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Destroy nothing, reveal everything: unveiling a century of pollution change in the Venice Lagoon (Italy) by non-invasive X-ray fluorescence biomonitoring of historical macroalgal specimens

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ABSTRACT

The Venice Lagoon is a highly biodiverse yet heavily anthropized coastal ecosystem that has undergone profound environmental transformations over the past century. Between 1930 and 1932, Aristocle Vatova conducted a comprehensive survey of the lagoon's algal flora, collecting specimens from 68 sites. These were later identified, mainly by Victor Schiffner, and are now preserved at the Natural History Museum of Venice. In this study, we compared the elemental composition of *Ulva* spp. specimens from this historical collection with that of samples collected in 2025 at 27 corresponding sites, using portable X-ray fluorescence (XRF) spectrometry. This fully non-destructive approach enabled a direct century-scale comparison of potentially toxic element (PTE) concentrations without damaging irreplaceable material. Overall, PTE concentrations in *Ulva* spp. decreased markedly from the 1930s to 2025, with median old/new ratios ranging from 1.2 for Ba to 7.4 for Zn and site-specific maxima exceeding 10 for P (11.6), Cr (10.6), Mn (17.0), Fe (14.5), and Zn (21.5). Localized increases in Cu and Cr were detected near urban (Venice, Chioggia) and industrial areas, suggesting possible point-source inputs. These findings demonstrate the value of historical macroalgal collections as quantitative archives for reconstructing long-term pollution trends and validate XRF as a robust, replicable analytical tool for non-invasive environmental monitoring. The century-scale decline in PTEs reflects a substantial improvement in the environmental quality of the Venice Lagoon and establishes a framework for applying similar retrospective biomonitoring approaches in other coastal ecosystems.

1. Introduction

Marine macroalgae are widely recognized as sensitive biomonitors, especially of metal pollution, owing to their sessile habit and rapid uptake kinetics (He and Chen, 2014; Rakib et al., 2021; Gubelit et al., 2023), which are strongly modulated by local water chemistry (AbouGabal et al., 2023). They are increasingly included in risk-assessment frameworks that integrate biomonitoring data with water and sediment chemistry (El-Mahrouk et al., 2023). Macroalgae are therefore suitable for both broad-scale environmental screening and quantitative monitoring, particularly when calibrated against water/sediment concentrations or used in deployed-biomonitor experiments (Ordóñez et al., 2023).

Among marine algae, several green (*Chlorophyta*) and brown taxa (*Fucales* and *Laminariales*) have been extensively used to assess contamination by metals, pesticides, and hydrocarbons. In particular, species of the genus *Ulva*, which includes taxa formerly assigned to *Enteromorpha*, are among the most effective biological sentinels for coastal pollution (García-Seoane et al., 2018). Their rapid growth, cosmopolitan distribution across salinity gradients, high surface area-to-mass ratio, and ability to accumulate dissolved and particulate contaminants through both passive and active mechanisms make them

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highly responsive to environmental variation (Wichard et al., 2015). *Ulva* thalli take up and release potentially toxic elements (PTEs; e.g. As, Cd, Cu, Pb, Zn) from seawater, reaching an equilibrium reflecting more or less recent inputs, depending on their physiological state and environmental conditions (Turner and Furniss, 2012; Bonanno et al., 2020). For this reason, their PTE concentration reflects spatial contamination gradients and point source emissions more reliably than instantaneous water samples (Pacín et al., 2025; Jönsson and Nordberg Karlsson, 2024). Consequently, *Ulva* species are widely regarded as effective biomonitors of coastal ecosystem health (Areco et al., 2021). Moreover, given their growing use as edible biomass, their PTE burdens are increasingly assessed in the context of food safety and value-chain regulation (Vargas-Murga et al., 2025).

Beyond single time point analysis, macroalgae can also serve for diachronic assessments of pollution trends (Miller et al., 2020). Two main approaches are typically adopted: *i*) repeated sampling through time using standardized protocols, and *ii*) retrospective analysis of preserved herbarium specimens compared with newly collected samples. The latter enables investigation of environmental change across long temporal scales, but is inherently invasive, as conventional analytical methods require destructive analysis (Miller et al., 2020). Given that historical specimens represent valuable cultural and scientific heritage, this approach can hardly be widely applied (Bakker et al., 2020; Seggi et al., 2024).

The Lagoon of Venice (LoV) represents a paradigmatic example of a highly biodiverse yet heavily anthropized coastal ecosystem. Its morphology and hydrodynamics have been profoundly shaped by centuries of human activities from Roman times to the industrial and post-industrial eras (Madricardo et al., 2019). Twentieth-century modifications, such as the construction of inlets, dredging, and increased boat traffic, have exacerbated sediment resuspension, salt-marsh erosion, tidal-flat deepening and seafloor alteration, driving large-scale habitat loss and geomorphological change (Carniello et al., 2009; Madricardo and Donnici, 2014; Sfriso et al., 2022). Despite these pressures, the LoV still sustains fisheries and aquaculture, providing key ecosystem services to surrounding communities and remaining central to ongoing efforts toward integrated management and conservation (D'Alpaos and D'Alpaos, 2021; Rova et al., 2022).

Between 1930 and 1932, Aristocle Vatova conducted an extensive survey of the lagoon's algal flora, collecting samples from about 70 sites (Schiffner and Vatova, 1938). These specimens, identified mainly by Victor Schiffner and preserved at the Natural History Museum of Venice (Seggi et al., 2024) are digitally accessible (https://dryades.units.it/MUVE_VS). This unique collection provides an unprecedented baseline for assessing a century-scale environmental change in the LoV through comparative biomonitoring.

X-ray fluorescence (XRF) spectroscopy is a multi-elemental, non-destructive technique that offers the great advantage of portable devices. It is based on the detection of characteristic fluorescence emitted by the element being measured, under excitation by radiation energy at a specific wavelength (Fedeli et al., 2024). Portable XRF devices enable non-destructive, on-site analysis, which is particularly valuable when dealing with precious or irreplaceable materials, such as artworks, as well as biological samples from historical collections. However, XRF instruments have limitations, notably lower sensitivity for certain trace elements compared with ICP spectrometers (Fedeli et al., 2024).

Multiple studies have already demonstrated the suitability of XRF techniques for assessing metal and metalloid accumulation in marine macroalgae. Carvalho et al. (1997) showed that XRF enables reliable, multi-element quantification of both essential and toxic metals in brown algae, supporting its application in pollution studies thanks to minimal sample preparation and high analytical precision. More recently, Rakib et al. (2021) applied the XRF technique to a broad range of macroalgal species from the Bay of Bengal, confirming the technique's effectiveness for biomonitoring, revealing species-specific patterns of metal bioaccumulation. Likewise, Navya et al. (2025) further validated the applicability of XRF for quantifying pollutants in macroalgae within coastal monitoring programmes.

The present study aimed at evaluating changes in the quality of the LoV environment in terms of PTEs from 1930 to 2025, using historical and fresh *Ulva* specimens and XRF as a non-destructive analytical method. Specifically, we tested whether the PTE concentrations in *Ulva* samples, as well as contamination levels and synthetic metrics expressing the overall ecological risk posed by PTEs, differed between the two periods.

2. Materials and methods

2.1. Study area

The LoV is a large, shallow transitional coastal ecosystem located along the northwestern Adriatic Sea in northeastern Italy. It covers approximately 549 km^2, of which about 432 km^2 is open water. The mean water depth ranges from 1.0 to 1.2 m, though navigation channels and inlets can reach depths of 8–15 m or more. The LoV is connected to the sea via three major inlets: Lido, Malamocco, and Chioggia.

Morphologically, the LoV is subdivided into three main basins (northern, central, and southern) that differ in hydrodynamics, salinity regimes, water renewal, and anthropogenic impact (Madricardo et al., 2019; Sfriso et al., 2025). The tidal range is modest (typically ~70–80 cm) but may exceed 1 m. Water renewal varies markedly across the lagoon, with residence times reaching up to ~40 days in semi-enclosed sub-basins (Sfriso et al., 2024). The lagoon includes extensive intertidal flats, salt-marshes, and shallow soft-bottom habitats, which play critical roles in sediment dynamics, primary production, and habitat complexity (Sorokin et al., 1996).

The LoV's hydrodynamics are strongly influenced by freshwater inflows, tidal exchange, wind- and wave-driven resuspension, and sediment transport processes, all of which are further modulated by anthropogenic alterations such as dredging, navigation, and inlet engineering (Sorokin et al., 1996; Madricardo et al., 2019). Consequently, the lagoon is highly sensitive to changes in sediment supply, hydrodynamic energy, salinity fluctuations, and anthropogenic perturbations (*i.e.*, pollutant input, nutrient loads, boating and ship traffic, dredging, etc.).

2.2. The Vatova-Schiffner herbarium

The algal herbarium assembled by Aristocle Vatova and Victor Schiffner, preserved at the Natural History Museum of Venice 'Giancarlo Ligabue', consists of 1406 sheets hosting 2209 dried specimens collected from 68 sampling sites in the LoV between 1930 and 1932. The labels, written by various authors, usually detail taxon names and collection localities (Seggi et al., 2024).

The collection was the basis for a comprehensive floristic report published by Vatova and Schiffner (1938) and is a detailed snapshot of the lagoon's algal community between the two World Wars. Thus it provides an invaluable resource for reconstructing temporal and spatial changes in the macroalgal flora of the Lagoon over the last century.

2.3. Selection of sampling sites

Across the historical 68 sampling sites, most specimens belonged to the genus *Ulva* (including taxa formerly referred to as *Enteromorpha*). Since *Ulva* species are abundant and widespread throughout the LoV, they were selected as the focus of the temporal comparative analysis. Since historical (OLD) samples cannot be subjected to destructive analyses, and species-level identification requires molecular markers, both OLD and newly collected (NEW) samples were treated conservatively as *Ulva* sp. Since *Ulva* species share similar thallus architecture, physiology and bioaccumulation traits (Vargas-Murga et al., 2025; El-Mahrouk et al., 2023), and since this study focused on broad patterns of PTE contamination rather than species-specific differences, we expected no or very limited systematic bias linked to the species composition of the samples.

At 27 of the original sampling sites (Fig. 1), at least 10 *Ulva* (mostly *U. rigida* C. Agardh) samples were available. These sites, distributed throughout the whole lagoon and encompassing both urbanized and relatively natural areas, were chosen for resampling (Fig. 1B). The coordinates of the original sampling sites were derived from the original map and the textual description published by Vatova and Schiffner (1938). Whenever possible, sampling was performed at the exact historical location. However, due to morphological characteristics or bathymetric alterations in the LoV over the past century, six sites were no longer accessible (S_3, S_{12}, S_{18}, S_{25}) or environmentally suitable (S_7, S_{13}); in these cases, sampling was conducted at the nearest feasible location (200–600 m from the original site) and under comparable environmental conditions.

The sampling campaign took place in May 2025. At each site, thalli of *Ulva* spp. were collected to obtain at least 10 replicate samples of dry material for elemental analysis. Two operators worked from a boat adapted for shallow-water navigation in the lagoon. Samples were collected manually or by gently scraping the seabed with a rake. Specimens were stored in sealed plastic bags, transported under refrigeration, washed for removing sediments, and then dried.

2.4. Thallus burden analysis

2.4.1. Historical specimens

Since historical (OLD) specimens can be neither damaged nor destroyed, a non-invasive portable X-ray fluorescence (XRF) instrument was used for elemental analysis (Olympus, Waltham, MA, USA). This instrument (Vanta series C), equipped with an Ag X-ray tube (15–40 kV) and a large-area silicon drift detector (resolution = 165 eV), was operated using the Vanta Desktop PC App v. 3.44. Measurements were conducted in both *Soil* and *Geochem* modes, each employing three sequential beams with an acquisition time of 20 s per beam. This configuration allows detection of a wide range of elements (Fedeli et al., 2024). For each site, based on availability, 6–10 OLD thalli were analyzed, with three measurements per thallus.

2.4.2. Recent specimens

The newly collected *Ulva* samples (NEW) were oven-dried at 40 °C for 24 h, then ground using a mortar and pestle. Approximately 1 g of dry material per sample was stored in tubes for analysis. To ensure cross-calibration between historical and modern materials, a subset of fresh *Ulva* thalli was air-dried and mounted on herbarium sheets following standard archival procedures. These mounted samples were first analyzed using the same non-invasive XRF protocol as the historical specimens, then ground and measured again as powdered material in plastic cups inserted into the device's sample chamber (Fedeli et al., 2024). This procedure allowed for calibration and direct comparability of the data obtained from historical and modern samples. The calibration procedure consisted in calculating sheet-mounted/powdered ratios both for *Geochem* and *Soil* measurements and selecting the calibration factor in the mode with the lowest uncertainty. The calibration factors used are reported in Table 1.

For each site, at least 10 NEW thalli were analyzed, with three measurements taken per thallus. The final data of both OLD and NEW samples are referred to as powder values, corrected according to Fedeli et al. (2024), and expressed as mg/kg dry weight. The limits of quantification (LOQ) for each element are reported in Table S1. Accuracy of analyses was verified with the certified reference materials IAEA-392 (Algae), IAEA-336 (Lichen) and GBW07603 (Bush branches and leaves), which showed recoveries in the range 93–113 % (Table S1). In case of values < LOQ, the LOQ value was used for calculations and statistical analysis.

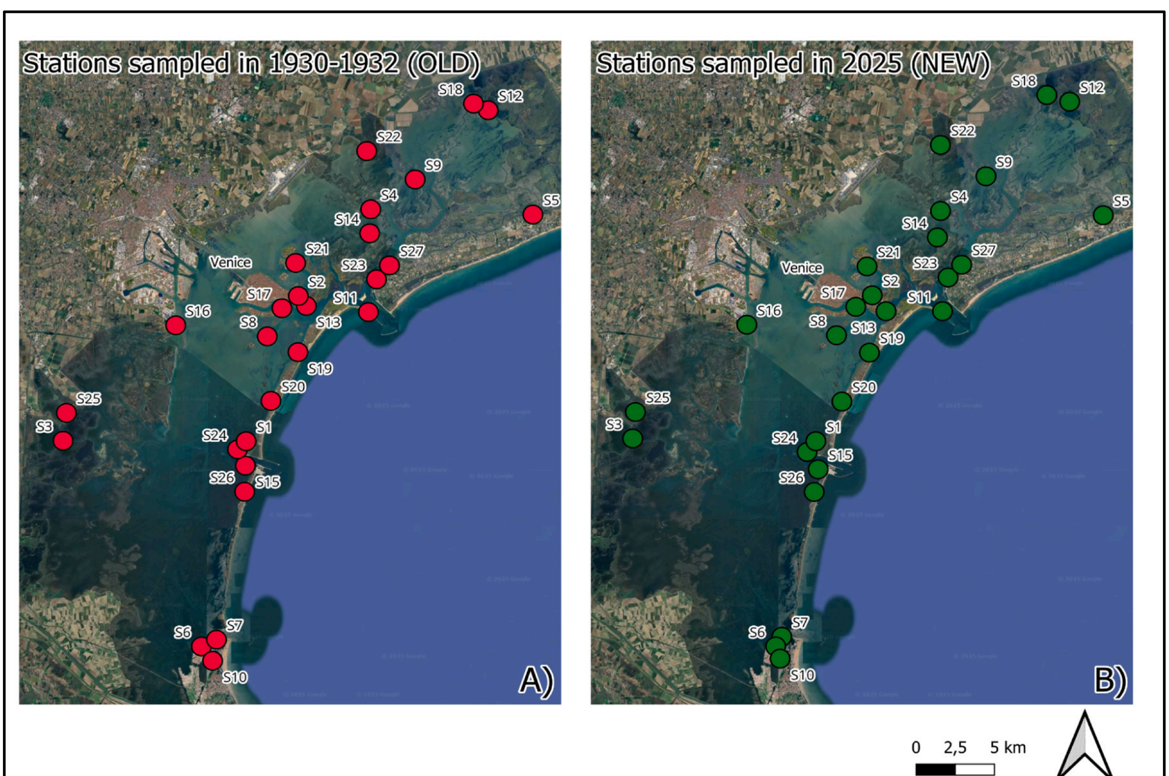

Fig. 1. Stations sampled in 1930-32 (**A**) and in 2025 (**B**).

Table 1
Calibration factor and uncertainty % for each element.

	calibration factor	uncertainty
Mg	1.8	15.2
Al	1.5	33.9
Si	1.6	34.5
P	1.1	3.2
S	1.1	19.6
Cl	0.7	32.5
Ca	3.6	23.4
K	1.3	43.0
Fe	0.6	13.2
As	1.2	5.9
Rb	0.2	16.7
Sr	0.4	11.1
Ba	5.1	53.3
Cr	2.9	35.7
Mn	2.1	15.8
Cu	2.6	26.3
Zn	0.7	15.2

2.5. Statistical analysis

Since the data largely failed to approach a normal distribution (Shapiro-Wilk test, $p > 0.05$), the OLD and NEW datasets are presented as median values ± error, the latter being calculated as the median absolute deviation (MAD) divided by the square root of the number of observations. To check for differences in the concentration of PTEs between OLD and NEW samples, a Wilcoxon signed rank test ($p < 0.05$) was run.

2.6. Contamination and ecological risk

To assess contamination by PTEs based on *Ulva* spp. accumulation, a Contamination Factor (*CF*) was calculated for each element using the formula:

$$CF = C_{element} / C_{background}$$

where $C_{element}$ = measured concentration and $C_{background}$ = the background concentration. Since direct $C_{background}$ values were not available, for each element these were assumed to be the median of the values below the 10° percentile of the frequency distribution of the data (European Commission, 2018).

CF values were interpreted according to the Håkanson (1980) scale: $CF < 1$ = low contamination; $1 < CF < 3$ moderate; $3 < CF < 6$ high; $CF > 6$ very high.

The Potential Ecological Risk Index (*PERI*), commonly used to evaluate whether a given level of contamination could pose a potential threat to the environment by integrating chemical and ecotoxicological information (Håkanson, 1980; Yüksel and Ustaoğlu, 2025), was determined using the following equation:

$$PERI = \Sigma ERF = \Sigma T \times (CF)$$

where *ERF* is the Ecological Risk Factor, *CF* is the contamination factor of each PTE, and *T* is the toxic-response factor assigned to each element (Rahman et al., 2019; Markert, 1992): Al = 2, As = 10, Ca = 1, Cr = 3, Cu = 5, Fe = 1, Mg = 1, Mn = 2, Rb = 2, Zn = 5.

Table 2
Interpretation of ERF and PERI values.

ERF	PERI	Risk
ERF <40	PERI <65	Low
40 < ERF <80	65 < PERI <130	Moderate
80 < ERF <160	130 < PERI <260	High
160 < ERF <320	260 < PERI <520	Very high
ERF >320	PERI >520	Critical

The resulting ERF and PERI values were interpreted following Håkanson (1980) and Iqbal et al. (2014)(Table 2).

3. Results and discussion

3.1. Calibration of sheet-mounted and powdered samples

The cross-calibration procedure consisted of analyzing newly collected (2025) *Ulva* samples first as mounted herbarium sheets and then as ground powder to calculate calibration factors. These factors were then applied to the 1930–1932 specimens, assuming a full transferability. We acknowledge that several factors could, in principle, act as confounding factors, e.g. long-term specimen degradation as well as possible diagenetic alterations thallus tissues; however these were regarded as minor issues, and no experimental approach was feasible to specifically evaluate them. One clear exception emerged for mercury. A marked chemical contamination of both OLD specimens and mounting papers was detected, consistent with the historical use of mercuric chloride ("sublimate"), a highly toxic compound widely employed in herbaria to prevent insect feeding. Consequently, Hg was excluded from the dataset. The calibration factors (Table 1) were affected by large (>30 %) uncertainties for some elements, namely Al, Si, Cl, K, Ba, Cr, and it must be considered that for these elements the outcomes are less robust. However, the significance of the differences between OLD and NEW samples (see further) for these elements was very high, and it is unlikely that they were affected. Nevertheless, these uncertainties may have affected the calculation of CF and PERI values.

3.2. PTE concentration in historical vs. recent Ulva samples

Across the 27 resampled sites, 17 elements were detected in both OLD and NEW *Ulva* samples (Fig. 2). Elemental patterns show marked temporal and spatial variations, consistent with mid-20th-century anthropogenic pressure followed by improvements associated with emission controls, wastewater treatment, and remediation measures (Zonta et al., 2020a; Soccio et al., 2018).

OLD samples exhibited significantly ($p < 0.05$) higher concentrations for all PTEs but Ca (Fig. 2), indicating a higher contamination status in the early 1930s, whereas NEW samples showed lower burdens, in line with recent improvements in water quality (Zonta et al., 2020b; Berti et al., 2020). Elements typically associated with industrial or urban inputs (*i.e.*, Fe, Zn, Cu, Cr, Mn, As) showed the largest differences, highlighting strong historical enrichment. Sulphur and Cl concentrations were notably higher in OLD specimens, particularly at sites S_1, S_9, S_{13}, and S_{24}, reflecting past organic and saline inputs from industrial and urban effluents (Pojana et al., 2003). Some PTEs showed the signature of historical pollution. Fe and Zn reached their highest OLD values at sites S_2, S_{13}, S_{19} (Fe) and S_2, S_{16}, S_{17} (Zn), consistently with impacts from the Porto Marghera industrial area (Zonta et al., 2007; Guarino and Sciarrillo, 2017). In OLD samples, Cu and Cr also peaked at sites S_6–S_{10} and S_8–S_9, respectively, consistently with the historical use of Cu-based antifouling and Cr-rich industrial processes (Berto et al., 2012; Guarino and Sciarrillo, 2017). Manganese (S_5, S_{22}) and As (S_2, S_{26}) showed OLD maxima indicative of past industrial and agricultural sources. Al and Si were higher in OLD samples (peaks: Al at S_7, S_{21}, S_{27}; Si at S_{11}, S_{22}, S_{25}, S_{27}), reflecting stronger historical sediment resuspension and particulate deposition in the more turbid lagoon waters (Zonta et al., 2007). In contrast, Ba, Sr, and Rb varied less over time: Ba showed a moderate OLD maximum at S_1; Sr peaked in NEW samples at S_6 and S_{26}; Rb remained higher in OLD samples (S_5, S_7, S_{20}), consistently with natural geochemical origins (Berti, 2018; Berti et al., 2020). Calcium was the only element not differing significantly ($p > 0.05$) between the two periods, and showing site-specific increases in NEW samples (*i.e.*, S_{22}, S_{25}), likely reflecting present-day salinity and water-exchange gradients.

The strongest OLD enrichment occurred for Fe, Zn, Cu, Cr, Mn, and As, often approaching or exceeding an order of magnitude, i.e., classic

Fig. 2. PTE concentrations (median ± error) at 27 stations: OLD (green), NEW (red). * indicates a statistically significant ($p < 0.05$) difference between OLD and NEW samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

urban-industrial tracers associated with metal processing, antifouling paints, and chemical manufacturing (Pavoni et al., 1987; Zonta et al., 2007). In contrast, Mg, Ca, Sr, and Ba showed ratios around 1, consistently with natural geochemical and marine processes (Berti et al., 2020). Moderate decreases were observed for S, Cl, and K, which may reflect a combination of shifting marine influence and reduced organic or nutrient loading in modern conditions(Berti, 2018; Pojana et al., 2003).

Overall, OLD specimens captured a high-impact industrial phase, whereas the NEW ones reflect a recovering lagoon. This demonstrates the value of algal biomonitors for long-term diagnostics and documents a clear ecological recovery following decades of interventions (Favero et al., 1996; Seggi et al., 2024).

Although brown algae and *Ulva* have marked biological differences making direct comparisons of accumulation efficiency inappropriate, the reduction trend observed is consistent with patterns increasingly reported for macroalgal biomonitors worldwide at multiple spatial scales. Globally, a recent meta-analysis showed widespread declines (60–84 %) in major metals in brown algae over the last 90 years, largely linked to improved environmental regulations and reduced bioavailable metal inputs (Aboal et al., 2023). Regionally, long-term observations in the NW Atlantic revealed significant decreases in Cu, Cr, Hg, Cd and Ni in *Fucus* spp. over three decades (Pacín et al., 2025), and similar trends were documented in Ussuri Bay following the shutdown of major pollution sources (Kozhenkova et al., 2021). Locally, near pollution hotspots, remediation interventions have led to marked reductions in metal accumulation in macroalgae, including at AMD-impacted Welsh sites monitored between 1987 and 2017 (Søndergaard and Mosbech, 2022; Chalkley et al., 2019). Overall, these convergent findings indicate that declining PTE levels in macroalgae reflect a broad-scale improvement in coastal environmental quality.

Site-wise patterns of OLD/NEW median ratios (Fig. 3) summarize century-scale trends. Spatially, the highest ratios occurred at central and inner lagoon sites (S_2–S_{10}, S_{13}–S_{17}), reflecting historical and residual urban-industrial pressure from Porto Marghera and Venice (Pojana et al., 2003; Zonta et al., 2007; Juhmani et al., 2021). Conversely, southern and outer lagoon sectors (S_{20}–S_{27}) showed ratios close to or slightly below 1, consistently with lower anthropogenic pressure and stronger marine influence (Berti et al., 2020).

Laminar and tubular *Ulva* species have been reported to differ in their metal-loading capacities (Ali et al., 2017; El-Din et al., 2014; Agrawal et al., 2022; Mohamed and Khaled, 2005), but we are confident that this issue does not compromise the robustness of our comparison between historical and recent samples since if differences in metal uptake exist, they are substantially lower than the century-scale differences (often ≥ an order of magnitude) observed between the historical and recent samples, and the measured XRF concentrations primarily reflect environmental contamination, not fine-scale species-specific differences. This conclusion is also supported by the spatial coherence of historical enrichment patterns with known industrial hotspots, such as Porto Marghera.

Several studies have documented intra-annual fluctuations in metal concentrations in *Ulva* spp. For example, Villares et al. (2002) reported significant seasonal oscillations across 22 sites along the NW Spanish

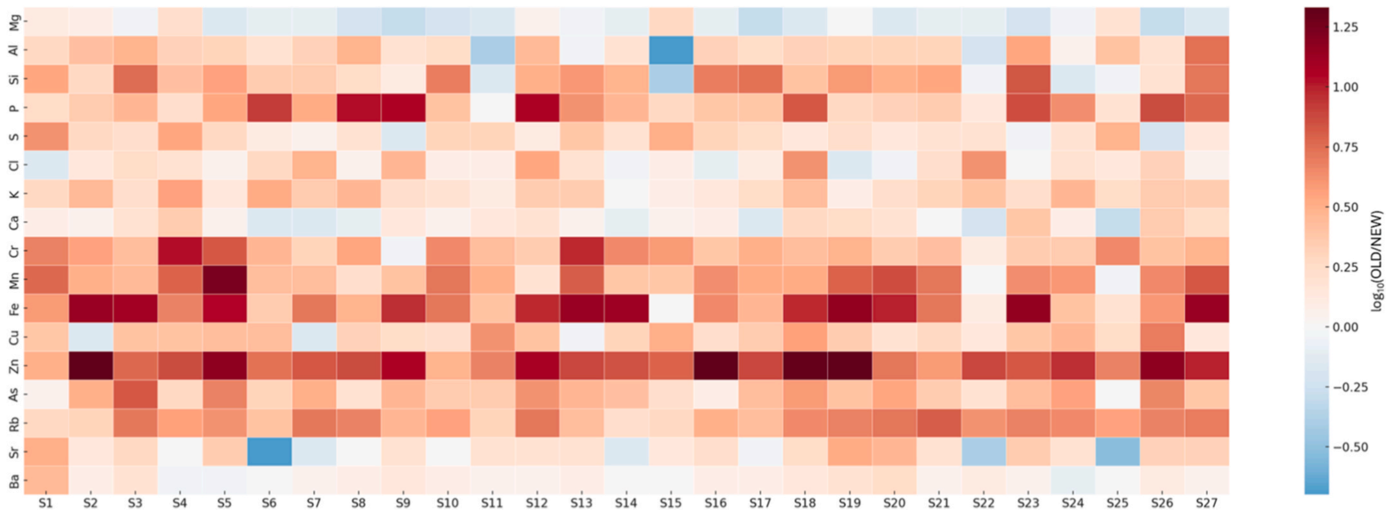

Fig. 3. Heatmap ($\log_{10}$) of OLD/NEW ratios by site and element.

coast, typically with lower values in summer and higher levels in autumn–winter. Similar patterns have been observed for *Ulva rigida* in the Dardanelles (Ustunada et al., 2011) and for *Ulva lactuca* along the Atlantic coast of Morocco (Kaimoussi et al., 2004). In the Venice Lagoon, Favero et al. (1996) reported highly significant seasonal variability in *U. rigida* over an 18-month period. However, even if seasonality is a recognized source of short-term variability in *Ulva* PTE concentrations, our results indicate that seasonal effects cannot account for the observed magnitude or pattern of the century-scale differences. OLD/NEW ratios frequently reach or exceed one order of magnitude at multiple sites for several elements, specifically P (11.6), Cr (10.6), Mn (17.0), Fe (14.5) and Zn (21.5). Such amplitudes are far greater than the seasonal fluctuations reported in the literature, which generally fall within much smaller intra-annual ranges. Seasonal variation would be expected to affect all sites similarly. Instead, we observe that the largest OLD enrichments occur at sites historically impacted by industrial and urban emissions, particularly those influenced by the Porto Marghera industrial complex. Conversely, outer-lagoon sites, less affected historically, show OLD/NEW ratios close to 1. This geographic structure is incompatible with a phenological or seasonal driver. The 1930–1932 herbarium specimens were collected across different months and years, inherently incorporating a natural range of seasonal conditions. This dilutes the possibility that a single seasonal bias explains the systematically higher OLD values observed across most stations. The NEW samples were collected within a narrow seasonal window, reducing intra-annual variability and yielding a coherent contemporary baseline. Taken together, these arguments strongly suggest that the OLD/NEW contrasts predominantly reflect long-term environmental change, not seasonal dynamics.

3.3. Contamination and ecological risk

Background values of PTEs accumulated by OLD and NEW *Ulva* samples differed markedly for several elements (Table 3). Concentrations were broadly comparable for Mg, Al, Si, S, Ca, Sr and Ba, but much higher (OLD/NEW >2) in OLD samples for Cl, K, Cu, As, Rb, and especially for P, Fe, Cr, Mn and Zn (Fig. 4). This clearly suggested that baseline pollution in the water and sediments of the LoV was definitely higher in the last century for many elements of urban/industrial concern, while elements largely linked to sediment geochemistry remained relatively constant (Berti et al., 2020). The higher concentrations of several PTEs (i.e., Fe, Zn, Cr, Mn, and Cu) in OLD samples confirm the significant influence of industrial and urban sources that affected the LoV during the 20th century (Guarino and Sciarrillo, 2017; Pojana et al., 2003). These PTEs are well-known tracers of metallurgical, chemical and shipyard activities that were predominant in the last century (Bernardello et al., 2006; Perin et al., 1997). In contrast, the significant reduction of most PTEs in the NEW samples indicates a substantial improvement in the LoV's environmental conditions. This shift is consistent with major environmental management actions, including waste treatment and sediment remediation programs implemented during the last two decades of the 20th century (Perin et al., 1997; SedNet Coalition, 2014).

Table 3
Background values of PTEs (mg/kg) accumulated by OLD and NEW samples expressed as median ± error.

	OLD	NEW
Mg	5936 ± 115	6515 ± 177
Al	316 ± 21	390 ± 6
Si	2478 ± 108	2066 ± 44
P	849 ± 33	284 ± 6
S	21205 ± 375	15945 ± 221
Cl	8245 ± 376	3676 ± 154
K	13812 ± 571	6803 ± 106
Ca	3847 ± 64	3528 ± 44
Cr	7.0 ± 1.0	1.7 ± 0.1
Mn	82.0 ± 2.0	22.0 ± 0.3
Fe	977 ± 79	330 ± 8
Cu	8.0 ± 0.2	3.7 ± 0.1
Zn	43.0 ± 1.0	8.7 ± 0.2
As	6.4 ± 0.1	2.6 ± 0.1
Rb	80.0 ± 0.4	35.0 ± 0.4
Sr	49.0 ± 1.0	52.0 ± 0.4
Ba	8.0 ± 0.1	7.4 ± 0.1

Using the background concentrations of NEW samples as reference, CF values (Fig. 5A) indicated very high contamination in OLD samples for Si, P, Cr, Mn, Fe, and Zn; high contamination for Al, Cl, K, Cu, As, and Rb; and moderate contamination for Mg, S, Ca, Sr, and Ba. In contrast, NEW samples showed a much-improved situation, with no element reaching the "very high contamination" category; only Cl and Cr exhibited high contamination, while all other elements fell within moderate levels. Among PTEs, Cr, Mn, Fe, and Zn clearly emerged as the most affected by industrial and urban discharges in the past (Berti et al., 2020). The persistently high contamination of Cl and Cr likely reflects the different ways these PTEs interact with the environment. Chromium tends to accumulate in sediments and can be remobilized under fluctuating redox conditions (Förstner and Wittmann, 2012; Perin et al., 1997), while Cl acts mainly as a conservative element, with its concentration mirroring the salinity gradient and the influence of past

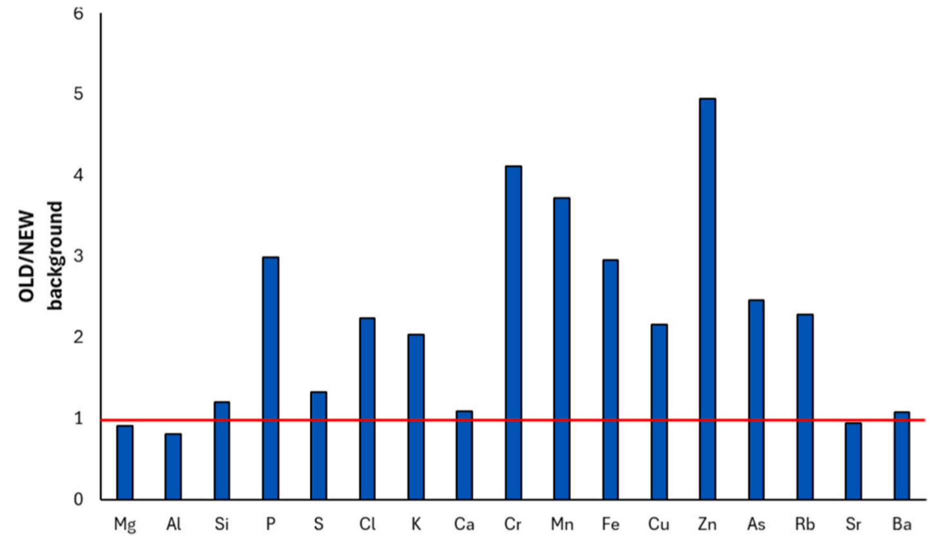

Fig. 4. Ratios between background values of PTEs accumulated by OLD and NEW samples. The red line indicates 1 (no change). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

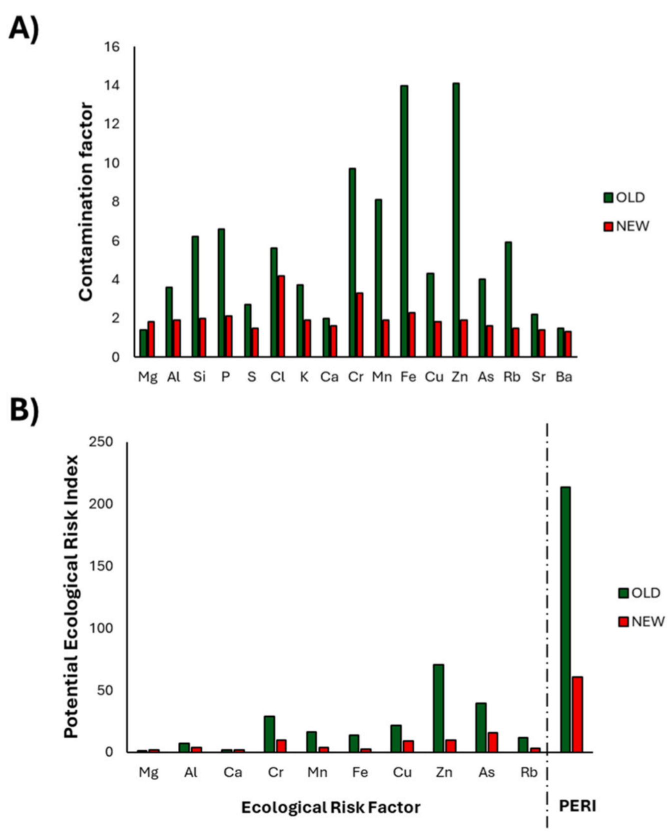

Fig. 5. Contamination factor (**A**) and ecological risk (**B**) of PTEs (median values) based on OLD and NEW samples.

industrial waste (Zancanaro et al., 2020).

ERF values for each PTE revealed a moderate risk only for As and Zn in OLD samples, and low risk for all remaining elements; the NEW samples showed low risk across all PTEs (Fig. 5B). The overall PERI was high for the OLD samples but low for the NEW samples (Fig. 5B). This indicates a clear temporal improvement in the environmental condition of LoV. The moderate ERF values for As and Zn in OLD samples identify these PTEs as the most critical and persistent contaminants in the historical lagoon, as similarly observed in other aquatic ecosystems (Chan et al., 2021; Hossain et al., 2025). Their strong reduction in the NEW samples confirms the decrease in bioavailable forms of these elements following the implementation of wastewater treatment, emission controls, and sediment remediation programs over the last 50 years.

Overall, the PERI results indicate that ecological stress in the LoV has been substantially reduced, reflecting the long-term success of environmental management and remediation efforts. The comparison between OLD and NEW *Ulva* specimens provides compelling evidence of the lagoon's century-scale recovery and validates the use of historical macroalgal collections as a robust tool for reconstructing past contamination patterns and assessing the outcomes of large-scale remediation measures.

4. Conclusions

To the best of our knowledge, this is the first study to use natural history collections for biomonitoring purposes with a fully non-destructive analytical approach. Portable-XRF proved to be a reliable, safe and replicable tool for quantifying PTEs in century-old algal specimens, preserving their integrity while unlocking their hidden chemical archive. These preserved macroalgae acted as trustworthy recorders of past marine conditions and can effectively support diachronic analysis of environmental change.

The comparison between historical and contemporary *Ulva* samples revealed a marked decrease in PTE concentrations across the LoV, demonstrating a substantial improvement in environmental quality and the long-term success of emission control, wastewater treatment, and remediation measures implemented.

Overall, the methodological framework developed in this research, combining natural history collections with modern non-invasive spectroscopy, offers a robust, replicable, and ethical tool for retrospective biomonitoring. It demonstrates that museum specimens can serve as time capsules of ecosystem health, providing a powerful and ethical pathway to trace environmental change, validate restoration outcomes, and inform global conservation strategies.

CRediT authorship contribution statement

Stefano Martellos: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Linda Seggi:** Writing – review & editing, Investigation. **Riccardo Fedeli:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Raffaella Trabucco:** Investigation. **Annalisa Falace:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Alessandra Metalli:** Investigation. **Stefano Loppi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability statement

Data are available on reasonable request from the corresponding author.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stefano Loppi reports financial support was provided by National Recovery and Resilience Plan. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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