 2.2 | 12.11 | 43 | < 0.8 | < 0.02 | 18 | 45 | 2998 | 3072 | 6366 | -253 | 0.84 |
| γ–09 | 5.3 | 4.82 | 25 | 21.07 | < 0.02 | 13 | 35 | 1616 | 1690 | 8937 | -329 | 0.45 |
| γ–10 | 3.5 | 35.14 | 130 | < 0.8 | < 0.02 | 59 | 153 | 12730 | 12977 | 17471 | -250 | 0.89 |
| γ–11 | 1.4 | 0.36 | 3 | < 0.8 | < 0.02 | 3 | 4 | 124 | 131 | 439 | -154 | 0.21 |
| γ–12 | 5.5 | 4.64 | 20 | < 0.8 | < 0.02 | 16 | 13 | 1528 | 1562 | 5240 | -368 | 0.54 |
| γ–13 | 4.0 | 1.51 | 27 | < 0.8 | < 0.02 | 8 | 19 | 398 | 427 | 2625 | -157 | 0.29 |

**Supplementary Material Table 3SI**

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Table 3SI. Toxicity thresholds calculated for other species and tests used in the Lagoon of Venice. Toxicity threshold was not evaluated for ELS mortality.

| Tests and species | MSD's 90$^{th}$ percentile | Threshold | *n* |
|---|---|---|---|
| *A. tonsa* larval development test (LDR) | 0.40 | 60 | 65 |
| Amphipod *C. orientale* survival (10-d test) | 0.10 | 90 | 135 |
| Amphipod *C. volutator* survival (10-d test) | 0.13 | 87 | 79 |
| Amphipod *C. volutator* survival (28-d test) | 0.13 | 87 | 24 |
| Sea urchin *P. lividus* sperm-cell test on elutriate | 0.08 | 92 | 212 |
| Sea urchin *P. lividus* embryotoxicity test on elutriate | 0.12 | 88 | 215 |
| Mussel *M. galloprovincialis* embryotoxicity test on elutriate | 0.11 | 89 | 142 |
| Oyster *C. gigas* embryotoxicity test on elutriate | 0.12 | 88 | 229 |