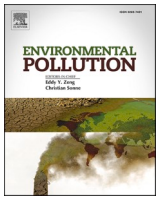
Environmental Pollution 342 (2024) 123095

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



A multibiomarker approach in clams (*Ruditapes philippinarum*) for a toxicological evaluation of dredged sediments☆

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ARTICLE INFO

Keywords:
Sediment toxicity
Lagoon of Venice
Clams
Biomarkers
Metals
Organic pollutants

ABSTRACT

The Lagoon of Venice is often dredged for channel maintenance. To avoid harmful consequences to the ecosystem, a proper disposal of bottom sediments requires a preliminary evaluation of its potential toxicity before excavation. Here we evaluated the effects of polluted sediments on clams (*Ruditapes philippinarum*) using a multibiomarker approach. Bivalves were exposed for 3 and 14 days to five sediment samples collected along a navigation canal between Venice historical centre and the industrial area of Porto Marghera. Immunological, antioxidant, detoxification, and neurotoxicity biomarkers were analysed in haemolymph, gill, and digestive gland. As a control, sediment collected far from pollution sources was used. Two experiments were performed to assess potential seasonal/gametogenic influence in clam sensitivity. A different response of clam biomarkers was observed during the two experiments and among sampling sites. Clams' digestive gland resulted to be the most sensitive tissue analysed showing significant differences among sites in all biomarkers analysed. Greater differences were present due to seasonality rather than exposure. The concentrations of metals and organic pollutants increased from the city centre to the industrial area, highlighting the influence that industrial activities had on the lagoon ecosystem. However, bioaccumulation in clams did not follow the same clear pattern, suggesting low bioavailability of compounds due to relatively high organic matter content. Biomarkers modulation was mainly driven by metals, both present in sediments and bioaccumulated. In comparison, effects of organic pollutants on the biomarkers tested were negligible. Other sources of contamination not investigated (e.g. pesticides) were suggested by neurotoxicity biomarkers alteration.

Abbreviations

As	Arsenic
Cd	Cadmium
Cr	Chromium
Cu	Copper
Hg	Mercury
Ni	Nickel
Pb	Lead
V	Vanadium
Zn	Zinc
PAHs	Polycyclic aromatic hydrocarbons
LightHs	Light hydrocarbons
HeavyHs	Heavy hydrocarbons
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
PCBs	Polychlorinated biphenyls

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☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

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https://doi.org/10.1016/j.envpol.2023.123095
Received 30 August 2023; Received in revised form 2 November 2023; Accepted 2 December 2023
Available online 7 December 2023

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OT	Organotins
HCB	Hexachlorobenzene
TEQ	Toxic Equivalent
DL-PCBs	Dioxin-like PCBs
WHO TEFs	World Health Organization; Toxic Equivalency Factors
SM1	Supplementary material 1 – Word document with descriptions of material & methods, and results
SM2	Supplementary material 2 – PDF file with detailed post hoc results per each biomarker.

1. Introduction

The Lagoon of Venice is a semi-enclosed Northern Italy basin in connection with the Adriatic Sea. Its sediments are composed of sand, silt, and clay from both alluvial and marine origin (Brambati et al., 2003). However, the morphology of the lagoon is changing over time, mostly due to anthropogenic alterations of the lagoon system linked to sediment movement (Molinaroli et al., 2009). Dredging is one of the main sources of sediment loss. Indeed, the extensive dredging activity carried out moves on average about 0.8–1 million m^3/year of sediments (Apitz et al., 2007; Madricardo et al., 2019; Sarretta et al., 2010). Various are the reasons for dredging, including navigation safety, opening of new canals, enlargement of ports, and most recently the construction of the MOSE (Experimental Electromechanical Module, projected to protect the city of Venice from flooding during extreme high tides) (Apitz et al., 2007; Madricardo et al., 2019; Sarretta et al., 2010; Teatini et al., 2017).

The reuse or disposal of dredged sediments may have an impact on the lagoon communities due to changes in sea-floor integrity, increased turbidity, loss of fine portion, enhancement of sediment-bound-contaminants exchange with water, and potential pollutants trophic transfers (Crocetti et al., 2022; Hoffman et al., 2003; Lammel et al., 2020; Madricardo et al., 2019; Pal and Hogland, 2022; Ruocco et al., 2020; Sturve et al., 2005; Teatini et al., 2017). In line with the regulation protocols (for Italy case, see Ministero delle Infrastrutture e dei Trasporti, 2023), various sediment toxicity tests have been implemented to assess the negative effects of contaminated sediments on the inhabiting biota (Burton and Scott, 1992; Hoffman et al., 2003). Different organisms can have different exposure modalities to contaminated sediments, depending on their burrowing behaviour, and feeding strategy.

Buried filter-feeders bivalves, such as the clam *Ruditapes philippinarum*, which lives in sandy-muddy bottoms of the Lagoon of Venice, are in direct contact with the sediments. *R. philippinarum* is widely recognized as suitable bioindicator species in biomonitoring studies and in ecotoxicological studies in controlled conditions (Boscolo et al., 2007; Cajaraville et al., 2000; Da Ros and Nesto, 2005; Fabrello et al., 2021a; Galloway and Depledge, 2001; Marisa et al., 2022; Sacchi et al., 2013; Volland et al., 2017).

The present study is part of a broad project designed to integrate several chemical and biological aspects in a Weight of Evidence approach for the environmental risk assessment of sediments prior to dredging. Regulation protocols to assess toxicity of dredged sediments usually focused on endpoints such as mortality, growth and fertilization impairment, or abnormalities (e.g. see Environment Canada, 2015; Toxics Cleanup Program, 2013; Ministero delle Infrastrutture e dei Trasporti, 2023), potentially overlooking sublethal effects at the biochemical and cellular levels. This work, in an explorative way, tries to include new analyses to attain a better evaluation for the purpose of decision making. Biochemical biomarkers, combined with chemical analysis, are powerful tools that can inform on the threat posed by contaminants even at low concentrations. Indeed, they have been widely used for environmental monitoring programs (e.g. see Matozzo et al., 2012b; Regoli et al., 2011; Sacchi et al., 2013; Sturve et al., 2005). A biomarker approach allows to define these effects at various levels of organization, from cellular to organism, while chemical analyses alone might not reflect the contaminant bioavailability and potential subsequent effects (Durou et al., 2007; Galloway et al., 2004; Sacchi et al., 2013).

The main aim of this study was to evaluate the potential toxicity of lagoon sediments prior to dredging by studying the biomarker response of clam *R. philippinarum* and if these effects may change with time (acute vs chronical exposure) and seasonality (animal resting phase vs early gametogenesis). To understand the impact of polluted sediments, clams were exposed in laboratory to sediments collected in 5 locations along a canal in the Venice Lagoon (Italy) subject to future dredging and characterized by different contamination levels. Sediment collected in a sixth site of a canal far from pollution sources was selected as a reference. A suite of biomarkers, as well as bioaccumulation, were measured in clams. Cellular and biochemical biomarkers chosen and described below have been used in previous studies to assess the effect of metals, organic pollutants, as well as polluted sediments (Fabrello et al., 2021b; Maria et al., 2009; Marisa et al., 2022; Matozzo et al., 2012b; Moschino et al., 2012; Stara et al., 2020, 2021; Wang et al., 2012; Won et al., 2016). Trace elements and organic pollutants were measured in sediment samples. It has also been suggested that research must explore the impact of season on the dynamic interaction between environmental stressors and the invertebrate biological response to fully understand and forecast how environmental stress will impact population dynamics (Ellis et al., 2011). Consequently, the experiments were performed twice exposing animals in late autumn (resting phase, Meneghetti et al., 2004) and early spring (at the beginning of gametogenesis, Meneghetti et al., 2004) to assess possible differences in biomarker responses due to clam reproductive cycle. The results provided herein will be integrated with other data in a Weight of Evidence approach with the purpose of achieving a higher level of sediment characterization before dredging activities take place.

2. Materials and methods

2.1. Study area and sediment sampling

Sediment samples from six sites of the Lagoon of Venice were collected in November 2020 and March 2021 (Fig. 1). Five sampling stations (I–V) were in the Canale Vittorio Emanuele III, a waterway that connects the city of Venice to the industrial area of Porto Marghera. The industrial area has been home of thermo-electrical powerplants, metallurgical, mechatronic, and chemical-producer companies, as well as a large commercial port. Site I was the closest to the city of Venice, whereas site V was the closest to the industrial area of Porto Marghera on the mainland. These sites are characterized by high anthropogenic influence, mainly due to urban input from the city of Venice and boat traffic (Site I-II), and industrial activities (Site IV-V).

Site VI, chosen as the reference site, was far from the other sampling stations as it was in the San Felice canal in the northern basin of the lagoon. This site is characterized by low anthropogenic influence, classified as a high-quality site due to the fast turnover of the water, and was used also in the past as reference site (Ghirardini et al., 2003; Losso et al., 2004; Melaku Canu et al., 2001).

Four samples of sediments were collected at each sampling site, within a circle of 1 m diameter, from a pontoon boat using a vibracore system. Each core tube had a diameter of 8.55 cm and a length of 1 m. After arriving in the laboratory, the 4 core samples from each site were extruded from the tubes, thoroughly mixed, and then stored in sealed containers at −80 °C for one week before experiments.

2.2. Sediment characterization

Sediment grain size was determined by laser diffraction analysis (Mastersizer 3000, Malvern Instruments, UK). The data are expressed as

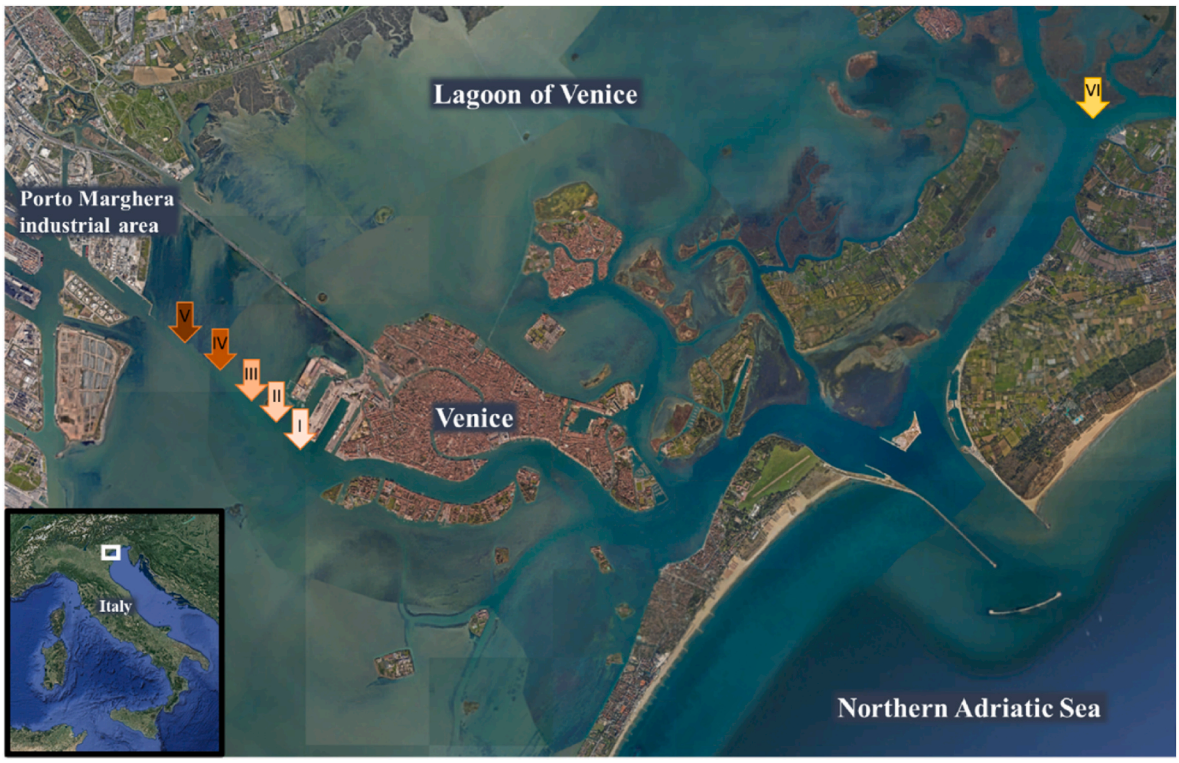

Fig. 1. Map showing the location of the sampling stations I-VI in the Lagoon of Venice.

volume percentages in three size classes, clay (d < 3.9 μm), silt (3.9 μm < d < 63 μm), and sand (d > 63 μm), according to the Udden-Wentworth scale.

Organic matter content was determined in triplicates by titration, following the method of Gaudette et al. (1974). The analysis was performed with sediments sampled in November 2020 only.

Chemical characterization of the sediments samples was performed by determining the total concentrations of trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, V, Zn) and of organic pollutants (PAHs, PCDDs, PCDFs, PCBs, HCB, light and heavy hydrocarbons, and organotin compounds). TEQ values for PCDD/Fs and DL-PCBs were calculated based on the 2005 WHO TEFs. The details about the analytical procedures used for sediment characterization are reported in the Supplementary material (SM1).

2.3. Exposure of clams to sediments

Specimens of the clam *R. philippinarum* were obtained from an aquaculture facility located in the southern basin of the Lagoon of Venice. Clams were exposed to sediment samples in two different experiments performed in late autumn 2020 and early spring 2021, during two different phases of the gametogenic cycle of clams, namely the resting phase (late autumn: 1st experiment, Exp 1) and early gametogenesis (early spring: 2nd experiment, Exp 2).

Before the exposure, clams were measured (mean size ± standard deviation: 1st experiment 39.0 ± 4.4 mm, 2nd experiment: 36.2 ± 3.9 mm) and acclimatized to the laboratory conditions for 10 days in large aquaria (70 L), with a 10–15 cm sandy bottom (collected in a site far from human activities and washed several times before being used) and aerated natural seawater (salinity of 35 ± 0.5 and temperature of 10 ± 0.5 °C (Exp 1) or 12 ± 0.5 °C (Exp 2). Water was renewed twice a week. Animals were fed with a microalgae mixture (*Isochrysis galbana*, *Phaeodactylum tricornutum, Tetraselmis chui*) by addition of laboratory cultures in tanks after the water change (target final concentration of 130000 cells/L). Acclimation and exposures were carried out at the Department of Biology of the University of Padova. Natural seawater pumped from the Lagoon of Venice at high tide and stored in the laboratory in dark was used.

For sediment toxicity test, sediment samples were placed in 28x50x30 cm (WxLxH) glass tanks. In both experiments, 2 replicate tanks were prepared for each sampling site. The sediment samples previously frozen at −80 °C were thawed and mixed thoroughly before being added into the tanks (5-cm layer) that were then filled up with seawater. Sediment:water ratio was about 1:6 (5 L of sediment and 30 L of water per tank). Each tank was provided with an aerator to properly oxygenate seawater and covered with a Plexiglas slab to reduce evaporation. One day after the sediments and water were added to the tanks, clams were placed on the sediment, ensuring animals of similar size in each tank. In both experiments, 58 animals for each sediment sample were added to every tank. Clams were exposed to sediments for 3 (acute response) and 14 days (chronic response).

Every 48 h the seawater in the experimental tanks was changed by renewing 15 L of water. Animals were fed every 48 h, after water renewal, by adding 200 mL of a microalgae mixture as described above. Seawater physico-chemical parameters were measured daily. Results are shown in Table S9 (SM1). Seawater temperature was determined with a thermometer with a precision of 0.1 °C. Density was determined using densimeters and Knudsen tables were used for the calculation of salinity, whereas pH was measured with a benchtop pH-meter (CRISON, mod. GLP 22). Dissolved oxygen (mg/L) was measured through the Fiber-Optic Oxygen Meter Piccolo2 (Pyro Science GmbH, Aachen, Germany) in glass vials provided with an oxygen sensor spot (OXSP5, Pyro Science GmbH). To analyse water quality, samples were collected before each water renewal during the exposure to measure the ammonia (NH_3), nitrite (NO_2^-), and orthophosphate (PO_4^-) content. To measure sulphides content in the sediments, a sample of water was collected after each water renewal. The details about these analytical procedures are provided in the SM1.

2.4. Clam tissue collection for biomarker analysis

After 3 and 14 days of exposure, 20 clams per sediment sample (10 from each replicate tank), were used for tissue collection in clams. Clams were pooled into 5 groups of 4 animals each. Haemolymph was collected from the anterior adductor muscle of each clam by a 1-mL plastic syringe and stored in Eppendorf tubes at 4 °C. A volume of pooled haemolymph was immediately used to measure total haemocyte count (THC), haemocyte diameter and volume, haemocyte proliferation and lactate dehydrogenase (LDH) activity. The remaining part of pooled haemolymph was then centrifuged at 780×*g* for 10 min, the supernatant was discharged, while the pellets (haemocytes) were resuspended in distilled water to obtain haemocyte lysate (HL) samples. HL samples were then collected, frozen in liquid nitrogen and stored at −80 °C until analyses (lysozyme, acid phosphatase and alkaline phosphatase activities and nitric oxide content assays). Gills and digestive gland from clams were then excised, pooled to obtain 5 different pools of 4 animals each, divided in aliquots, frozen in liquid nitrogen, and stored at −80 °C until analyses.

2.5. Haemolymph biomarkers

THC, as well as haemocyte diameter and volume, were determined using a Scepter™ 2.0 Automated Cell Counter (Millipore, FL, USA). Haemocyte proliferation and cytotoxicity were evaluated using the commercial kits, as described in a previous study (Matozzo et al., 2012a). For the cell proliferation endpoint, the *Cell proliferation* Kit II (Roche) was used, normalising the results to THC values of each experimental group and expressed as optical density (OD) at 450 nm. For the cytotoxicity endpoint, the *Cytotoxicity Detection* Kit (Roche) was used to measure LDH in cell-free haemolymph. The results were expressed as OD at 490 nm.

The lysozyme activity (LYS) was measured in HL from pooled haemolymph (500 μL) following the method described in Fabrello et al. (2021a), expressing the results as μg lysozyme/mg of protein. Nitric oxide (NO) content was measured in haemocytes according to the in-house Griess method proposed by Yucel et al. (2012), expressing the results as nM of NO/mg protein. The acid and alkaline phosphatase assay have been performed according to the method of Mazorra et al. (2002) and the results were expressed as units (U) of enzyme/mg protein.

For all analysis, protein concentrations were quantified according to Bradford (1976). Details for each analysis are provided in the SM1.

2.6. Gill and digestive gland biomarkers

Before analyses, gill and digestive gland samples were homogenized in homogenization buffer (TRIS-HCl 10 mM, pH 7.6, KCl 0.15 M, Sucrose 0.5 M, with protease inhibitors) using a homogenizer (TissueLyser LT, Qiagen) for 5 min at 50 Hz oscillation frequency. After 30 min of centrifugation at 12000*g* and 4 °C, the supernatant (SN) was used for analyses.

Total superoxide dismutase (SOD) activity was measured following the method proposed by Crapo et al. (1978). Enzyme activity was expressed as U SOD/mg protein.

Catalase (CAT) activity was measured following the method proposed by Aebi (1984). Results were expressed as U CAT/mg protein.

Glutathione reductase (GR) activity was evaluated according to Smith et al. (1988). The enzyme activity was expressed as U GR/mg protein.

The method of Ellman et al. was used to measure acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities (Ellman et al., 1961). The results were expressed as nmol thiocholine/min/mg of protein.

Glutathione S-transferase (GST) activity was measured according to the method described in Habig et al. (1974). GST activity was expressed as nmol/min/mg protein.

The glyoxalase type 1 (GLYOX) activity was measured according to the method proposed by Principato et al. (1983). The results were expressed in μmol/min/mg protein.

Oxidative damage was measured through the protein carbonyl content (PCC) assay. PCC was measured using the method of Mecocci et al. (1999). Results were expressed as nmol carbonyl group/mg protein.

The protein concentration in SN samples was quantified according to Bradford (1976). In gills, SOD, CAT, GR, AChE, BChE activities and PC content were measured. In digestive gland, SOD, CAT, GR, GST, and GLYOX activities and PC content were measured. Details for each analysis are provided in the SM1.

2.7. Bioaccumulation of trace elements and organic pollutants in clams

To evaluate the extent of bioaccumulation of the different pollutants, clam soft tissues (whole body from 10 clams per sediment sample) were excised immediately before exposure (T0) and after 14 days of exposure (T14). Samples were then frozen, lyophilized, homogenized, and stored until analysis.

Metal concentrations in clams were determined via wet acid digestion with HNO_3 and H_2O_2 and analysis by ICP-MS, while the analysis of organic pollutants was carried out according to the methods reported in Table S2 (SM1). The details about the analytical procedures used are reported in the SM1.

2.8. Statistical analysis

Statistical differences in the biomarker responses have been investigated by a three-way analysis of variance (ANOVA) to include the effect of the factors 'site' (6 levels), 'exposure duration' (2 levels: acute T3 and chronic T14), and 'seasonality' (2 levels: resting phase Exp1 and early gametogenesis Exp2). Before the analysis, the data were tested for normality and homoscedasticity with the Shapiro-Wilk test and the Breusch-Pagan test, respectively. Not normal data were log-transformed. Pair-wise comparisons of the biomarker responses among times and sites were performed using the Tukey HSD test. The differences in biomarker responses between sediment sampling time, sites and day of exposure were visualized with a principal component analysis (PCA). Since chemical results of sediments and bioaccumulation were not available for T3, an additional PCA was performed on the whole T14 dataset that included results of biomarkers, water quality, chemical analysis of the sediments and bioaccumulation data. To test whether differences among clusters visualized with PCA were significant, a non-parametric permutational multivariate analysis of variance (PERMANOVA, with 9999 permutations) was performed, applying the Euclidean distance matrix on the raw data. The significant threshold was set at $p < 0.05$. The analyses and graphics were done using RStudio software (version 4.1.2, R Core Team, 2023).

3. Results and discussion

3.1. Clams and seawater parameters

No clams died throughout the two experiments. The water quality parameters analysed (Table S3, Fig. S1, in SM1) every 48-h pre-renewal presented differences based on the seasonality in the nutrient content of seawater from exposure tanks. In the first experiment, there is a gradual decrease in the concentrations of nitrite, phosphate and ammonia over time, while an increase of sulphides was observed (Fig. S1). The exposure to sediment V showed the highest concentrations of nitrite, phosphate, and ammonia in seawater.

In the second experiment, seawater nitrite concentrations gradually increased during the exposure, while phosphate and ammonia decreased. Sulphide content showed high variability along the second experiment. In general, results showed that higher values of nitrite,

phosphate, ammonia, and sulphides were present in the second experiment compared to the first one. This could reflect a seasonal-based natural variation of the nutrients' content in seawater. A differential contribution of clam metabolism among sites may also be present. Seawater parameters analysed in control tanks containing sediment VI showed intermediate conditions concerning the concentration of nitrites, phosphates and sulphides, while it showed the lowest and most constant concentrations of ammonia (below the guidelines for this type of experiment (Nordin and Pommen, 2009).

3.2. Sediment characterization

The grain size and organic matter content of the sediments collected in the two sampling campaigns are reported in Table SM1-S4. The five sites of the Canale Vittorio Emanuele III showed a quite similar composition, with a predominance of fine particles with a diameter below 63 μm (silt + clay), in the range 84.3–92.3%, except for site II (71.8%), which had a higher sand content (28.2%). On the other hand, the sediments from the reference site (site VI) was mostly sandy (average 90.8%) with a 5.1–13.7 times lower organic content (average 0.24%). The differences in sediment characteristics between site I–V and site VI are to be found in the different origin of the sediments. Site I–V have a more alluvial source from the inland, while site VI is closer to the sea hence the higher percentage of sand. Organic matter was analysed only in the first sampling campaign, however, since the grain size analysis yielded comparable results in the two sampling campaigns for all sampling sites, we anticipate a similar organic matter content between the two sampling campaigns.

Total concentrations of trace elements and organic pollutants measured in sediments collected in the two campaigns are reported in the SM (Table SM1-S4). Different concentration trends are observed according to the element under exam. Zn, Cd, Pb, Hg, and Cu exhibited a similar trend, with increasing concentrations from the site I to site V, that is, from the town of Venice to Porto Marghera, while the reference site VI showed relevantly low concentrations. This suggests that the considerable enrichment in sediments originates mainly from the industrial area. Other sources, such as the urban area of Venice and the drainage basin, are not to be excluded. For Hg and Cd, the industrial origin seems prevalent since concentrations at site I are sensibly lower and like those measured at reference site.

Conversely, the concentrations of Ni, Cr, and V, which may have mostly a lithogenic origin, do not vary significantly along the Canale Vittorio Emanuele III but are anyhow at the lowest in site VI. The total concentrations of trace elements were higher in sediment cores from the second campaign, except for Hg. Total concentration, mobility and bioaccessible fractions (geospeciation) of trace elements may be affected by temporal variations in anthropogenic activities, seasonal fluctuations of the drainage basin, and changes in water parameters (e.g. temperature, dissolved oxygen, organic matter content) (Bancon-Montigny et al., 2019; Corami et al., 2020; Khaled-Khodja et al., 2018; Najamuddin et al., 2016; Tham et al., 2019).

Organic pollutants were detected in all sites (Table SM1-S5). However, the concentrations of PCDD/Fs, PCBs, dioxin-like PCBs, PAHs, and hydrocarbons at the reference site were extremely low or under the method detection limit (MDL). PCDD/Fs, PCBs, and dioxin-like PCBs showed increasing trends going from Venice to Porto Marghera, with slightly higher values at some sites in the second campaign (Table SM1-S4).

PAHs' trend in the two campaigns were not univocal (Table SM1-S5). This suggests that additional sources, besides the industrial area, may contribute to the presence of these pollutants in the Canale Vittorio Emanuele III sediments, such as the city harbor of Venice, located close to site II. The presence of this class of organic pollutants in environment is mainly due to incomplete combustion of petroleum products (Mojiri et al., 2019). As recently summarised by Mojiri et al. (2019), sources are multiple, either domestic (burning oil, gas, cigarette, etc), or industrial (transports, waste products), or agricultural (pesticides formulation, burning of plant material), making the overall picture of PAHs presence in lagoon sediments quite complicate.

Light hydrocarbons did not show differences among sites I–V. In contrast, heavy hydrocarbons exhibited maximum concentrations at the sites IV and V, i.e. closer to the industrial area, which is likely to represent a significant source of these pollutants. Lastly, HCB and organotins (only TBT) were present only in a few sediment samples in the first campaign and were below MDL in all the other samples (1st and 2nd sampling campaigns).

Overall, contamination levels for site VI were very low compared to the those in Canale Vittorio Emanuele III, confirming this site as suitable for reference.

3.3. Bioaccumulation in clams

The results concerning bioaccumulation of metals and organic pollutants in clams are reported in the SM1 (Table S6). The discussion refers to values in Table S6 where a cutoff value of 20% is applied to discriminate a decrease or increase at the end of 14-day exposure in comparison to the T0 concentrations.

Regarding metals, in the first experiment, the concentration of most trace elements in the organisms showed no changes or a decrease at the end of experiment, excluding Ni and Zn in animals exposed to sediments from site V, and Cd to sediments of site VI. Exception is represented by Pb, whose concentrations remarkably increased (≥50%) in animals exposed to sediments from most sites (I, II, III, and V).

Like the first experiment, in most cases of the second experiment the concentrations of trace elements showed no changes or a decrease after 14 days of exposure and Pb concentrations presented the highest increase in most sites (II, III, IV, and V). Animals exposed to sediments from sites II and IV also reported an increase (≥50%) of Cr and V concentrations.

Despite the different initial concentrations in clams between experiments, Cu and As fell generally within the 20% variation from the T0 concentrations. Limited variation from T0 concentrations is also shown for Zn. Micronutrients such as Cu and Zn have been shown to be regulated in various aquatic invertebrates (Rainbow, 2002). Differences observed in the concentrations of most of the trace elements at the start of the two experiments might be related to the diverse phase of the gametogenic cycle of the organisms when they were sampled. This would explain the different concentration at the beginning of the second experiment, compared to the first one.

During the exposure, clams may encounter favourable conditions (lack of predation and competition) that potentially enhance the activation of detoxification biomarkers, which play a crucial role in regulating bioaccumulation levels. However, it is somewhat challenging to compare total concentrations in sediments and bioaccumulation, confirming the long-known difficulty to relate metal(loid) bioaccumulation to environmental concentrations, which is an even more complex task for exposure under laboratory conditions (Luoma and Rainbow, 2005; Moschino et al., 2012). Besides, the bioaccessibility, hence the bioavailability, and the mobility of trace elements in sediments may significantly affect their uptake by biota (Corami et al., 2020).

In a recent paper, it has been highlighted that specific molecular pathways have been activated in clams exposed to polluted sediments which could trigger defence mechanism ultimately reducing metal accumulation. Indeed, ABC transporter pathway which are involved in detoxification processes showed upregulation during the 14 days exposure (Bernardini et al., 2023).

For the organic pollutants, the PCDD/Fs and DL-PCBs results indicate an increasing bioaccumulation trend in clams exposed to sediments from site I to site V, reflecting partly the increasing levels of sediment contamination. Non-dioxin-like PCBs showed a similar bioaccumulation behaviour, though with slightly less pronounced differences among sites. For PAHs, the first experiment provided similar results for all

sediments collected in the Canale Vittorio Emanuele III, while in the second experiment higher concentrations were observed in clams exposed to sites II and V. Similarly to what was observed in sediments, concentrations in clams did not show a clear spatial or temporal trend. TBT, HCB and hydrocarbons were always undetectable in clam soft tissues.

Clams exposed to sediments from the reference site VI showed lower bioaccumulation levels of organic pollutants compared to site II-III-IV-V. However, interestingly, even if the sediments from Canale Vittorio Emanuele III had higher levels of organic pollutants compared to the reference site, site I bioaccumulation levels were like those observed in animals exposed to sediments from site VI. The difference in organic matter in sediments would likely play a role in the metal and organic compounds bioaccumulation results. Indeed, the relationship between organic matter presence and bioavailability has been investigated deeply for both metals and organic compounds (Akkanen and Kukkonen, 2003; Haitzer et al., 1998; Hamoutene et al., 2018; Maranger and Pullin, 2003; Reijonen et al., 2016). For example, it was tested that a 6-times difference in organic matter reduced the bioavailability of V in soils (Reijonen et al., 2016). As reported in Table S3 (SM1), sites I to V had 5 to 13.7 times more organic matter than the control site VI. Hence, the organic matter could have a buffer effect mediating the bioavailability of contaminants at sites I–V. Sediment pH and redox potential are other parameters that can influence metals bioavailability; however, they were not included in this study (Chapman et al., 1999; Simpson et al., 2012).

3.4. Haemolymph biomarkers

Immunomarkers are crucial for understanding and monitoring the health status of marine invertebrates, aiding in the assessment of animal immunosurveillance and overall well-being. They can provide valuable insights into the impact of stressors on organism immunocompetence in laboratory exposure (Ayhan et al., 2021; Balbi et al., 2021; Cotou et al., 2013; Volland et al., 2017) and can be used as sentinels for xenobiotic presence in biomonitoring programs (Cajaraville et al., 2000; Galloway and Depledge, 2001).

In the present study, all haemocyte biomarkers showed statistically significant differences between the two experiments (Fig. 2, Table SM1-S7), suggesting seasonally differences. In this study, THC was slightly higher in the first experiment, mainly in clams exposed to sediments I and VI, while cell diameter and volume were lower. Cytotoxicity (LDH assay) and haemocyte proliferation (XTT assay) were lower in the first experiment compared to the second one, whereas higher NO content was detected in the haemocytes from clams used in the first experiment.

THC, cytotoxicity, haemocyte proliferation, acid and alkaline phosphatase activity and NO content were significantly different among sites, but a clear pattern of variation was not revealed. Indeed, the interactions between the factors experiment:site, experiment:day and site:day were often statistically significant, complicating the general evaluation of the results (Table SM1-S7).

At T14 of the second experiment, THC values of clams exposed to sediment IV were significantly lower, when compared to all other sites (SM2). Moreover, haemocyte proliferation was significantly higher compared to that of bivalves exposed to sediment VI (SM2).

In the first experiment, the activity of acid phosphatase increased over time (from T3 to T14) in clams exposed to sediments I and II, whereas it remained stable for the other sediments. In the second experiment, no significant variations were detected between T3 and T14, and sediment III showed the lowest values.

At T3 and T14 of the first experiment, haemocytes of clams exposed to sediment I showed significantly higher NO levels when compared to those of clams exposed to remaining sediments (SM2).

The post-hoc test revealed that values of cytotoxicity and acid phosphatase measured in clams exposed to sediment VI varied significantly between the two experiments (SM2). As for the other biomarkers

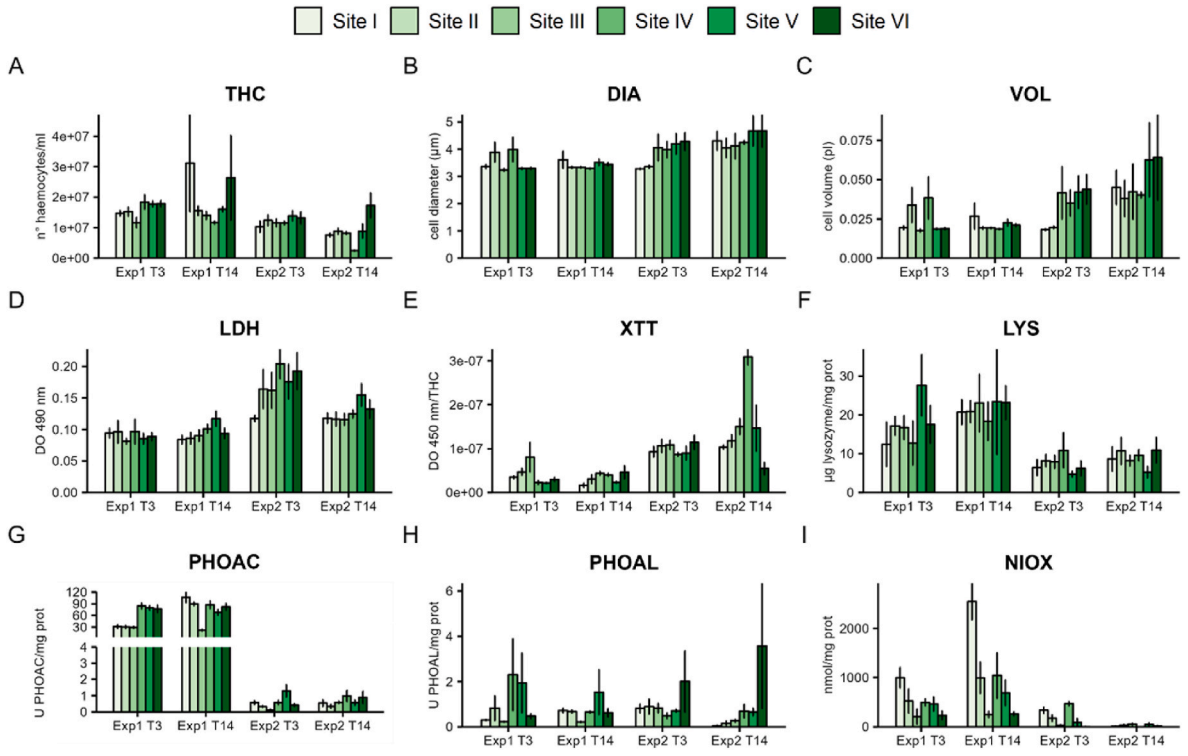

Fig. 2. Results of cell biomarkers measured in haemocytes/haemolymph of clams (mean ± standard error) exposed for 3 (T3) and 14 (T14) days to sediments from the different sites (I-VI) during clams' resting phase (Exp 1) and early gametogenesis (Exp 2). Total haemocyte count (THC); haemocyte diameter (DIA); haemocyte volume (VOL); cytotoxicity (LDH assay); cell proliferation (XTT assay); lysozyme activity (LYS); acid phosphatase activity (PHOAC); alkaline phosphatase activity (PHOAL); nitric oxide content (NIOX). Results of the three-way ANOVA analysis are reported in Table SM1-S7.

analysed, differences between the two experiments or days of exposure (T3 and T 14) were not significantly different in clams exposed to sediment VI. Exposure to other sediments caused variations in haemocyte biomarkers (SM2). For example, the activity of acid phosphatase was significantly higher after 14 days of exposure when compared to 3 days of exposure in clams maintained in sediments I and II.

Bivalves highlight high sensitivity to both legacy and emerging contaminants, and the haemolymph is one of the first barrier that organisms have against pollutants (Fabrello et al., 2021a; Iori et al., 2020; Marisa et al., 2022; Mazorra et al., 2002). Having an open circulatory system, haemocytes are free to move between tissues and haemolymph and vice versa (Cima and Matozzo, 2018; Pipe and Coles, 1995). In the second experiment of the present work, lower THC values were correlated to higher levels of cytotoxicity (LDH), however, they were also correlated to higher levels of cell proliferation (XTT). Overall, such results suggested that haemocytes moved to other tissues of animals but were affected by the exposure to polluted sediments. Consequently, new cells were produced to counteract the loss of cells from the haemolymph (Cima and Matozzo, 2018; Matozzo et al., 2012a; Pipe and Coles, 1995).

3.5. Gills biomarkers

A possible consequence of exposure of aquatic organisms to contaminants is oxidative stress, generally due to an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences. To evaluate the capability of sediments to cause oxidative stress and damage, and to induce detoxification enzymes, several biomarkers were evaluated in this survey. Biomarkers of oxidative stress are reliable indicators of the stress caused by various pollutants in aquatic ecosystems, even though not specific (Canesi et al., 2012; Hagger et al., 2006; van der Oost et al., 2003). For instance, metals can induce or inhibit antioxidant enzyme activities. It has been demonstrated that Pb (10–100 μg/L) could cause an increase of SOD gene expression in bivalve gills (Xie et al., 2018), but it suppressed SOD activity (Aouini et al., 2018). Differences between results obtained from gene expression and biochemical analyses are common (Regoli and Giuliani, 2014). Therefore, a multi-biomarker approach followed by multivariate analyses is a suitable methodology, as it can catch the effects of various pollutants and overall evaluate environmental quality and health status of marine and freshwater organisms (Aouini et al., 2018; Kim et al., 2014; Marisa et al., 2022; Matozzo et al., 2012b). In the present study, antioxidant defences and neurotoxicity biomarkers were induced in the second experiment compared to the first one, whereas oxidative damage was higher in the first experiment.

SOD activity was the only biomarker analysed in the gill that did not differ between experiments (Fig. 3, Table SM1-S8). CAT was significantly higher in the trials carried out in March 2021, whereas PCC values were significantly lower in the second experiment when compared to the first one (Fig. 3, Table SM1-S8). The post hoc test revealed significant differences in CAT activities of clams during the second experiment within T3 and T14, even if with an opposite trend. While the CAT activity of clams exposed to sediments I, II, and III increased, that of clams exposed to sediments IV, V, and VI significantly diminished (SM2).

GR is an important enzyme that plays a key role in antioxidant defences, converting oxidized glutathione to reduced glutathione. In this study, GR activity was significantly different between the two experiments, but a lot of significant variability was also found within each experiment (Table SM1-S8). The highest GR activity was recorded at T3 in clams exposed to sediment II during the first experiment, which was significantly higher than those found in clams exposed to sediments IV, V, and VI (SM2). In the second experiment, at T14, the GR activity was significantly higher in clams exposed to sediments IV, V, and VI compared to the others (SM2).

In the first experiment of our study, at T3, AChE in gills of clams exposed to sediments I–V showed values like those from the reference site VI, while a decrease of activity was generally recorded at T14 (except for site II). On the other hand, BChE showed an opposite pattern of variation, with lower vales at T3 compared to T14. With regards to the second experiment, an induction of both AChE and BChE activities was recorded at T3 in clams exposed to all sediments, when compared to those exposed to sediment VI (Fig. 3). At T14, activity of the two enzymes recorded in clams exposed to sediments I–V were not statistically different compared to that of animals exposed to sediments of the reference site (Fig. 3; SM2).

Pesticides concentrations were not measured in the present study. Nevertheless, the observed alterations in AChE and BChE, particularly prominent at site 1 during the second experiment, provide evidence of

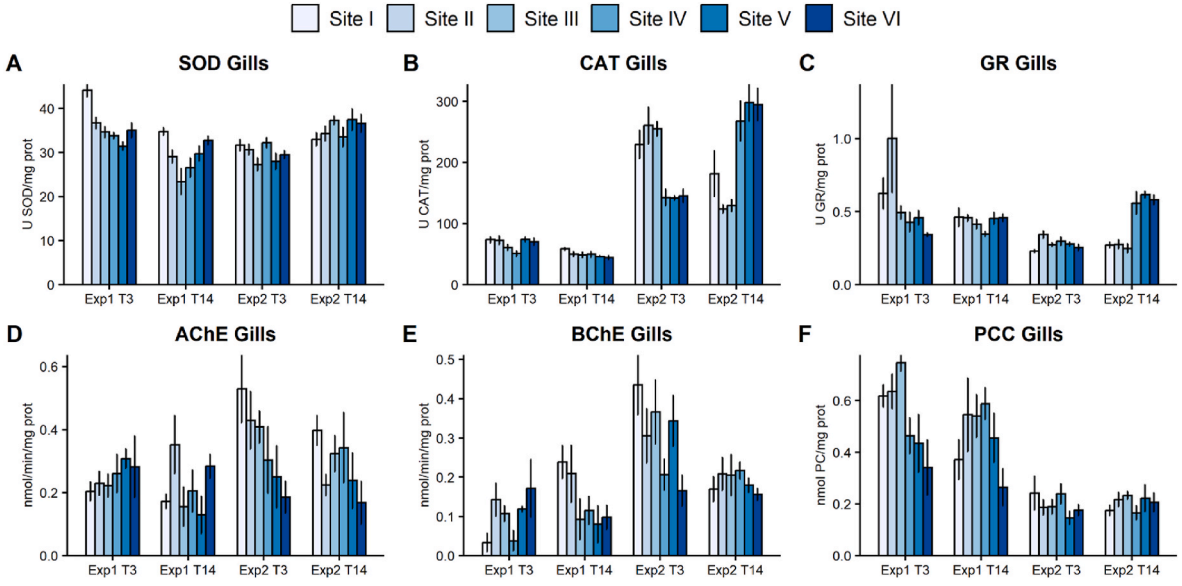

Fig. 3. Results of gill biomarkers measured in clams (mean ± standard error) exposed for 3 (T3) and 14 (T14) days to sediments from the different sites (I-VI) during clams' resting phase (Exp 1) and early gametogenesis (Exp 2). Superoxide dismutase (SOD) activity; catalase (CAT) activity; glutathione reductase (GR) activity; acetylcholinesterase (AChE) activity; butyrylcholinesterase (BChE) activity; protein carbonyl content (PCC). Results of the three-way ANOVA analysis are reported in Table SM1-S8.

the potential presence of neurotoxic compounds or pesticides within the sediments of the Venice lagoon. This inference aligns with previous studies that have demonstrated the occurrence of such substances in this environment (Cappello et al., 2015; Lomartire et al., 2021; Matozzo et al., 2005). While the observed effects are indicative, it is important to acknowledge that attributing a single definitive source within the complexity of the lagoon is challenging.

The post hoc test highlighted differences within sediment VI between T3 and T14, as well as between experiments concerning the activity of CAT and GR. In both cases, enzyme activity was significantly higher in the second experiment after 14 days of exposures (SM2). This reflects the seasonal variability often detected in biomarkers analysed in bivalves (Chahouri et al., 2022; Leiniö and Lehtonen, 2005; Silva et al., 2021).

3.6. Digestive gland biomarkers

The oxidative stress-related enzymes showed a different pattern of variation between the two exposures (Fig. 4). SOD activity was significantly lower in the first experiment (Table SM1-S9), mainly at T14. In particular, its activity was significantly lower in clams exposed to sediment IV compared to sediments VI and I (SM2).

CAT activity was significantly lower in the first experiment (Table SM1-S98). However, within experiment, differences were also present between the sampling days (Table SM1-S9). CAT activity of clams exposed to sediments II and III increased over time, while the activity lowered over time in clams exposed to sediments IV, V and VI.

An opposite trend of variation was detected for GR activity and the oxidative damage (PCC levels) compared to that of CAT. Indeed, levels of the two biomarkers were significantly lower in the second experiment. Clams exposed for 14 days to sediment IV had the lowest GR activity, significantly lower than that of clams exposed to sediment I, II, and III (SM2).

The two detoxification enzymes showed an opposite pattern of variation, with higher GST levels in the first experiment and higher GLYOX levels in the second one. In this study, GST activity remained stable during the first experiment, while it lowered over time in the second one, especially in clams exposed to sediments IV, V, and VI, showing statistical differences compared to clams exposed to sediments I, II and III (SM2). GST activity seems more likely to be modulated by the presence of organic pollutants rather than metals (Fitzpatrick et al., 1997; Lomartire et al., 2021; Regoli and Principato, 1995), although activation of this detoxification enzyme has been linked to metals presence as well (Wang et al., 2012). Indeed, levels of PCDDs, PCBs, and heavy hydrocarbons were higher in the second campaign, where the GST activity was at the highest.

GLYOX activity increased over time between T3 and T14 in clams exposed to most sediments during the first experiment, except in clams exposed to control sediments, which was significantly higher than that of clams exposed to sediments I and II (SM2). On the other hand, GLYOX activity remained stable during the second experiment (Fig. 4).

Clams exposed for 3 days to sediment V during the first experiment showed the highest levels of protein oxidative damage (Fig. 4, PCC), with a statistically significant difference with those recorded in clams exposed to sediments I and III, which had among the lowest levels (SM2).

Like to the results obtained in gills, the post hoc test highlighted differences within sediment VI between T3 and T14, as well as between experiments (SM2), reflecting the importance of considering the season/gametogenic period in clams' exposure.

3.7. Multivariate analyses

The PERMANOVA and the PCA analyses confirmed the significant differences between the two experiments (Fig. 5). The PCA showed that the two experiments are clearly separated along the PC1 (33.74% of the variance explained). Along the PC2 (10.06%), the two sampling times (T3 and T14) were only slightly separated as many dots overlapped. It is noteworthy that the two sampling times (T3 and T14) are separated in an opposite way regarding the experiments (i.e., along the PC2) (Fig. 5). This means that the biomarker response pattern varied in a different way between days of exposure when comparing the two experiments. For example, the SOD activity in gills was lower at T14 compared to T3 in the first experiment but was higher at T14 compared to T3 in the second one. The overlapping of the areas is more pronounced in the data of the second experiments compared to the first one. This suggested a slightly broader difference in the values of the biomarkers investigated in clams during the first experiment, when compared to the second one. However, the PERMANOVA analysis did not reveal a significant effect neither of the factor "Day" nor of the interaction "Experiment:Day" (Fig. 5). The specific variation of the other biomarkers has been

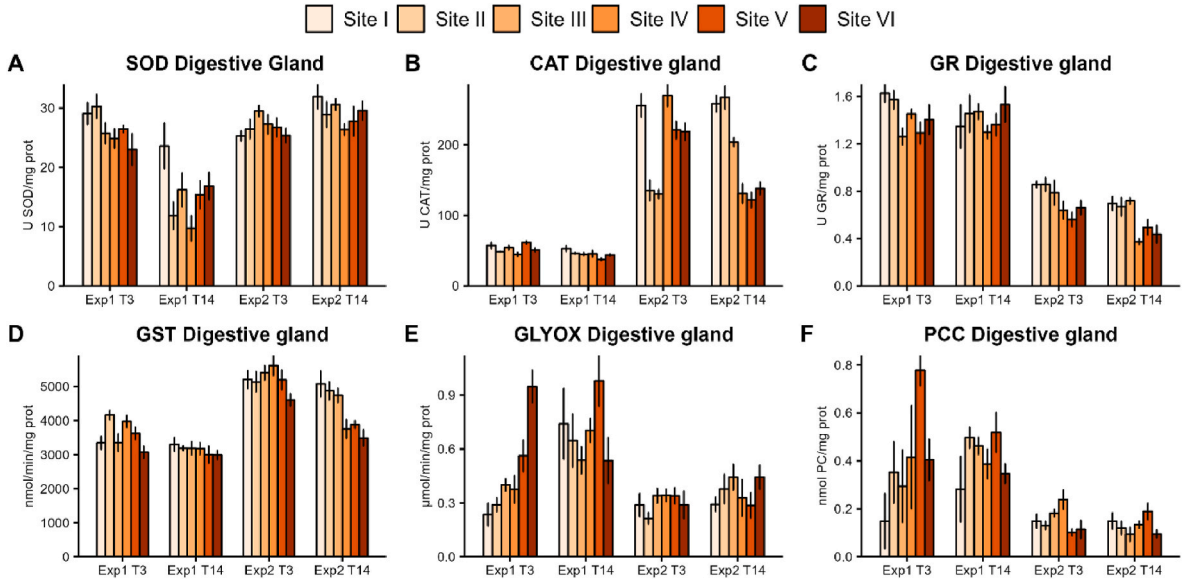

Fig. 4. Results of digestive gland biomarkers measured in clams (mean ± standard error) exposed for 3 (T3) and 14 (T14) days to sediments from the different sites (I-VI) during clams' resting phase (Exp 1) and early gametogenesis (Exp 2). SOD activity; CAT activity; GR activity; GST activity; glyoxalase type 1 (GLYOX) activity; protein carbonyl content (PCC). Results of the three-way ANOVA analysis are reported in Table SM1-S89.

Factors	Df	F value	R^2	p value
Experiment	1	13.987	0.104	< 0.001
Site	5	1.616	0.060	0.126
Day	1	0.001	0.000	0.978
Experiment:Site	5	0.698	0.026	0.701
Experiment:Day	1	2.982	0.022	0.083
Site:Day	5	1.547	0.058	0.145
Experiment:Site:Day	5	0.471	0.018	0.850
Residuals	96		0.713	

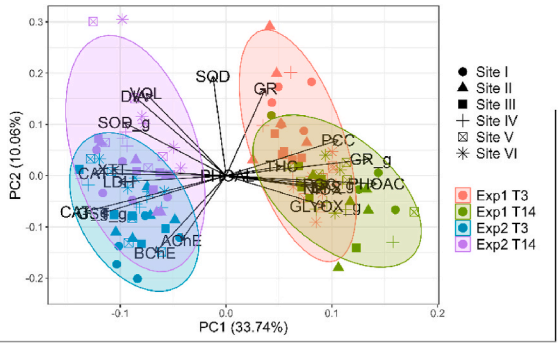

Fig. 5. PERMANOVA and PCA analyses performed on the whole dataset of biomarkers analysed in haemolymph, gills, and digestive gland of clams exposed for 3 (T3) and 14 (T14) during clams' resting phase (Exp 1) and early gametogenesis (Exp 2) to sediments from different sites (Sed I-VI). Results of the PERMANOVA are reported in the table next to the plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

described in the previous chapters. Notably, clams exposed to sediments during the first experiment had a higher protein damage (PCC) in both gills and digestive gland, suggesting that their antioxidant system was less able to protect proteins from ROS (reactive oxygen species).

However, as indicated above, the response of each biomarker was not univocal. In Fig. 5, among sites within each experiment, a separation between exposure day is only slightly visible and not clear. But, if chemical analysis of the sediments and bioaccumulation are added to the multivariate analysis (Fig. 6), a significant distinction among sites is revealed. Since the chemical data were not available for T3, the multivariate analyses in this case were performed only for T14.

As for the PCA in Fig. 5, also in Fig. 6 the two experiments form two clusters divided along the PC1 (31.16% of variance explained), mainly driven by biomarkers and metal bioaccumulation. Clams of experiment 1 had higher activity of immunological enzymes (LYS, NIOX, PHOAC), GLY, GR, and protein damage in gills and digestive gland, which seems related to the higher bioaccumulation of Ni, Pb, and Cr. On the other side, clams of the second experiments showed higher activities of most of the enzymes related to oxidative stress, neurotoxicity, and detoxification, as well as haemocytes sizes, viability, and proliferation ability. Even though those clams showed bioaccumulation of a larger variety of metals (Cu, As, Cd, Zn, and Hg), the antioxidant enzymes could be able to protect the potential damage posed by trace elements to the macromolecules. The bioaccessibility, hence the bioavailability, and the mobility of the trace elements should be considered to better comprehend whether the observed biological effects could be caused by metal (loid)s (Corami et al., 2020).

Along the PC2 (23.96% of variance explained), the separations among sites create a gradient with sediments IV and V on top part of the panel, followed by sediments III and II in the middle part and sediments I and VI in the lower part. This separation is due to compounds detected in the sediments (both metal and organic) and organic pollutants bioaccumulated in clams exposed to sediments for 14 days. Indeed, as described in the previous paragraphs, the site with the lowest concentration of investigated contaminants was site VI followed by the others with a gradient from the city centre to the industrial area (site I to site V). The PERMANOVA confirmed this separation, as the factor site is statistically significant. Also the interaction Experiment:Site turned out statistically significant (Fig. 6). Indeed, the position in the plot of sediments V and IV is slightly switched between the two experiments (Fig. 6). The same happened for the sediments III and II. However, the position remained close and the gradient of contamination from the sites closer to the city to those closer to the industrial area remained also in the second experiment. As it can be seen in Fig. 6, biomarkers had little influence on the differentiation among sites, whereas they have a great power in discern seasonality differences along the PC1 which best describe the variation in the data (31.13%).

To better understand the results reported above, a correlation

Factors	Df	F value	R^2	p value
Experiment	1	11.430	0.149	< 0.001
Site	5	1.848	0.120	0.020
Experiment:Site	5	1.657	0.108	0.039
Residuals	48		0.624	

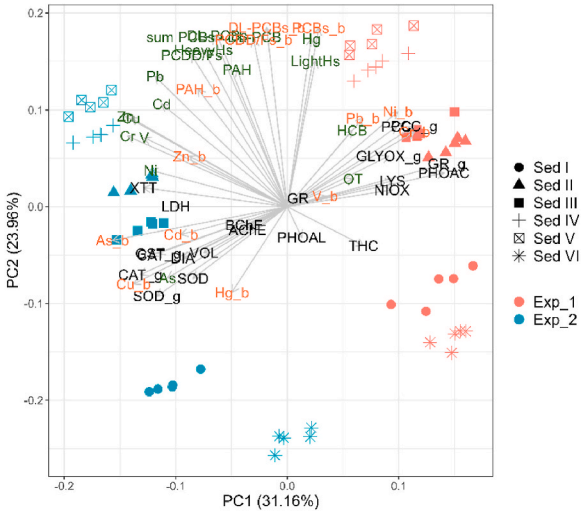

Fig. 6. PERMANOVA and PCA analyses performed on the whole dataset of chemicals analysed in sediments (green) and bioaccumulated by clams (orange), as well as biomarkers (black) analysed in haemolymph, gills, and digestive gland (highlighted with "_g") of clams exposed for 14 (T14) days during clams' resting phase (Exp_1) and early gametogenesis (Exp_2) to sediments from different sites (Sed I-VI). Results of the PERMANOVA are reported in the table next to the plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

analysis (function "cor.test" on RStudio) was performed on the dataset used for PCA and PERMANOVA putting in relationship each biomarker with chemical and bioaccumulation analysis and selecting the strong correlations (absolute Pearson's r coefficient >0.5) (Table S10 in SM1).

Among immunological parameters, no correlation with organic pollutants occurred and only two endpoints strongly correlated with metals analysed in sediment: XTT (positively correlated with V, Cr, Cu, Zn, Cd, and Pb) and acid phosphatase (negatively correlated with Cr, Cu, Zn, and As). Cell diameter, cytotoxicity, XTT and acid phosphatase further positively correlated with Cu, As, Zn, Cr, and Ni bioaccumulated in clam soft tissues, whereas acid phosphatase and NIOX negatively correlated with Cu, Zn, and As bioaccumulated. As summarised by Weng et al. (2022), metal toxicological effects on bivalves' immunological parameters can be expressed in many ways and, here, changes in size, number, cytotoxicity as well as enzymatic modulation in *R. philippinarum* were observed due to metals in sediments and bioaccumulated.

Antioxidant biomarker response (SOD, CAT) in the gills was negatively influenced by HCB in the sediments, and by Cr and Ni bioaccumulated, and it was positively influenced by Cu and As bioaccumulated in the clam tissues. Oxidative damage (PCC) was positively associated with HCB in the sediments, and negatively with Cu, As, and Cd bioaccumulated. Presence of HCB in the sediments lowered the activity of SOD, hence leading to higher damage to protein in the gills. On the other hand, Cu and As bioaccumulated increased the activity of SOD, which in turn lowered the damages, as highlighted by PCC values. Cu and Cr are redox-active metals that can directly create free radicals (Nuran Ercal et al., 2001), which could explain the enhanced response of SOD and CAT after exposure to these metals. HCB negative correlates with antioxidant capacity in gills and positively with oxidative damage (Table S10 in SM1). To the best of our knowledge, relationship between HCB presence in sediments and oxidative stress biomarkers in clams was not observed in other studies, even though its effect on vertebrates biochemical defences is known (Bebianno et al., 2015; Benedetti et al., 2022; Faria et al., 2010; Parolini et al., 2013; Turja et al., 2014; van der Oost et al., 2003).

In the digestive gland, the activity of SOD and CAT was negatively correlated with presence of Hg in the sediments, and with Cr, Ni, and PCBs bioaccumulated in the clams. SOD activity in this tissue was also positively correlated with presence of As in sediments and Cu, and As bioaccumulated, whereas CAT activity positively correlated with V, As, Cr, Ni, and Cu and Cu, As, and Hg bioaccumulated.

As in gills, Hg in the sediments lowered the activity of SOD, hence leading to higher damage to protein (PCC). Sediments with high As content enhanced SOD and CAT activities, lowering the PCC levels. Also, as in gills, high levels of Cu and As bioaccumulated increased SOD and CAT activities enhancing the protection of proteins from oxidative damage, whereas high levels of Cr, Ni and PCBs bioaccumulated lowered SOD and CAT protective action, exposing proteins to higher damages.

GR in digestive gland presented an alternate narrative compared to SOD and CAT as its activity positively correlated with Cr and Ni bioaccumulated, and negatively with V, Cr, Ni, Cu, Zn, and As in the sediments, and Cu and As bioaccumulated in the clam soft tissues. Differences in this antioxidant enzyme response compared to SOD and CAT might look confounding, however its action is different and is more related to other enzymes, such as glutathione peroxidase which was not analysed here (Martín-Díaz et al., 2007). Differently from this study, sediments with comparable concentrations of Cr induced the activity of GR in *R. philippinarum* (Martín-Díaz et al., 2007) whereas in the present study it was inhibited. Experiment duration, however, was sensibly different as the clams were exposed for 28 days in the Martín-Díaz et al. study.

SOD, CAT and PCC responses were consistent in the two tissues analysed. Other studies highlighted that a tissue specific response of antioxidant biomarkers to metals might occur (Bebianno et al., 2015; Cantú-Medellín et al., 2009). Copper analysed in clam digestive gland and muscle, for example, positively correlated with SOD (Cantú-Medellín et al., 2009), but GST correlated differently depending on the tissue analysed.

In the present study GST activity was analysed only in digestive gland and was positively correlated with V, Cr, Ni, Cu, and As in sediments, and with Cu, As, and Hg bioaccumulated in clams, but negatively with Cr bioaccumulated. Previous studies showed induction of GST activity in relation to presence of metals in polluted sediments (e.g. Cantú-Medellín et al., 2009; Martín-Díaz et al., 2007). As in Wang et al. (2012), the presence of As in sediments was positively related to GST activity in the present sediments. In the same paper however, significant correlations with bioaccumulated As, Cu, Hg and Cr were not highlighted.

GLYOX was negatively correlated to Cu and As bioaccumulated in the digestive gland. Literature about GLYOX in marine molluscs is scarce. Relationship between metals or organic pollutants and GLYOX activity was highlighted in other species, such as *Scapharca inaequivalvis* (Antognelli et al., 2006a, 2006b), *Mytilus galloprovincialis* (Regoli and Principato, 1995), *Chamaelea gallina* (Rodríguez-Ortega et al., 2002) and *Scrobicularia plana* (Romero-Ruiz et al., 2003). The present is the first study highlighting a relationship between this enzyme and metals bioaccumulation in *R. philippinarum*.

AChE and BChE did not significantly correlate with anything, further emphasizing that the differences in activity observed might have been caused by pesticides or other neurotoxic compound in sediments or water column that were not analysed in the present study. Interestingly, a biomarker can have an opposite correlation with a given compound if the latter is found in sediments or bioaccumulated. This happened with Cr and acid phosphatase (negatively correlated if in sediments, positively correlated if bioaccumulated), Cr and Ni with GR in the digestive gland (negatively correlated if in sediments, positively correlated if bioaccumulated), and Cr and GST both in the digestive gland (positively correlated if in sediments, negatively correlated if bioaccumulated).

It is important to notice that a similar pattern highlighted by the PCA in Fig. 6 is obtainable by doing the PCA analysis with only biomarker and bioaccumulation data (Fig. S2). In this case, both a very marked division between the two experiments and the trends in sites differences are present. All in all, even without chemistry, by coupling bioaccumulation and biomarker it is possible to distinguish between sites. If the sediment chemistry data are added (as in Fig. 6 of the paper), a further stretch along PC2 is given, thus further differentiating between sites. In conclusion, the mere quantification of compounds in the sediments must not be considered alone when doing risk assessment, as not all compounds present in the sediments might be bioavailable and influence organisms.

4. Conclusion

Intentional or accidental release or movement of hazardous sediments in the environment can pose serious risk to aquatic life. Our chemical analysis confirmed the role played by the industrial area as a major source of persistent pollutants in the lagoon sediments. Indeed, results suggested a potential for bioaccumulation of PCDD/Fs and DL-PCBs when clams are exposed to the most contaminated sediments, i.e., collected at the sites closer to the industrial area. Bioaccumulation was significantly lower in clams exposed to sediments from site I, close to Venice. Nonetheless, PAH bioaccumulation levels suggested the presence of more diffused sources and different patterns of bioaccumulation. The bioaccumulation patterns of metals in clams were variable in site and time, and due also to relatively high organic matter content influencing the compounds bioavailability, it was not possible to identify a relationship with the concentrations measured in sediments. Indeed, sediments form a complex matrix trapping various pollutants whose impact on the biota is challenging to anticipate or interpret. Employing a multi-biomarker approach becomes valuable in assessing the overall sublethal toxicity of sediments especially if considering

seasonal changes due to metabolic/gametogenic stage of animals. Here, we highlighted a strong effect of metal presence in sediments and bioaccumulation on all the biomarkers considered, whereas organic pollutants had little influence on the biomarker modulation. The digestive gland was the tissue most impacted, showing more significant differences among sites. However, overall, more difference in biomarker response was highlighted between the two experiments (clams' resting phase and early gametogenesis) rather than among sampling sites. In conclusion, results suggest that the sole measurement of pollutants concentration to evaluate their possible threat to aquatic organisms is not always sufficient, since it may not provide a proper estimate of their bioavailability and effects.

Funding

Scientific activity performed in the Research Programme Venezia 2021, with the contribution of the Provveditorato for the Public Works of Veneto, Trentino Alto Adige and Friuli Venezia Giulia, provided through the concessionary of State Consorzio Venezia Nuova and coordinated by CORILA.

Author statement

All the authors: Conceptualization, Methodology, Software. Davide Asnicar: Data curation. Davide Asnicar, Jacopo Fabrello, Maria Ciscato, Luciano Masiero, Fabiana Corami, Cinzia Bettiol: Investigation. Davide Asnicar: Formal analysis. All the authors: Validation. All the authors: Writing - Original Draft. Valerio Matozzo: Supervision, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Valerio Matozzo reports financial support was provided by Provveditorato for the Public Works of Veneto, Trentino Alto Adige and Friuli Venezia Giulia, provided through the concessionary of State Consorzio Venezia Nuova and coordinated by CORILA. Valerio Matozzo reports a relationship with Provveditorato for the Public Works of Veneto, Trentino Alto Adige and Friuli Venezia Giulia, provided through the concessionary of State Consorzio Venezia Nuova and coordinated by CORILA that includes: funding grants. Valerio Matozzo has patent pending to none.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.123095.

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