An extended quantitative weight of evidence with uncertainty evaluation for the risk assessment of dredged sediment

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Abstract

The sustainable management of dredged material requires robust risk characterization of contaminated sediment to protect both dredging areas and destination sites. However, integrated frameworks for assessing sediment quality prior to management are rarely applied. This study presents a paradigmatic case using a quantitative Weight of Evidence (WoE) approach on sediments from a canal in the Venice Lagoon (Italy), designated for future dredging. The aim is to assess whether integrating biological and chemical lines of evidence (LoEs) provides a more robust framework for dredged material risk assessment and management. Multiple LoEs were integrated, including chemical analyses of inorganic and organic contaminants, ecotoxicological bioassays, bioaccumulation tests, biomarker responses, and a novel transcriptomics LoE. Uncertainty in LoE integration was addressed probabilistically to quantify confidence in the final risk assessment. Results revealed a spatial gradient in sediment quality, with higher degradation near the industrial area. However, biological data indicated potential toxicity in sediments far from non-industrial sites that chemical analyses alone failed to detect. This discrepancy suggests the possible presence of not-targeted contaminants or other unknown factors, warranting further investigation. The study emphasizes the importance of assessing sediment effects across biological levels, tracing hazards to specific LoEs, and explicitly addressing uncertainty: these are three key steps for effective sediment risk assessment and informed decision-making.
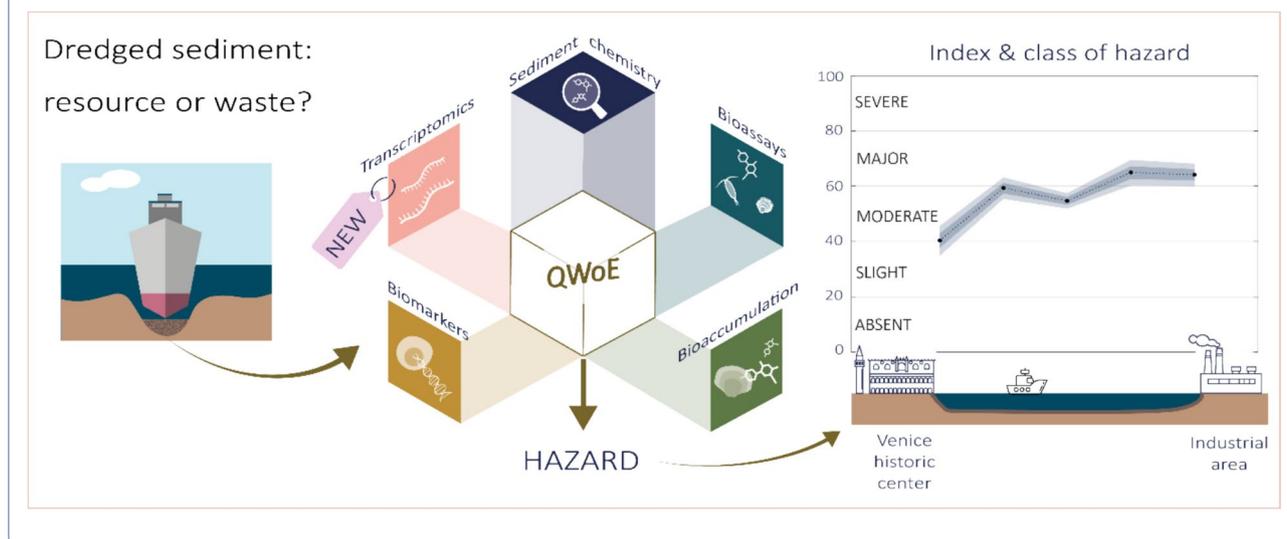
Keywords Quantitative weight of evidence, Probabilistic modelling of uncertainty, Dredged sediment management, Multiple lines of evidence, Transcriptomics line of evidence

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Graphical Abstract

Introduction

Management of coastal areas requires addressing both unwanted sediment deposition and threatening erosion [1]. Deposition necessitates dredging to maintain navigable waterways, whereas erosion can lead to the loss of coastal areas and natural habitat (e.g. wetlands). In response to this issue and to the 17 United Nations SDGs (Sustainable Development Goals)—in particular, Goal 14 asking for a sustainable use of oceans, seas and marine resources—dredging can become a source of sediment. This sediment can be used in nourishment and restoration projects for the protection of coastal resources and to support the mitigation of habitat loss [2].

Therefore, one of the most important aspects in the sustainable management of dredged sediment is the assessment as to whether the sediment is suitable for a particular beneficial use, and to what extent contamination may limit the use of the excavated material for beneficial purposes [3]. As these choices can have far-reaching consequences in space, the potential impacts that dredged sediment can exert on the ecosystem as a whole should be assessed through a broad spectrum of indicators [4] via a structured risk assessment [5]. To this end, an integrated sediment quality assessment based on a Weight of Evidence (WoE) approach may provide an effective assessment framework [6–8].

The Venice Lagoon (Italy) is presented here as a paradigmatic example of challenging sediment management. In this coastal wetland, channel aggradation occurs at the cost of sediment loss in natural salt marsh areas. This ongoing morphodynamic evolution, a result of the intricate interaction between natural and anthropogenic forcing [9], might be further exacerbated in the future by the operation of the mobile barriers (MoSE) for flood prevention of the Venice historic centre [10, 11]. In the context of increasing sediment loss [9] and considering that the last comprehensive restoration of the canal network resulted in approximately $3.5 \cdot 10^5$ metric tons of removed sediment [12], a sustainable management of dredged sediment is required. A broader collection of data on sediment quality and a profitable use of the information is the first step in this direction. While traditional dredged material management regulatory frameworks rely primarily on measures of total sediment concentration and results from standard ecotoxicological bioassays, recent advancements in the assessment of sediment quality have created opportunities for the incorporation of additional lines of evidence (LoEs) to improve the management decisions [13–15]. These advancements include cellular biomarker analysis [16] and genomics technologies [17]. Biomarkers and transcriptomics can serve as screening-level indicators of potential ecosystem stress and inform the need for further evaluation to determine potential causes of the perturbations, which might include contaminants that are not routinely measured or are unknown [4, 18]. Given their increased affordability, these analyses offer a practical additional LoE to further inform and improve current regulatory practices [19]. However, most frameworks and practices in dredged material management have struggled to complement sediment chemical analysis with biological effect-based tests; typically, only ecotoxicological bioassays have been

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incorporated into national regulatory frameworks [4]. This gap between the potential of advanced analytical techniques and current regulatory practices highlights the need for a more integrated approach to sediment assessment and management.

In an attempt to achieve a more accurate assessment of the impacts of future dredging activities, a quantitative WoE approach is demonstrated through its application on sediment samples collected along an important canal of the Venice Lagoon. This study aims to assess whether integrating multiple LoEs, incorporating biology and analytical chemistry, provides a more robust framework for dredged material risk assessment and management. Sediment chemical characterization, sediment toxicity tests, bioaccumulation tests, and biomarker analyses complemented by transcriptomics were utilized to provide a more wholistic assessment of sediment quality. A new line of evidence based on transcriptional data was recently proposed [20] and is here integrated to capture early warnings signals that emerge before adverse effects manifest at higher levels of biological organization.

In the application of integrated WoE frameworks, disagreement between evidence types may arise, potentially impacting the reliability of the outcome. To address this issue and improve the understanding of the effectiveness of an integrated WoE methodology based on five independent LoEs, a probabilistic approach was explored to express and discuss confidence in the hazard classification.

Materials and methods
Study area
With a surface of 550 km^2, the Venice Lagoon is the largest coastal transitional ecosystem in the Mediterranean area. Because of its favourable characteristics, the lagoon has been extensively exploited for waterborne transport, fishing, and aquaculture farming, and tourism. The consequences of these activities are traceable on the seabed, marking the Venice Lagoon as a good example of "ecosystem alteration in the Anthropocene" [9]. After transport restrictions imposed by the COVID19 pandemic in 2020, maritime traffic has significantly grown in this area [21]. If, as expected, the commercial and touristic traffic is going to steadily increase [22], dredging navigable waterways and ports will soon become more intensive.

For the past 30 years, dredging material in the Venice Lagoon had been regulated based on sediment concentrations ("Protocollo Fanghi" by the Italian Ministry of the Environment, 1993). Only recently (Decree 86/2023, effective from 19/07/2023) has the evaluation of dredged material reuse or disposal also included ecotoxicity testing to account for the effect of contaminants in relevant aquatic species, but the new regulatory framework has

not yet been implemented in routine sediment management operations. It is crucial to emphasize that the present study was not designed to evaluate the newly proposed regulatory framework, which was still undergoing the approval process at the time of field data collection. Rather, this research focuses on implementing an extended WoE assessment framework for scientific purposes, aiming to assess its potential to enhance scientific understanding and serve as a steppingstone towards optimizing routine sediment management procedures in the future.

For this reason, one of the main waterways of the lagoon, i.e. Vittorio Emanuele III Canal (hereafter VEIII Canal), was selected as a case study (Fig. 1). The canal, which connects the Venice historic centre to the industrial area of Porto Marghera, is planned to serve as a passage for cruise ships that are no longer allowed to navigate through the historical centre of Venice. This would require the dredging of the canal bed, whose actual depth varies from about 6 m in the industrial area to 13 m in correspondence with the city centre.

Experimental campaign
The experimental campaign started in November 2020 with the collection of sediment in five sampling stations along VEIII Canal. As visualized in Fig. 1, sites are numbered consecutively from the historic centre (S1) to the industrial area (S5). A sixth site was selected far from the other sampling stations in San Felice Canal (hereafter SanF Canal), in the lagoon's northern basin. This site (S6) is removed from known urban and industrial sources of pollution. It then represents a valid reference site with low chemical and ecological impact to evaluate the quality of sediment collected along VEIII Canal.

The sampling of sediment cores (five cores per site) was performed starting from the bottom plane down to a depth of 1.0 m from a pontoon boat using a vibracore system. Sediment cores were opened under a nitrogen atmosphere to minimize oxidation and preserve sample integrity. The entire core was then thoroughly homogenized, and aliquots were taken only afterward for subsequent analyses. By doing this, the tested sediment resembles the typical characteristics of sediment grabbed in dredging activities. Sediment was also used in laboratory-controlled experiments for ecotoxicological evaluation through a suite of bioassays, bioaccumulation tests, biomarkers and transcriptomic analyses.

Quantitative WoE approach for risk assessment
Data collected in the experimental campaign are presented hereafter. The different types of analysis fell into five separate LoEs (i.e. chemistry, bioassays, bioaccumulation, biomarkers, and transcriptomics), each LOE
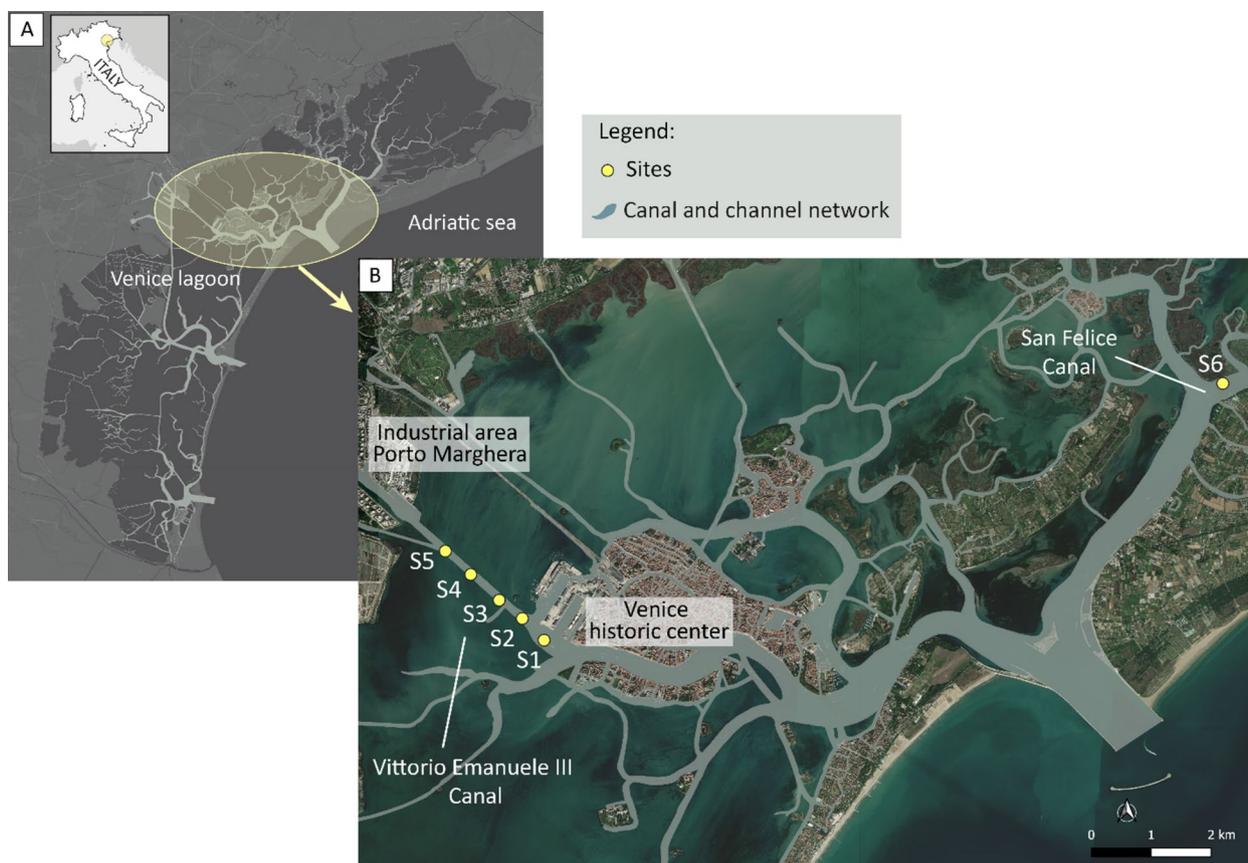
Fig. 1 **A** Map of the Venice Lagoon with **B** location of sampling sites. Sites S1 to S5 in Vittorio Emanuele III Canal and site S6 in San Felice Canal

providing a separate source of information to inform characterization of ecological risk associated with the dredged sediment under evaluation. Data from each LOE were processed following the methodology presented in Piva et al. [23] and Regoli et al. [24], which was adapted to include the addition of transcriptional effect LoE and uncertainty analysis to support decision-making.

The methodology, briefly outlined in the next sections, consists of mathematical algorithms that translate data from each LoE into synthetic indices of hazard (Hazard Index, HI). Such indices, indicating the degree of hazard associated with each LoE measurement (classified from *absent* to *severe*), are then integrated to infer the level of risk associated with each site (as per each LoE, from *absent* to *severe*). The methodology has been widely used to monitor the temporal evolution of the quality status of a site [14, 15], to study the ecosystem health in harbour areas [25], to compare multiple sites spatially distributed over an area of interest [24], and, ultimately, to assess the potential effects of largely detected pharmaceuticals in marine species [26]. The flowchart, reported in the SM (Figure SM1), summarizes the logical steps of this approach with regard to the LoE data collected in this study.

Chemistry LoE

Sediment samples collected for chemistry were freeze-dried and analysed following the procedures detailed in the SM. Briefly, chemical characterization included the determination of total concentrations of nine trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, V, Zn) by ICP-MS after microwave-assisted acid digestion of sediment samples, while the analysis of organic pollutants—polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls, including both the dioxin-like (DL-PCBs) and other regulated polychlorobiphenyls PCBs, hexachlorobenzene (HCB), hydrocarbons (C > 12 and C ≤ 12) and organotin compounds—was carried out according to the methods reported in Table SM2a.

To evaluate the chemical hazard, concentrations of contaminants in sediment were compared with sediment quality guideline (SQG) values, here set according to the Italian Ministerial Decree 173/2016 [27] for dredged sediment. The mathematical procedure for the chemistry

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hazard index (HI_c) calculation is detailed elsewhere [23] and it can be adapted to the SQGs of interest. Tables SM2b,c in SM report the measured concentrations of the analysed contaminants. SQG values were available for all trace elements with the exception of vanadium, for which the background concentration was considered as a reference value in this study. The averaged concentration in the European marine ecosystem proposed by Tulcan et al. [28], that is 53 mg/kg$_{dw}$, was considered for reference since it well aligns with previous investigations of vanadium concentration in the Venice Lagoon sediment [29]. With regard to organic pollutants, Decree 173/2016 includes SQG values for the sum of PCDD/Fs and DL-PCBs (expressed as TEQ values), and for ΣPCBs (considered as the sum of the 13 congeners 28, 52, 77, 81, 101, 118, 126, 128, 138, 153, 156, 169, 180). In the case of PAHs, Decree 173/2016 provides a threshold for the sum of the 16 PAHs classified by US EPA as "High Priority Pollutants", as well as threshold values for 13 individual PAHs. All these SQG values were used to classify sediment contamination for the Chemistry LoE.

The number of contaminants exceeding the SQGs and the extent of the exceedance contributed to the calculation of HI_c, to which a class of hazard (from *absent* to *severe*) is ultimately scored.

Ecotoxicological bioassays LoE

The ecotoxicological characterization of sediments was performed using a suite of four bioassays: the acute lethality test on the whole sediment with the amphipod *Grandidierella japonica* (ISO, [30]); a sub-chronic toxicity test on the water–sediment interface with the copepod *Acartia tonsa,* using the larval development from the egg to the copepodite-I stage as the endpoint [31]; a sub-chronic toxicity test on elutriate with the bivalve molluscs *Mytilus galloprovincialis* using the larval development as the endpoint [32]; and a chronic toxicity test with the amphipod *Monocorophium insidiosum* using a set of sub-lethal endpoints, including growth rate and survival, after 28 days of exposure to the sediment [33].

Amphipods and mussels were wild-caught in the Lagoon of Venice in sites previously identified as a reference area for test organism collection [33, 34], whilst copepods were obtained from an in-house laboratory culture [31].

The test protocols are detailed in the SM. All tests were performed according to the CRED (Criteria for Reporting and Evaluating ecotoxicity Data) evaluation method for the reliability and relevance of toxicity data [35]. Quality Assurance/Quality Control (QA/QC) procedure included testing a negative control and performing a toxicity test with a reference toxicant (positive control). The results of the QA/QC procedure are reported in detail in

the SM. For amphipod and copepod tests, water quality parameters (temperature, salinity, dissolved oxygen, pH) were monitored at the beginning and during the test. For mussel testing, physico-chemical parameters in the elutriates (temperature, ammonia, salinity, pH) were measured at the beginning of the exposure.

One-way analysis of variance and Kruskal–Wallis non-parametric test were used to check for differences between the reference site S6 and the sediments collected in VEIII Canal. Factor analysis was used to verify possible correlation between sediment toxicity and chemistry (see SM).

According to Regoli et al. [24], an effect was calculated for each bioassay by comparing the variation to a control condition with the relative threshold derived from literature data based on the species sensitivity [23, 36, 37]. Effects are then mathematically integrated, depending on their biological endpoint, the tested matrix, the exposure time (as reported in the SM Table SM3c), and the statistical significance of variation from the control. To the so-derived bioassay hazard index, HI_{bs}, a class of hazard is assigned.

Bioaccumulation LoE

The evaluation of possible bioaccumulation of contaminants by benthic invertebrates in the sampled sediments was studied through laboratory-controlled exposure of Manila clams (*Ruditapes philippinarum*) as further detailed in SM. The same set of experiments was used for the analysis of biomarkers and transcriptomic changes that contribute to the next two LoEs. To this end, clams from an aquaculture facility were transported to the laboratory and, after a period of acclimatization, exposed to sampled sediments for 14 days. At the end of the 14-day exposure, concentrations of PCDD/Fs, PCBs, PAHs, HCB, organotin compounds, hydrocarbons and trace elements were measured in clam tissues. A laboratory-based assessment of bioaccumulation, biomarkers, and transcriptome was chosen due to the study area's conditions, which are unsuitable for passive assessment using native clams and active monitoring using transplanted organisms. Heavy naval traffic, strong tidal currents, sediment resuspension and turbidity limit the possibility of placing cages with transplanted clams and make sampling of native clams complicated.

Following the steps of LoE bioavailability [24], a ratio was calculated for each parameter as the average concentration measured in tissues of exposed clams versus the concentration in control organisms. Clams exposed to sediments from site S6 acted as reference organisms given the good quality status of the collected sediment, as discussed in the Results section. Similarly to previous LoEs, differences relative to the reference contributed to

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the calculation of the bioaccumulation hazard, HI_{ba}, to which a class from *absent* to *severe* is assigned.

Biomarkers LoE

The results of a battery of biomarkers performed on clams are used here to calculate the hazard index associated with the fourth line of evidence. Biomarkers of immune responses, antioxidant defenses, and detoxification of pollutants were measured in haemolymph, gills, and digestive gland after a 14-day exposure. Details of the experimental procedure and biomarker analysis are presented in Asnicar et al. [38] and briefly summarized in SM.

Following the methodology of the two reference studies [23, 24], each biomarker was evaluated based on the relevance of the biological endpoint in light of the current mechanistic knowledge, and a threshold for changes of biological significance. Variation in biomarker response measured in organisms exposed to VEIII Canal sediments from that measured in the reference sample (organisms exposed to S6 sediments) was corrected for statistical significance via a Mann–Whitney test. The weighted effects, which discriminate inhibited from induced responses, are used to calculate the hazard index, HI_{bm}, and class of hazard. It is important to note that only a selective range of biomarkers analysed in Asnicar et al. [38] were employed in the current investigation. The decision to use this subset of biomarkers (SM, Table SM5a) was driven by the availability of established thresholds.

Transcriptomics LoE

The line of evidence that translates analysis of transcriptional alterations into a transcriptomic hazard index (HI_t) suitable to be incorporated into a QWoE is presented in Cecchetto et al. [20]. The methodology, hereafter briefly outlined, finds one of its first applications to a weighted assessment in the present work. Experiments investigated changes at a molecular scale by studying gene expression profiles (RNA-seq) in clams exposed to sediments collected from the six sampling sites, as reported in Bernardini et al. [39]. Similar to the biomarker analysis, clams' digestive glands were collected at 14 days of exposure and organisms exposed to sediment from site S6 were used as control samples. The analytical procedure, data processing, and results at the end of the 14-day exposure are summarized in SM. To improve the biological interpretability of transcriptomic changes and reduce noise associated with individual gene fluctuations, a Gene Set Enrichment Analysis (GSEA) approach [40] was applied. While not all observed molecular responses can be unequivocally attributed to contaminant exposure, the derivation of the transcriptomic hazard index (THI) considers the biological relevance of the perturbed gene sets.

This helps reduce uncertainty in the interpretation of transcriptomics-based assessments. The transcriptional alteration, together with the relevance of the biological endpoint triggered by the change in transcripts, contributes to the derivation of the HI_t. The index and the corresponding class of hazard allow the integration of this LoE within the framework of the other lines.

Logical steps forming the conceptual framework of the transcriptomics LoE are detailed in Cecchetto et al. [20] and reported in the SM (Figure SM6).

LoE integration

The results obtained from the different LoEs are integrated by first normalizing the hazard indices to a common scale, i.e. from 0 to 100. In a quantitative WoE, the indices are eventually harmonized together using weighing [41] as an indicator of the degree of reliability and relevance of the conclusions reached within each line [42]. Instead of assigning a single value to each LoE, weights are here assumed to be triangularly distributed to account for the uncertainty related to the complexity of establishing the evidence strength. Triangular distributions are obtained by considering an arbitrarily chosen variation of 30% around the weight value established by experts. With a Monte Carlo simulation consisting of 10,000 random samplings from the weight distributions, the global HI_{WoE} for each site is calculated. Justification of the selected weights and discussion of the uncertainty associated with each line are provided below, particularly when multiple LoEs are integrated. The generated hazard is then a stochastic distribution of probable hazard indexes. Figure SM1 illustrates the overall process.

Results and discussion
LoE 1: chemical characterization

Chemical characterization confirmed the good quality status of sediments from the reference site S6 (SM, Table SM7), where all parameters reported concentrations well below the reference SQGs with the exception of Cd.

Along VEIII Canal, a slight gradient in sediment concentration was observed for some inorganic and organic contaminants. Concentrations of Cd, Cu, Hg, Pb, Zn, sum of PCDD/Fs and DL-PCBs, and heavy hydrocarbons gradually increased from the historic centre to the industrial area (i.e. from S1 to S5). This resulted in Cd and Hg exceeding the SQGs in sediments from S2 to S5, with the addition of Zn and Pb in sites S4 and S5.

The sum of PCDD/Fs and DL-PCBs exceeded the normative limit in all sites, exception made for S1, while sites S4 and S5 were the only ones reporting excess heavy hydrocarbons. Among the other organic contaminants, a more evident gradient is visible for ΣPCBs (as previously

defined), with concentrations greater than the quality standard measured in sites S3, S4, and S5.

The sum of the measured PAHs peaked in sites S2 and S4, where higher concentrations of benzo(a)pyrene, benzo(b)fluoranthene, and benzo(k)fluoranthene exceeded the SQGs.

Exceedance of the SQG also resulted for HCB in S3 and S4, located in the central part of the canal, while it was not detected in the other sites.

Among organotins, only TBT was found exceeding the SQG, at site S2 with a concentration of 0.37 mg/kg. This concentration is in line with the order of magnitude of those measured in channels located in the southern part of the Venice Lagoon but below values reported for harbours by Berto et al. [43]. The presence of TBT in the core sample from S2 could be the legacy of the contamination from the nearby port of Venice. According to the low rate of TBT degradation in marine anoxic deep sediments [44–47], the estimated TBT half-time decade could explain why TBT metabolites were not here detected. Further investigations are required to confirm the occurrence and relevance of this contamination.

Unlike other trace elements, As, Ni, and V showed the highest concentration in S1. However, their concentrations in S1 comply with the relative SQG values (as in the case of all other pollutants).

As a result, the application of the chemistry LoE algorithm to the concentration data indicated *absent* hazard for S1. *Severe* hazard characterized sites S2, S4, and S5, with the worst situation registered in S4. The *severe* hazard of S2 is mostly a result of the organotin TBT. Although Cd, Hg, PCDD/Fs, PCBs, and HCB exceed the SQGs, the lower PAH levels at site S3 compared to neighbouring sites 2 and 4 contribute to a *major* overall hazard at this location (instead of a *severe* hazard).

Table 1 presents the results of the analysis in terms of class of hazard and the normalized hazard index (on a scale of 0–100) for each site. Additional information about the distribution of parameters according to their threshold exceedance and the contaminants that majorly contributed to the HI_c is reported too, whereas concentrations of single contaminants are presented in SM.

LoE 2: ecotoxicological bioassays

Toxicity tests confirmed the good quality status of reference site S6. The reference sample S6 showed effects comparable to or even lower than the negative controls used for quality assurance and quality control (see SM) in the tests with copepods and amphipods. Only for the mussel test, elutriates extracted from sediment collected in S6 showed a slightly higher effect than the artificial water used as the negative control. This effect was, however, expected due to the different nature of the two

matrices. The results of the QA/QC procedures and tests are reported in detail in SM.

The tests evidenced a diffused toxicity in VEIII Canal, with sediment samples significantly affecting all the biological indicators used for the survey. In the acute test with *G. japonica,* four samples out of five significantly reduced the survival compared with the reference site, with the lowest survival observed in samples S1 (47%) and S4 (53%). Sample S3 was the least acutely toxic sediment for amphipods. Samples S1, S4, and S5 significantly inhibited the larval development of the copepod *A. tonsa*. As in the lethality test with amphipods, the toxic effects were more marked in samples S1 and S4 than in the other sediments, and the inhibition compared to the reference sample was 61% and 58%, respectively, for S1 and S4. In the larval development test with *M. galloprovincialis,* all the tested undiluted elutriate, except S1, caused a significant inhibition compared to the reference sample, and none of the exposed larvae reached the prodissoconch-I larval stage. Samples S2, S3, S4, and S5 fully inhibited the larval development after a 1:2 dilution with artificial seawater, and significant inhibition also occurred after a 1:4 dilution. Sample S4 was the elutriate toxic toward *M. galloprovincialis* larvae, with a calculated toxicity unit of 12.1 (see SM). Ammonia is expected to have influenced the results of the *M. galloprovincialis* development test, as further discussed in SM under the section "Ammonia analysis on elutriates".

In the amphipod chronic test, both the survival and growth rate of *M. insidiosum* were significantly affected by the exposure to sediments sampled in S1 and S4. Conversely, no significant effects on both survival and growth compared to the reference sample were observed in samples S2 and S3.

Effects on mussels were correlated with sediment concentrations of PAHs, PCBs, PCDD/Fs, alkanes, and metals (all but V, Ni, and As), particularly in samples S4 and S5, while effects on copepods and amphipods were mostly related to As and Ni sediment concentrations, particularly in samples S1 and S4. An analogous hazard trend characterized the chemistry and bioassays LoEs (Fig. 2A), with the exception of site S1. Despite the results of the chemical characterization (HI_c *absent*), the exposure to sediment from S1 (closer to the Venice historic centre) caused a significant reduction in the survival of *G. japonica*, the inhibition of larval development of *A. tonsa*, and a reduction in *M. insidiosum* survival and growth rate. Sediment from S1 presented greater toxicity at the organism level, a result that might be attributed to contaminants or other factors not analysed in this study. For the chronic test with amphipods, the texture of this sediment might have affected the results of the analyses. The sediment was a very compact loam, and amphipods

Table 1 Results of the elaboration of chemistry (C), bioassays (Bs), bioaccumulation (Ba), biomarkers (Bm), and transcriptomics (T) LoE for the five sites along VEIII Canal. Additional information is reported for each LoE. Final HI_{WoE} and class of hazard is reported too. These values refer to the deterministic weight attribution. Colours are associated with classes as follows: white/*absent*, light blue/*slight*, yellow/*moderate*, red/*major*, dark grey/*severe*

		S1	S2	S3	S4	S5
LoE C	Class	ABSENT	SEVERE	MAJOR	SEVERE	SEVERE
	HI_c	5.1	86.2	74.9	94.0	88.6
	Distribution of contaminants based on ratio to the reference SQG					
	Contribution of contaminants with max exceeding of SQG	-	37%, TBT	27%, PCDD/Fs + DL PCBs	20%, PCDD/Fs + DL PCBs	34%, PCDD/Fs + DL PCBs
LoE Bs	Class	MAJOR	MAJOR	MODERATE	MAJOR	MAJOR
	HI_{bs}	65.1	61.5	50.4	72.5	72.2
	Test and species with major influence on HI	Acute mortality test with *G. japonica*	Sub-chronic toxicity test on the water-sediment interface with *A. tonsa* \n\n Sub-chronic toxicity test on elutriates with *M. galloprovincialis*	Sub-chronic toxicity test on the water-sediment interface with *A. tonsa* \n\n Sub-chronic toxicity test on elutriates with *M. galloprovincialis*	Chronic toxicity growth test with the amphipod *M. insidiosum* \n\n Acute mortality test with *G. japonica* \n\n Sub-chronic toxicity test on elutriates with *M. galloprovincialis*	Acute mortality test with *G. japonica* \n\n Sub-chronic toxicity test on elutriates with *M. galloprovincialis*
LoE Ba	Class	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE
	HI_{ba}	41.1	44.5	44.2	41.4	46.8
	Moderate parameters	Naphtalene, Hg	PCDD/Fs, Pirene, Benzo(b)fluorantene, V, Hg	PCDD/Fs, Pirene, Benzo(b)fluorantene, Benzo(a)pirene, Benzo(g,h,i)Perilene	PCDD/Fs, Pirene, Benzo(b)fluorantene	DL-PCBs, Fluorantene, Pirene, Benzo(b)fluorantene
	Major par.					Dioxins
	Severe par.					
LoE Bm	Class	MAJOR	SLIGHT	SLIGHT	SLIGHT	SLIGHT
	HI_{bm}	60.2	29.6	39.3	29.6	29.6
	Moderate par.	CAT (gill)	BChE (gill)	SOD (gill), AChE (gill)	SOD (dig. gland)	AChE (gill)
	Major par.	AChE (gill)				
	Severe par.	BChE (gill)				
LoE T	Class	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE
	HI_t	41.1	40.1	45.7	42.6	44.7
	Moderate par.	Development, Cellular component	DNA damage, Immune	DNA damage, Proliferation	DNA damage, Development, Signaling	DNA damage, Signaling
	Major par.			Development		Proliferation
	Severe par.					
QWoE	Class	MODERATE	MODERATE	MODERATE	MAJOR	MAJOR
	HI_{WoE}	40.4	59.4	54.7	64.9	64.2

encountered difficulties during burrowing. The higher costs for building the burrow might have reduced the energy budget for maintenance and growth, increasing the mortality and slowing the growth. For the other sites (from S2 to S5), the hazard derived from the suite of bioassays well reflects the toxicity classification of the
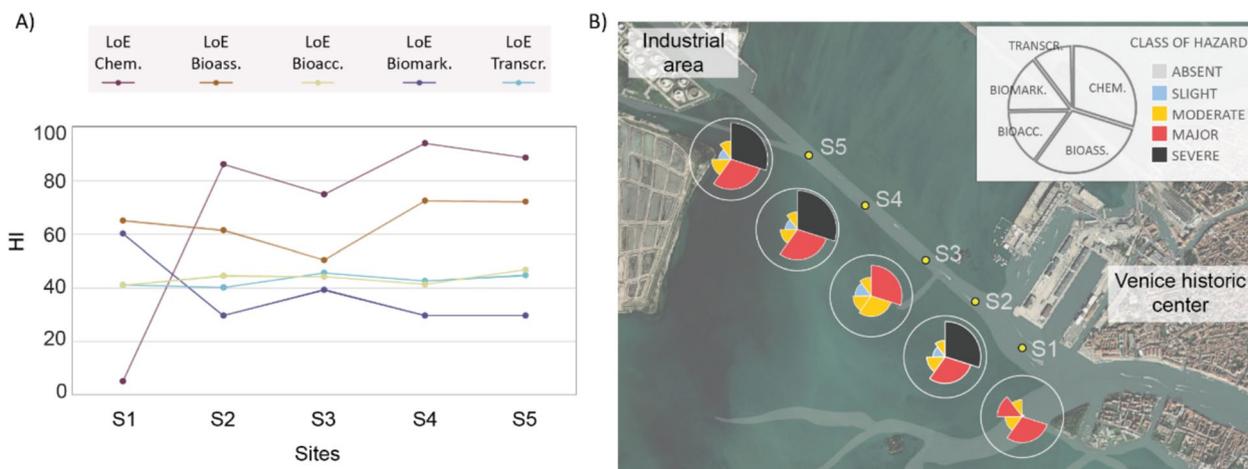
Fig. 2 **A** HI trends of the five LoEs along the VEIII Canal. **B** Aggregated results for each site with pie chart representation, where the sector radius indicates the magnitude of hazard (with regard to the maximum achievable value of 100—outer white circle) and the sector width denotes the average weights assigned to each line (i.e. 1.2 for chemistry and bioassays, 0.6 for bioaccumulation and biomarkers, and 0.4 for transcriptomics). Sector colours correspond to the five classes of hazard

sediment samples, with greater hazard, i.e. *major*, at sites closer to the industrial area (S4 and S5). A trend inflection is presented at site S3 where a lower toxicity in both tests with amphipods resulted in a *moderate* hazard class. This may be attributed to the lower PAHs and As concentrations in S3 sediment, which resulted in a significant correlation with *G. japonica* survival and (only in the case of As) with *M. insidiosum* survival and growth (see SM).

The outcomes are summarized in Table 1, which reports both hazard index and respective class of hazard for each site.

LoE 3: bioaccumulation

Concentration of organic and inorganic contaminants in clam soft tissues is presented in the SM (Tables SM4b,c). As reported in SM4b, the bioaccumulation pattern of trace elements was spatially variable and dependent on the specific element so that the relationship with the concentrations measured in sediments could not be identified. Some trace elements of major anthropogenic origin, such as Cd, Hg, Pb, showed an increasing trend in sediment from the Venice historic centre to the industrial area, while this was not observed for concentrations in clam soft tissues. These findings confirm the long-established difficulty in linking trace elements bioaccumulation in aquatic organisms to environmental concentrations, especially under laboratory conditions [48].

For PCDD/Fs and PCBs, the results showed an increased bioaccumulation from S1 to S5, with significantly lower levels for site S1 and the reference S6, whose concentrations remain comparable to the initial ones (i.e.

T0 in SM Table SM4c). In this case, results seem to reflect the increasing spatial trend of contamination observed in the sediments from the Venice historic centre to Porto Marghera, eliciting the role played by industrial activity as a major source of these persistent pollutants in the past. The sum of PAHs concentrations in clams did not show a clear spatial trend. However, it should be noted that the majority of the PAHs analysed in clams showed concentrations below the limit of quantification (LOQ) values, which could partly affect the significance of the results. Among those detected, concentrations of pyrene (S2 to S5) and naphthalene (S1) in clams were notably higher than concentrations measured in clams exposed to sediment from the reference site S6. TBT, HCB, and hydrocarbons were not detected in clam soft tissues.

When compared to the reference sample S6, a *moderate* hazard is obtained for the five sites along VEIII Canal (Table 1). However, a slight spatial trend is still visible (Fig. 2), with bioaccumulation of contaminants from the S5 sample reporting the highest hazard index due to dioxins concentration in clam tissue.

LoE 4: biomarkers

The response of clams exposed to the collected sediment (SM, Table SM5b) showed that antioxidant defence and neurotoxicity biomarkers were variously induced and inhibited. As reported in Asnicar et al. [38], biomarkers response highlighted the effect of trace elements Pb and Hg presence in sediments and, at a lower degree, of Pb and Ni in clam tissues. Sediment and tissues concentrations of PCBs, PAH, and PCDD influenced a different biomarker modulation among sites. Interestingly, AChE

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and BChE alterations were significantly more important in organisms exposed to S1 sediments. Given the low contamination level of S1 sediment, the inhibition of AChE and the induction of BChE in gill tissue, as well as the induction of SOD, CAT, and GST in the digestive tract, might imply the presence of neurotoxic compounds not considered in this study, such as carbamates and organophosphate pesticides.

The application of the LoE algorithm, which implies calculating the variation of biomarkers response in S1–5 relative to S6, corrected for statistical significance, produced the outcomes presented in Table 1. For sites S2, S3, S4, and S5, the moderate effect of some antioxidant defence and neurotoxicity biomarkers was attenuated by the general low response of the biomarker battery. The *slight* hazard for these sites (i.e. a slight difference in biomarkers response compared to S6) reflects the finding of Asnicar et al. [38], who reported that biomarker results do not significantly support the differentiation among the S2–S5 studied sites. The highest significance in variation from the reference site was obtained for S1, characterized by *major* hazard. The result is ascribable mainly to the BChE and AChE alterations in gill and, to a moderate extent, to CAT activation.

LoE 5: transcriptomics

The application of GSEA analysis to the Hallmark gene sets revealed uniform transcriptional alteration across sites (SM, Table SM6). The most important transcriptional changes occurred in molecular pathways involved in proliferation, cell cycle regulation, development, immune response, and DNA damage. The highest number of altered gene sets was found in S5, as widely discussed in Bernardini et al. [39]. In that study, clams exposed to the contaminated sediments collected at site S5 showed upregulation of several pathways involved in proliferation and cell cycle progression and regulation ("MYC targets", "p53 pathway", "G2M checkpoint" and "E2F target"), "DNA repair", "protein folding", "mTORC1 signalling" and immune response.

Similar to the evidence from bioassays and biomarkers, important alterations were also identified in clams exposed to S1 sediments. For this sediment, the modification of gene sets responsible for the regulation of development and cellular processes aligns with the findings of Bernardini et al. [39] who reported upregulation of pathways involved in signalling and development ("WNT signalling", "myogenesis"), "proliferation", "apoptosis", "cellular response to stress" and "immune response" (e.g. "NOD-like receptor signalling"; "innate immune response").

Once converted into a hazard index, transcriptional evidence showed limited variability across sites, which all scored *moderate* hazards (Table 1). Despite the little difference, sites S3, S4, and S5 reported the highest HI_t, an outcome that, as detailed in Bernardini et al. [39], could be ascribed to the presence of organic pollutants in the sediment.

LoEs aggregation

Pooled visualization of the HIs trends in Fig. 2A shows a correspondence between hazards generated by chemistry data and toxicity tests. For these LoEs, trends along the VEIII Canal are similar, with a slight decrease in hazard observed for site S3 and a confirmation of good quality status of the reference site S6 (presented in SM, Table SM7); only in S1 the LoE bioassays exhibited a HI significantly greater than the LoE chemistry. The presence of unmeasured toxicants in sample S1 is suggested by the high mortality observed in the bioassay with *G. japonica,* as well as by effects on copepod and amphipod growth. It is also corroborated by the biomarker LoE results, which indicated significant effects consistent with exposure to esterase inhibitors (i.e. carbamates and organophosphate pesticides). Evidence gathered from the biological analyses revealed a possible hazardous situation at the organism level for sediment collected close to the Venice historic centre that the chemistry LoE failed to identify. The new LoE transcriptomics well aligned with the outcome from the bioaccumulation evidence, indicating a moderate hazard across all sites.

The primary goal of this study was to integrate individual pieces of evidence into a quantitative WoE approach, assigning specific weights to each line of evidence. Among other considerations, weighting factors can account for the relevance of the results in relating effects to the exposure, the quality of the analyses and methods, and the uncertainty of the evidence as a function of data quantity and quality [42]. Based on expert judgement, a lower weighting was given to the evidence from bioaccumulation and biomarker ($w_{ba} = w_{bm} = 0.6$), and transcriptome ($w_t = 0.4$) analyses compared to weights assigned to chemistry data and ecotoxicological bioassays ($w_c = w_{bs} = 1.2$).

The choice of lower weights was primarily driven by uncertainties in the experimental results. As shown in Tables SM4b,c (SM), contaminant concentrations in organisms at the start of the experiments (T0) were often similar to those measured in clams after 14 days of exposure. This could be attributed to detoxification mechanisms, such as ATP-binding cassette transporters, observed at the transcriptional level [39], though further investigation is needed. While the Manila clam is widely used as a biomonitor species [49], it remains challenging to link trace element bioaccumulation in aquatic organisms to environmental concentrations, particularly under

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laboratory conditions [48]. Long-term and active monitoring using transplanted organisms could have provided more relevant data. However, as anticipated in the Methods section, these options were not viable due to the conditions of the study area. Besides the experimental design, other natural and analytical factors can influence bioaccumulation results [5, 50]. Being aware of these limitations, LoE Bioaccumulation was assigned a low weight in the overall WoE evaluation. Biomarkers LoE was given a similar weight based on the fact that thresholds of response are available for a limited number of biomarkers [51]. Of all 21 analysed biomarkers [38], only those 9 for which a threshold is available could contribute to the assessment. Biomarkers measures are also impacted by non-contaminant stressors, and changes at the molecular and biochemical level do not necessarily induce effects at higher levels of biological organization. As this consideration applies also to the transcriptional results, the weight was further lowered, and transcriptomics data were included as supportive evidence. Figure 2B aggregates in the same chart the individual hazards by visually converting this weighting system into the LoEs' sector width and the magnitude of hazard in the sector radius.

Combining the different LoEs results for site S1 reinforces the consideration above. It seems that contaminants not measured in sediment or other factors not considered in the analysis might have caused the discrepancy between the sediment chemical characterization and the organisms' biological response. This disagreement is also reflected in the calculation of the final HI_{WoE} (Table 1). The final risk estimate of S1 is borderline: the class is *moderate*, but the score of 40.4 is just above the boundary between the *slight* and the *moderate* class (i.e. 40). A similar borderline situation takes place in site S2, where a HI_{WoE} of 59.4 results in *moderate* hazard despite the fact that such score falls close to the threshold between the *moderate* and the *major* class (i.e. 60). In S2, the chemistry LoE is majorly driven by an anomalous spike in the organotin TBT concentration, which, alone, raised the chemistry hazard class from *major* to *severe*. However, the biomarkers LoE and, to a lesser extent, bioassays and transcriptomics LoEs scaled down the impact of the *severe* hazard obtained from the sediment chemical characterization. Disagreement among LoEs influenced the characterization of overall risk for sites S1 and S2, but the borderline classification depended also on the importance given to each line in the integration step. By varying the weights determined by experts by a ±30% and randomly selecting them according to the Monte Carlo approach, it was possible to evaluate the confidence in the reached conclusions. The final risk assessment is presented in Fig. 3. Dark and light-coloured bands around the mean hazard index, HI_{WoE}, express the confidence

intervals between the 5th and 95th percentiles and between the minimum and maximum values of the hazard index distribution, respectively.

Figure 3 reveals a gradient of hazard that increases when moving from the historical centre of Venice towards the Porto Marghera industrial area, showing a slight reduction in correspondence of site S3 that was supported by both chemical data and biological measures. A *major* hazard denoted sites closer to the industrial area, i.e. S5 and S4, while the hazard for site S3, which is located halfway through the canal, was *moderate*. The hazard classes assigned to these three sites remain reliably the same even when the weights vary, so the confidence in those hazard classifications is strong and consistent. In the case of S1 and S2, already identified as borderline situations, the distribution of hazard indexes is falling across two classes of hazard. Here, the variation of weights can give a quantitative estimate of the representativeness of adjacent classes in the definition of the conclusive risk. In S1, hazard is defined as *moderate* with a confidence of 58% and *slight* with 42% confidence; S2 hazard was instead 32% *major* and 68% *moderate*. While for sites S3, S4, and S5 agreement was found in the integration of the five LoEs, a univocal conclusion could not be reached for sediments from site S1 and S2.

Applicability of an integrated assessment approach in support of dredging activities

When several types of evidence can be collected and exploited for an environmental risk assessment, discrepancies across the outcomes are likely to arise. A critical point in quantitative WoE approaches is then establishing the correct strength of the evidence [52] by carefully choosing the LoE's weight. Despite the use of a well-established and transparent conceptual framework [24], the decision of the most appropriate weights inevitably involves subjective judgment, with implications on the reproducibility of the results.

In this work, a Monte Carlo approach has been selected to incorporate uncertainty around weights into the final evaluation, and probability is thus used to express the confidence in the risk assessment outcome. A sensitivity analysis of the importance attributed to the evidence types can be a practical solution for determining the variability of the hazard [42]. Risk assessment, by definition, deals with uncertainty [53]; yet, among decision-makers and other stakeholders, probability is often met with scepticism.

It is important to communicate uncertainty correctly when presenting the aggregated results for use in the next phase, i.e. risk management [54]. If the hazard cannot be classified with a high degree of confidence as for sites S1 and S2, this should be seen as an opportunity for
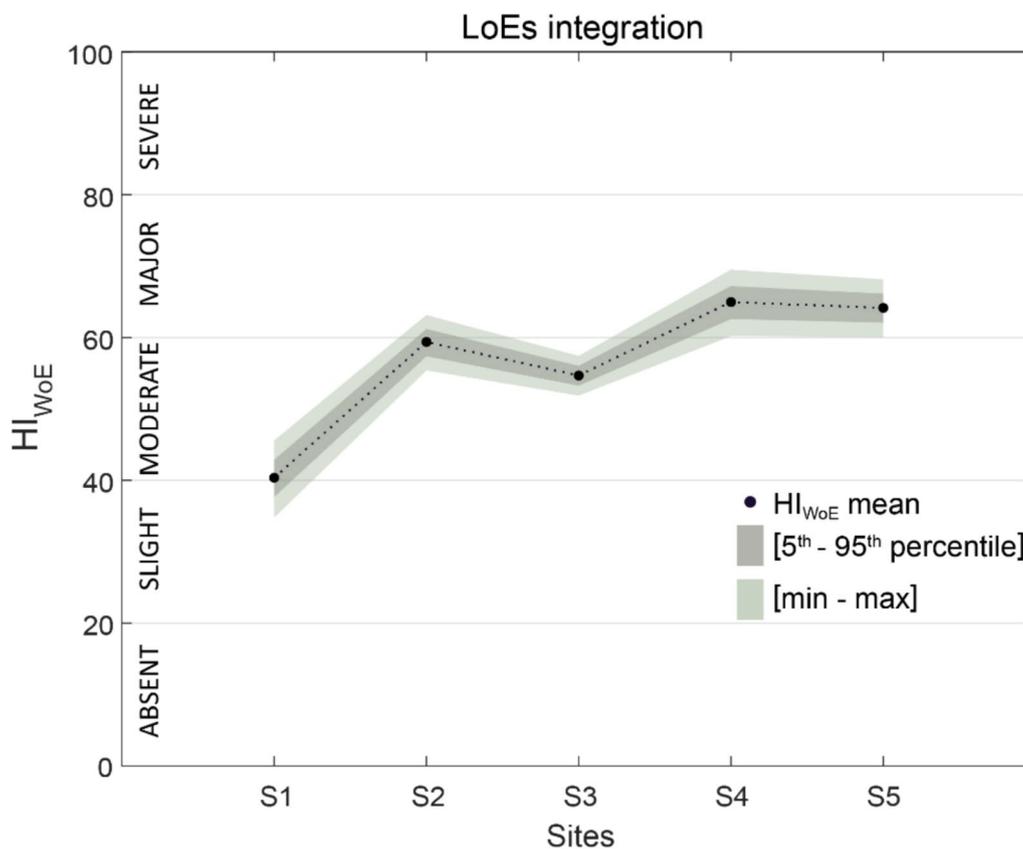
Fig. 3 Hazard trend along VEIII Canal. Bands around the mean hazard index, indicated with a dot, express the confidence intervals between the 5th and 95th percentiles and between the minimum and maximum values of the hazard index distribution. Classes of hazard are reported in the plot

further evaluation (e.g. tracing back to the single LoEs and exploring more deeply potential links between lines as well as possible factors of stress). If a univocal decision is not possible with the existing information, additional investigation may be warranted to facilitate a more comprehensive assessment. Additional investigation might include additional measurements, incorporation of new lines of evidence, or, in case of dredging activities, incorporation of a denser sampling mesh of sediment cores to account for the high spatial variability in sediment characteristics and depositional patterns. The last aspect is often taken into consideration in typical dredged material assessments.

Compared to the most used frameworks and practices for sediment management, new levels of detail have been included in the present sediment assessment framework. The results of this study show that in dredged sediments the measurement of pollutants concentration as a criterion to evaluate their possible threat to aquatic organisms is not always sufficient. Exemplary is the case of S1 in this work. By adding additional types of data, strong evidence of potential stress emerged for organisms exposed to sediment of S1. These findings underscore the importance

of broadening the range of targeted contaminants (e.g. starting from a non-target analysis) considered within the chemistry LoE to ensure a better understanding of cause–effect relationships. It is worth noting that expanding the set of contaminants included in the Chemistry LoE would require having appropriate SQG/threshold values for their evaluation.

A new element introduced to support the sediment quality assessment is the evaluation of chronic toxicity. In natural environments, chronic exposure to contaminants is often more representative relative to acute exposure, and moderately contaminated sediments are more common than highly contaminated ones. However, chronic toxicity tests have been used far less extensively than acute exposures for assessing marine sediments [55]. The assessment of sub-lethal effects on the growth of relevant benthic bioindicators, such as *M. insidiosum*, can offer a better picture of the long-term effects of dredging activities at the individual level, in particular the long-term consequences of the placement of the dredged sediment on the receiving area. In general, for delineating future assessment frameworks, the methods for evaluating ecotoxicity, bioaccumulation, and early warnings should

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be tailored to consider the potential impacts of all the phases of dredging, including excavation, lifting, transportation and placement.

Conclusions

Dredging is essential for maintaining navigation in canals and ports, but the contamination of sediments poses a challenge to the sustainable management of dredged material. To address this challenge, a QWoE approach was proposed and demonstrated through its application to sediments from a navigable canal of the Venice Lagoon, under a scenario of possible dredging activities. The experimental investigation led to the construction of five independent lines of evidence (LoEs) that considered chemical data, bioassays, bioaccumulation, biomarkers and transcriptomics.

The integration of chemistry-based evidence and adverse effects at the organism level confirmed the good quality status of the reference site. Results also indicated a quality worsening for sediment sampled closer to the Porto Marghera industrial area. The QWoE approach acted as an effective method to integrate large and complex amounts of data and to include an additional line associated with transcriptional analysis, promoting the possibility of adding other types of evidence to improve the robustness of the assessment. The incorporation of uncertainty, here associated with the determination of the evidence strength, provided a preliminary way to evaluate the degree of confidence in the risk characterization. The expression of confidence in the final hazard and the extended framework proposed in this study offers an improved tool that can assist in making more informed management decisions about dredged sediment.

Supplementary Information

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> Supplementary Material 1.

Author contributions

M.C.: conceptualization, methodology, software, formal analysis, data curation, writing—original draft, writing—review and editing, visualization. E.G.: conceptualization, methodology, writing—original draft, writing—review and editing, supervision, project administration. M.P.: formal analysis, investigation, data curation, writing—original draft, writing—review and editing. A.V.G.: supervision, project administration. C.B.: formal analysis, investigation, data curation, writing—original draft, writing—review and editing, visualization. F.C.: formal analysis, investigation, data curation. I.B.: investigation. M.M.: investigation, writing—review and editing, supervision, project administration. T.P.: supervision, funding acquisition. V.M.: supervision, project administration. D.A.: investigation, writing—review and editing. A.M.: writing—review and editing, project administration, funding acquisition. E.S.: conceptualization, methodology, writing—review and editing, supervision, project administration, funding acquisition.

Data availability

The datasets generated and used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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