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Research article

Does transplanted *Posidonia oceanica* act as a sink or source of trace elements? Ecological implications for restoring polluted coastal areas

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ABSTRACT

Despite the high potential of seagrass restoration to reverse the trend of marine ecosystem degradation, there are still many limitations, especially when ecosystems are severely degraded. In particular, it is not known whether restoring polluted ecosystems can lead to potentially harmful effects associated with contaminant remobilisation. Here, we aimed to investigate the role of *P. oceanica* transplanted from a pristine meadow to a polluted site (Augusta Bay, Italy, Mediterranean Sea) in two seasons of the year, as a sink or source of trace elements to the environment. The main results showed i) higher accumulation of chromium (Cr), copper (Cu) and total mercury (THg) in plants transplanted in summer than in winter, as well as an increase in Cr and THg in plants from sites with higher trace element loads; ii) an increase in leaf phenolics and a decrease in rhizome soluble carbohydrates associated with As and THg accumulation, suggesting the occurrence of defence strategies to cope with pollution stress; iii) a different partitioning of trace elements between below- and above-ground tissues, with arsenic (As) and Cr accumulating in roots, whereas Cu and THg in both roots and leaves. These results suggest that *P. oceanica* transplanted to polluted sites can act as both a sink and a source, sequestering trace elements in the below-ground tissues thus reducing their bioavailability, but also potentially remobilising them. However, the amount of trace elements potentially exported from *P. oceanica* to the environment through transfer into food webs via leaves and detritus appeared to be low under the specific conditions of the study site. Although further research into seagrass restoration of polluted sites would improve current knowledge to support effective ecosystem-based coastal management, the benefits of restoring polluted sites through seagrass transplantation appear to outweigh the potential costs of inaction over time.

1. Introduction

Nature-based solutions, such as ecosystem restoration, help to halt and reverse the degradation of ecosystems worldwide caused by ongoing anthropogenic pressures, as they can increase biodiversity, combat climate change, and improve human well-being. The United Nations (UN) Decade of Ecosystem Restoration, which runs between 2021 and 2030 aims to raise global awareness of the need to massively accelerate the restoration of degraded ecosystems.

Coastal marine ecosystems are among the most threatened globally, yet they play critical ecological roles and provide multiple ecosystem services. The need for effective restoration approaches capable of promoting the long-term recovery of the complexity, functionality, and attributes of coastal ecosystems in the face of increasing anthropogenic pressures, including climate change, has never been greater. Although restoration is a priority, especially where the natural recovery of degraded areas is slowed or prevented by physical or biological factors (e.g. persistent chronic stress, large-scale spatial disturbance, habitat fragmentation, low genetic diversity and habitat connectivity), it remains a major challenge due to environmental, technical, socio-economic, and political barriers (Stewart-Sinclair et al., 2020). This is particularly true in highly degraded and stressed environments, where the cumulative or even synergistic effects of multiple stressors reduce ecosystem resilience and drive them towards regime shifts (Unsworth et al., 2015).

Large- and local-scale disturbances, i.e., water quality degradation due to multiple pollutants, as well as hypersedimentation and burial, are among the main drivers of seagrass regression and loss in favour of fast-

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growing opportunistic primary producers (Unsworth et al., 2015), and are therefore recognised as the main environmental barriers that can compromise the success of seagrass ecosystem restoration (Pirrotta et al., 2015; Stewart-Sinclair et al., 2020).

Posidonia oceanica is one of the seagrass species that has suffered large-scale regression and loss in many Mediterranean areas since the last century. On the other hand, its great restoration potential can enhance the provision of a wide range of ecosystem services, from the sequestration and storage of blue carbon in the *matte* (i.e., below-ground living and dead tissues and associated organic sediments), the protection against coastal erosion, contribution to fisheries by supporting food webs, and water purification by filtering and absorbing nutrients and pollutants (Campagne et al., 2015; Macreadie et al., 2021). In addition, active restoration interventions of *P. oceanica* can trigger the re-establishment of positive feedback mechanisms, such as sediment trapping and oxygenation, as well as local and long-distance multitrophic facilitation, which further accelerate ecosystem recovery after disturbance and ensure its long-term success (Valdez et al., 2020). Indeed, successful examples of *P. oceanica* restoration report the reestablishment of meadow performances in relatively short time periods (e.g. Calvo et al., 2021; Piazzi et al., 2021). At the same time, this seagrass species is recognised as a good bioindicator of environmental pollution, as it can also reflect pollution levels in the surrounding environment due the uptake capacity of its root and leaf systems. Under this framework, the use of *P. oceanica* as a bioindicator (Bonanno and Borg, 2018; Pergent-Martini and Pergent, 2000) and the processes of trace element uptake, accumulation and partitioning in seagrasses growing in sites with different pollution levels (Malea et al., 2019; Pergent-Martini and Pergent, 2000; Schlacher-Hoenlinger and Schlacher, 1998) have been the most studied aspects. Given the role of *P. oceanica* in trace element cycling, the transfer of trace elements from seagrasses to ecosystem components is another aspect that has received attention in the literature (Cosio et al., 2014; Lewis and Devereux, 2009; Sanz-Làzaro et al., 2012). However, the experimental response of the plants transplanted to polluted areas has rarely been investigated. To our knowledge, only Capiomont (2000) transplanted *P. oceanica* from a pristine to a polluted site and vice versa to study the variation of Hg concentration in adult leaves. On the other hand, Ferrat et al. (2002) and Richir et al. (2013) experimentally exposed *P. oceanica* to high trace element contamination in seawater to investigate the uptake of dissolved TEs and partitioning mechanisms. In both cases, the effect of seasonality was not tested.

In this context, it is crucial to understand how to manage seagrass restoration at chemically polluted sites, an aspect that is almost completely unexplored today. Recently, only Fonte et al. (2023) and Oliveira et al. (2023) evaluated the response of another seagrass species used in restoration projects, *Zostera noltei,* to transplantation to a Hg polluted area, to assess its potential use as a Nature-Based Solution to minimize erosion, promote sediment accretion, and increase biodiversity in the area. In fact, while restoration can help to reestablish ecosystem functionality, resilience and the provision of ecosystem services, it is unknown whether it could lead to potentially detrimental environmental effects associated with seagrass mediated contaminant remobilisation.

In the central Mediterranean, Augusta Bay is a highly polluted basin due to long-term inputs of trace elements (mainly Hg, but also As, Cd, Co, Cr and Cu) and organic compounds (mainly PAH, PCB, and HCB) from the surrounding industrial area (Bellucci et al., 2012; Signa et al., 2015). The area is internationally recognised as an Hg hotspot in the Mediterranean basin (Sprovieri et al., 2011) and is included in the National Remediation Plan of the Italian Ministry of the Environment. Previous studies have provided evidence of bioaccumulation and biomagnification processes of trace elements (Signa et al., 2017a, 2017b), which pose serious risks to marine organisms and human health (Ausili et al., 2008; Scopelliti et al., 2015).

In this study, we investigated the ecological implications of restoring polluted coastal areas by transplanting *P. oceanica.* The main aim was to investigate the potential mobilisation and release of trace metals into the environment. To this end, we analysed (i) the accumulation and partitioning of trace elements (As, Cr, Cu and THg) in below- and aboveground tissues, (ii) the relationship between trace element contamination in *P. oceanica* and sediment, (iii) the biochemical response (in terms of changes in the concentration of phenolic compounds and soluble carbohydrates) of *P. oceanica* to trace element exposure and accumulation. We compared a transplanted area and a donor area during two transplanting campaigns, summer and winter, to take into account the seasonal metabolism of the plants.

2. Materials and methods

2.1. Study area

Augusta Bay is a semi-enclosed basin in the central Mediterranean Sea (eastern Sicily, Italy) that includes the heavily industrialised port of Augusta in the northern sector and the adjacent coastal system called Priolo Bay in the southern sector (Fig. 1). Uncontrolled industrial discharges from adjacent petrochemical plants over the last century have led to dramatic contamination of sediments with inorganic and organic compounds (Ausili et al., 2008; Bellucci et al., 2012; Sprovieri et al., 2011). In addition, dredging and illegal dumping of sediments outside the port of Augusta and the resulting high sediment resuspension have led to a massive regression of *P. oceanica* seagrass beds (Di Leonardo et al., 2017), with the result that the seabed is now covered by dead *matte* mixed with sandy and macroalgal patches.

The study is part of a more extensive transplantation campaign of *P. oceanica* in the southern part of Augusta Bay (Priolo Bay) using innovative biodegradable bioplastic supports (Mater-Bi ®) characterised by a 5-arm radial structure capable of anchoring to the seabed cuttings bearing plagiotropic rhizomes and multiple shoots (Bacci and La Porta,

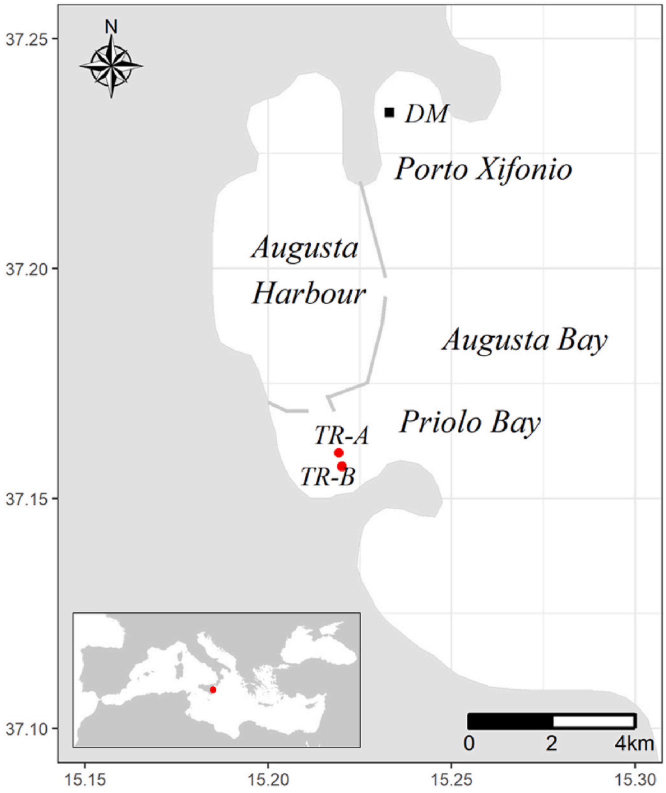

Fig. 1. Map of Augusta Bay showing the Donor Meadow area within the coastal area of Porto Xifonio (where the DM and Proc Ctrl sites are located) and the transplantation area within Priolo Bay with the two transplantation sites (TR-A and TR-B).

2022; Calvo et al., 2014). In summer (SUM, starting in June) and winter 2014 (WIN, starting in November), a total of 20,000 cuttings growing on *matte* with at least three shoots each were collected from the healthy seagrass meadow (hereafter referred to as donor meadow DM) located at a depth of approximately 13 m in the north-eastern part of the Bay of Augusta (Porto Xifonio) and classified in good status according to the PREI index (Bellissimo et al., 2020) and were transplanted onto dead *matte* at two sites (TR-A and TR-B) about 200m apart in Priolo Bay (Fig. 1). Specifically for this study, at the beginning (M0) of both transplantation campaigns, three cuttings from DM were taken to the laboratory in the dark and in a refrigerated environment, to record the initial conditions of the seagrass (hereafter referred to as DM_0) in terms of trace element, phenolic and soluble carbohydrate concentrations. Further ten cuttings were transplanted onto dead *matte* within DM as a procedural control (Proc Ctrl) to test for the transplantation effect. After five months (hereafter referred to as M5), corresponding to November 2014 and April 2015 for the summer (SUM) and winter (WIN) transplantation campaigns respectively, three cuttings were also collected from the transplanted sites in Priolo Bay (TR-A_5 and TR-B_5), and also from the donor meadow (DM_5) and the procedural control (Proc $Ctrl_5$). In addition, at M0 and M5 of both the SUM and WIN campaigns, surface sediments were collected in triplicate from all study sites using PVC hand corers (Ø 4 cm; length: 25 cm).

2.2. *Laboratory analysis*

In the laboratory, *P. oceanica* shoots were divided into leaves, rhizomes and roots. To reduce the variability associated with tissue age, the third youngest leaves from the shoots of each cutting were selected for analysis and, after removal of the apical part and epiphytes, were pooled to form one of the three replicates per site for laboratory analysis (Romero et al., 2007). The same procedure was followed for the first centimetre of rhizome and for roots from the same shoots. Surface sediment (~3 cm) was sliced from the cores, homogenised and subsampled for subsequent laboratory analysis. All samples were then lyophilised and ground using a micro mill (Retsch MM200).

Ground samples were mineralised in a microwave system (MARS 5, CEM) with a solution of 67–70% HNO_3, 30% H_2O_2 and Milli-Q water for *P. oceanica* tissues, and of 67–70% HNO3, 30% HF, 30% H_2O_2 and Milli-Q water for sediment samples according to Signa et al. (2013) and 1996 method. Mineralised samples were then analysed by ICP-OES (Optima 8000, PerkinElmer) for the quantification of trace elements (TE: As, Cr, Cu, THg). Concentrations of As and THg were determined using a hydride generation system linked to the ICP-OES with the addition of a reducing solution consisting of 0.2% Na borohydride and 0.05% Na hydroxide. Analytical quality control was performed using certified reference materials (CRMs): *Lagarosiphon major* BCR-060 (Institute for Reference Materials and Measurements) for *P. oceanica*, marine sediment NIST-2702 (National Institute of Standards and Technology) for sediments. The recovery ranged from 84 to 101%. The detection limit was calculated as three times the standard deviation of the blanks ($n > 20$) and was 0.003 mg kg^{-1} dry weight (dw) for all TEs. All results are expressed in mg kg^{-1} dw.

P. oceanica tissues were also analysed for total phenolic compounds (TPC) and soluble carbohydrates (SC). TPC extraction from leaves and rhizomes was performed according to a modified protocol of Bolser et al. (1998) and Harrison and Durance (1989) by addition of MeOH 80%, followed by incubation for 24 h in dark and cold conditions. After centrifugation, the TPC in the supernatant fraction was determined colorimetrically at 765 nm following a 2-h reaction with 20% Na_2CO_3 and Folin-Ciocalteu reagent. Results are expressed as mg g^{-1} dw. Soluble carbohydrates (SC) were extracted only from rhizomes by incubation with 80% ethanol and then determined by colourimetric reaction at 626 nm (Alcoverro et al., 1999; Carnal and Black, 1989). The results are expressed in %.

2.3. *Statistical analysis*

Trace element concentrations were expressed as mean values (± standard deviation SD). To avoid distribution assumptions, the Kruskal-Wallis test was applied to compare trace element concentrations in *Posidonia oceanica* i) between the three tissues (leaves, rhizomes, roots) within each transplantation campaign (SUM, WIN) and site (DM_0/DM_5, Proc $Ctrl_5$, TR-A_5, TR-B_5); and ii) between sites within each transplantation campaign and tissue. In this last case, we tested for change in trace element concentration i) due to the experimental procedure (transplantation effect) by comparing DM_5 and Proc $Ctrl_5$; ii) naturally occurring after the 5 months from the start of the transplantation campaigns, by comparing DM_0 and DM_5; iii) due to transplantation in the polluted area by comparing TR-A_5 and TR-B_5 with Proc $Ctrl_5$ as a transplantation effect occurred in some cases (see paragraph 3.2 for details).

Multivariate analysis was carried out using Principal Component Analysis (PCA) in the three *P. oceanica* tissues for each site in the two transplantation campaigns (SUM, WIN) based on trace element data.

To assess the change in trace element concentrations after 5 months of transplantation, the mean change from baseline (DM_0) in sites by transplantation campaign and *P. oceanica* tissue, as well as the comparison between sites and transplantation campaigns, was estimated based on the least squares mean change (LSmc) using the *emmeans* R package (Lenth, 2022). To study the relationships between variables, linear models (LM) were applied when the normality assumption was retained according to the Shapiro-Wilk test (Royston, 1982); otherwise, generalized linear models (GLMs) were applied, as they allow the use of any distribution belonging to the Natural Exponential family (Lovison et al., 2011; Tomasello et al., 2016). To assess whether the concentration of trace elements in *P. oceanica* depends on that in the sediment, and whether this effect is different in the two transplantation campaigns (interaction effect), GLMs with Gamma family and identity link were run for each tissue of *P. oceanica* as follows:

$$\text{P-TE} = \beta_0 + \beta_1\,\text{S-TE} + \beta_2\,\text{SUM} + \beta_3\,\text{S-TE SUM}$$

where trace elements in *P. oceanica* are the dependent variables (response variables), and trace elements in sediments and "transplantation campaign" are the independent variables.

Linear models (LMs) were used to investigate the relationship between the biochemical response of *P. oceanica* and the concentration of trace elements in the respective tissues. The full model included all trace elements. Parsimonious models were obtained using a stepwise approach based on the lowest Akaike Information Criterion (AIC). GLMs and LMs were performed using data from transplantation sites only (Proc $Ctrl_5$, TR-A_5, TR-B_5). Analyses were performed using R 4.1.2 software (R Foundation for Statistical Computing: Vienna, Austria, 2020). A p-value lower than 0.05 was considered statistically significant.

3. Results

3.1. *Trace element concentration in surface sediments*

Trace element concentration (As, Cr, Cu, THg) in surficial sediment varied between sites at the beginning of the two transplantation campaigns (SUM and WIN), with transplantation sites (TR-A_0 and TR-B_0) showing overall higher values than the donor meadow (DM_0), except for As, which showed higher values only in TR-B_0 in both campaigns, and for Cu, which showed higher values only in TR-A_0 in WIN (Table 1). When comparing the values with national sediment quality guidelines SQGs (G.U.R.I., 2016) and international thresholds suggested by the literature (Long et al., 1995; MacDonald et al., 1996), Cr and Cu showed values well below all SQGs, except for Cu, which exceeded the threshold effect level (TEL) at TR-A_5 in SUM. Arsenic slightly exceeded both the

Table 1

Mean trace element concentration (standard deviation SD) (mg kg^{-1} dw) in the surface sediment of the donor meadow site (DM_0) and the two transplantation sites in Augusta Bay ($TR\text{-}A_0$ and $TR\text{-}B_0$) at the beginning (M0) of the two transplantation campaigns (summer SUM, winter WIN). Sediment quality guidelines (SQGs) and international thresholds suggested by the literature, i.e., effect range low (ERL) and effect range mean (ERM) values according to [b]Long et al. (1995), threshold effect level (TEL) and probable effect level (PEL) values according to [c]Macdonald et al., (1996) and reference chemical levels (L1 and L2) according to [c]G.U.R.I., (2016) are also indicated. Values exceeding the above thresholds are shown in bold.

TE_{sed}	SUM			WIN			ERL[a]	ERM[a]	TEL[b]	PEL[b]	L1[c]	L2[c]
	DM_0	$TR\text{-}A_0$	$TR\text{-}B_0$	DM_0	$TR\text{-}A_0$	$TR\text{-}B_0$						
As	**11.18 (0.18)**	**11.23 (0.89)**	**12.50 (0.55)**	**8.55 (0.89)**	**8.02 (0.48)**	**11.19 (0.99)**	8.2	70	7.24	41.6	12	20
Cr	3.94 (0.67)	20.82 (2.87)	12.32 (2.68)	4.28 (1.33)	16.82 (1.78)	6.21 (0.54)	81	370	52.3	160	50	150
Cu	6.29 (0.39)	**27.68 (3.32)**	14.98 (2.58)	3.43 (0.53)	11.63 (1.33)	2.11 (0.54)	34	270	18.7	108	40	52
THg	0.07 (0.04)	**1.80 (0.40)**	**1.12 (0.41)**	0.05 (0.01)	**1.62 (0.03)**	**0.37 (0.05)**	0.15	0.71	0.13	0.70	0.3	0.8

effect range low (ERL) and the TEL at all sites in both transplantation campaigns and the L1 reference chemical level only at $TR\text{-}B_0$ in SUM, while THg exceeded, and even doubled, the effect range medium (ERM), the probable effect level (PEL) and the L2 reference level at $TR\text{-}A_0$ in both campaigns and at $TR\text{-}B_0$ only in SUM indicating a high level of pollution, as well as bioaccumulation and toxicological risk.

3.2. *Trace element concentration in Posidonia oceanica*

Arsenic and Cr were generally found in higher concentrations in roots than in leaves and rhizomes in both SUM and WIN transplanting campaigns (Table S1). In contrast, Cu (at all sites) and THg (at transplantation sites only) generally showed higher concentrations in both leaves and roots than in rhizomes (Table S1).

The comparison between sites highlighted in some cases the influence of the experimental procedure on the trace element concentrations detected in *P. oceanica* (transplantation effect). In particular, the concentration of As in the three tissues and Cr in leaves and roots was significantly different between DM_5 and Proc $Ctrl_5$ in the summer campaign (Fig. 2 a). In contrast, Cu concentration in leaves and rhizomes was significantly different between DM_5 and Proc $Ctrl_5$ in both summer and winter campaigns, while no transplantation effect was observed for THg (Fig. 2 b-d). With regard to natural variation (DM_0 *vs*. DM_5), the concentrations of As and Cu in the leaves, and only As also in the rhizomes, showed a significant increase during the five months of the summer transplantation campaign, in contrast to what was observed for As in the roots in SUM and Cu in the leaves in WIN. Similarly, Cr and THg concentrations in rhizomes and roots decreased significantly in SUM, and THg also in WIN.

Regarding the effect of transplantation in the polluted area, all four

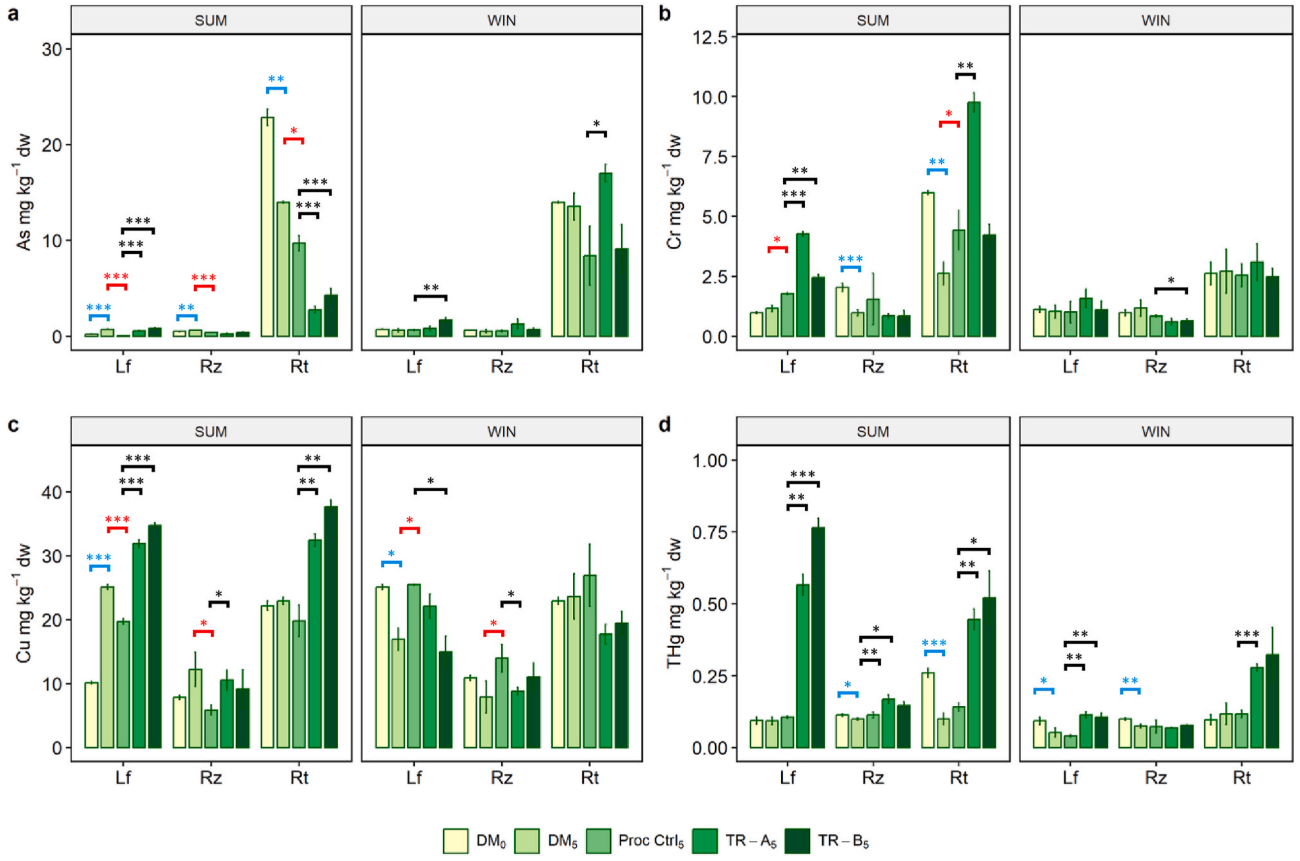

Fig. 2. Trace element concentration (mg kg^{-1} dw, mean ± standard deviation) in the three tissues (leaves Lf, rhizomes Rz, roots Rt) of *Posidonia oceanica* from the donor meadow at the beginning (DM_0) and after 5 months (DM_5) of transplantation, the procedural control (Proc $Ctrl_5$) and the two transplantation sites ($TR\text{-}A_5$ and $TR\text{-}B_5$) in the two campaigns (summer SUM and winter WIN). The results of the Kruskal Wallis test between sites (DM_0 *vs*. DM_5 in blue; DM_5 *vs*.Proc $Ctrl_5$ in red; $TR\text{-}A_5$ and $TR\text{-}B_5$ *vs*. Proc $Ctrl_5$ in black) are also shown. p-values: <0.05 (*); <0.01 (**); <0.001 (***). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

TEs analysed showed higher concentrations in the leaves collected in the summer campaign from both transplantation sites (TR-A_5 and TR-B_5) than in the procedural control (Proc $Ctrl_5$). The same pattern was observed in the leaves of the winter campaign only for As and THg concentrations, while Cu showed the opposite pattern and Cr did not differ between treatments (Fig. 2 a-d). As for the rhizomes, significantly higher concentrations in the transplanted sites than in Proc $Ctrl_5$ were recorded only in SUM for Cu and THg, while the opposite trend was found in WIN for Cr and Cu. Finally, regarding the roots, Cr and Cu showed significantly higher concentrations in the transplanted sites than Proc $Ctrl_5$ in SUM, THg in both campaigns, while As in WIN, and an opposite trend in SUM (Fig. 2 a-d).

Principal component analysis (PCA) revealed contrasting patterns among *P. oceanica* tissues, especially between leaves and roots compared to rhizomes. The first two principal components accounted for 87 and 81% of the explained variance in leaves and roots, respectively (Fig. 3 a, c). In both tissues, Cr, Cu and THg were highly correlated variables, and they were also strongly correlated with the first PC (represented by the horizontal axis), while As was more correlated with the second PC (represented by the vertical axis), especially in leaves. Moreover, in both leaves and roots, TR-A_5 and TR-B_5 SUM were well separated from Proc $Ctrl_5$ and DM_0 SUM, mainly because of the higher concentration of Cr, Cu and THg. All other cases (same sites in winter or different sites in both campaigns) were close to the centre (origin) of the plot, which indicates that they have an average value of trace elements, except for TR-B_5 WIN in leaves and DM_0 SUM in roots due to the higher As concentration. For rhizomes (Rz), the first two principal components accounted for 72% of the explained variance (Fig. 3 b). As and THg were correlated with the first PC, while Cu and Cr were correlated with the second PC. TR-A_5 and TR-B_5 SUM were on the opposite side of TR-A_5 WIN due to the higher concentration of THg in the formers, and the higher concentration of As in the latter. TR-B_5, Proc $Ctrl_5$ and DM_0 WIN, as well as DM_5 SUM were located together in the middle of the plot, representing a group with some similarity in trace element concentration.

Estimation of the mean change in trace element concentration in *P. oceanica* at each site compared to baseline (DM_0) by transplantation campaign and tissue showed that the concentration of all trace elements in the leaves of *P. oceanica* transplanted to TR-A and TR-B in the summer campaign increased significantly from baseline, and that both changes were greater than those observed in the Proc Ctrl site (Table S2). The same trend was observed in rhizomes and roots only for Cu and THg. In contrast, As and Cr concentrations in rhizomes and roots decreased significantly from baseline in both TR-A_5 and TR-B_5, and this decrease was in most cases significantly greater than that observed in Proc $Ctrl_5$. In the winter campaign, significant changes in trace element concentrations from baseline were observed only in a few cases, and in particular Cu and THg concentrations in leaves and roots showed a significant decrease and increase respectively from baseline in both transplantation sites. These changes were significantly greater than those observed in Proc $Ctrl_5$ (Table S2). Regarding the inter-seasonal comparison of the change in trace element concentration from baseline, a significantly greater increase was observed in the summer than in the winter campaign in *P. oceanica* leaves from both transplantation sites for all trace elements, but especially for THg. The same was observed for THg in rhizomes and Cu in roots, while the opposite trend, i.e., a significantly smaller increase in SUM than in WIN, was observed for As and THg in roots (Table S3).

3.3. *Relationship between trace element concentration in sediment and Posidonia oceanica*

The analysis of the interaction effect between sediment trace element concentration and transplantation campaign on trace element concentration in the different tissues of *P. oceanica* (leaves, rhizomes and roots) showed different responses depending on the element. In particular, for a unit increase in the concentration of trace elements in the sediment, the concentration of the same trace elements in the plant showed the following responses: i) As did not vary in leaves, whereas it increased (summer campaign) or decreased (winter campaign) in rhizomes and roots; ii) Cr increased more in the summer than in the winter campaign only in leaves and roots; iii) Cu decreased more in the winter than in the summer campaign in rhizomes, whereas it decreased in roots regardless of the transplanting campaign; iv) THg showed a significantly greater increase in the summer than in the winter campaign in all three *P. oceanica* tissues (Fig. 4, Table S4).

3.4. *Biochemical response of Posidonia oceanica to TE accumulation*

The concentration of total phenolic compounds (TPC) and soluble carbohydrates (SC) showed a uniform distribution among the sites in the winter transplantation campaign. In contrast, in the summer campaign, TPC in leaves and SC in rhizomes were higher and lower, respectively, in TR-A_5 and TR-B_5 than in Proc $Ctrl_5$ (Fig. 5). The best linear regression models, obtained through a stepwise procedure starting from a full model including all trace elements, highlighted some statistical associations (Tables S5 and S6). More specifically, in the summer campaign, at a unit increase in As concentration in leaves, TPC in the same tissue significantly increased, while, at a unit increase in THg concentration in

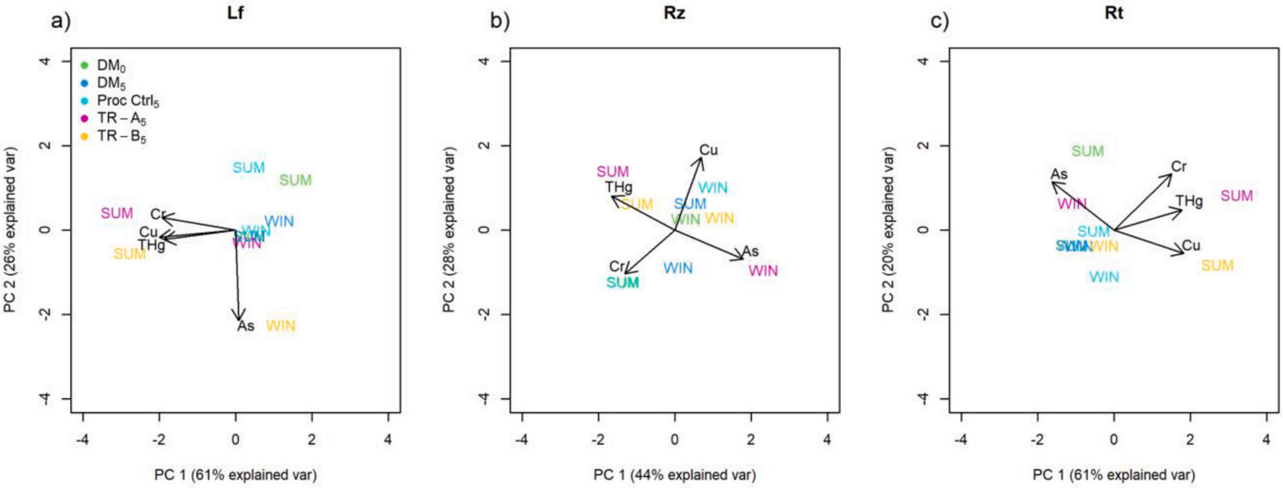

Fig. 3. PCA of trace elements in the three tissues (leaves Lf, rhizomes Rz, roots Rt) of *Posidonia oceanica* for each site in the two transplantation campaigns (summer SUM and winter WIN). For the sake of simplicity only the centroids are represented.

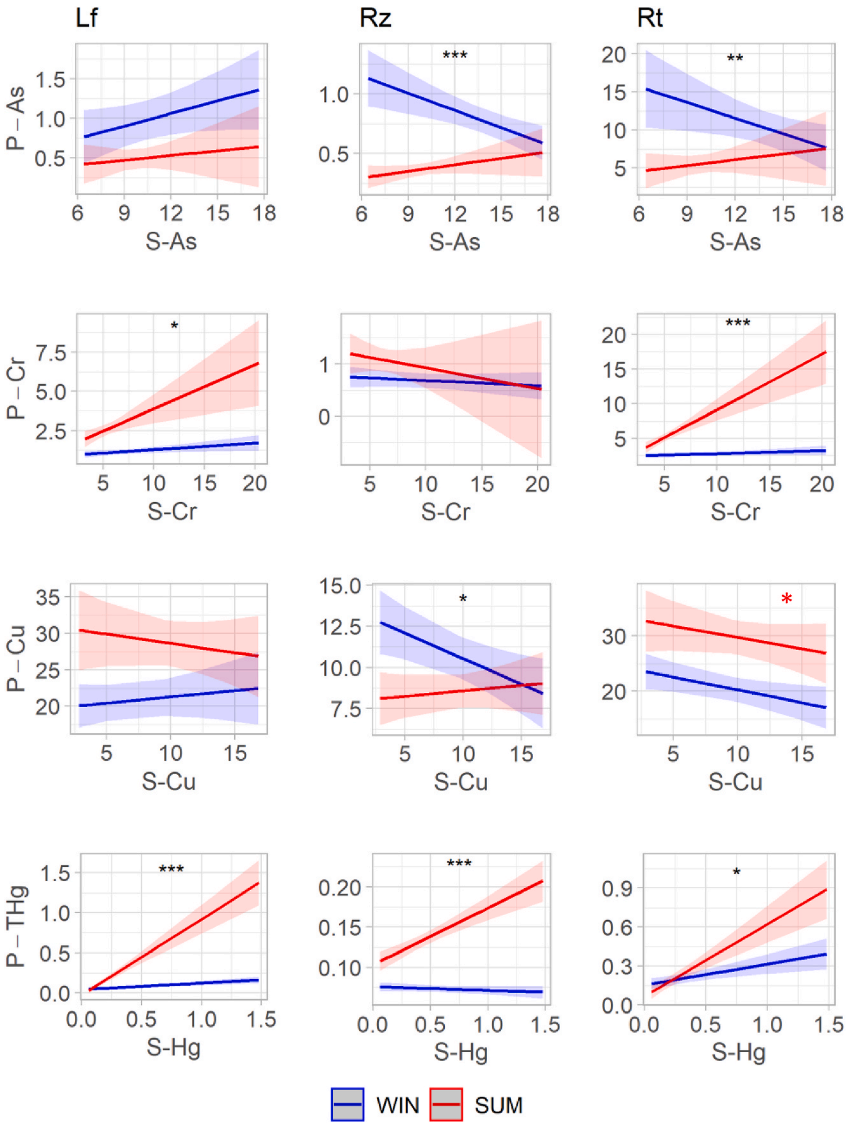

Fig. 4. Interaction effect between sediment trace element concentration (S-TE mg kg^{-1} dw) and season on trace element concentration in the different *Posidonia oceanica* tissues (P-TE mg kg^{-1} dw): leaves (Lf), rhizomes (Rz), roots (Rt). Black stars indicate significant interactive effects, red star indicates main effect. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rhizomes, SC significantly decreased. On the contrary, in the winter campaign, at unit increases in Cu concentration in rhizomes, SC significantly decreased (Table S5).

4. Discussion

Posidonia oceanica transplanted in Augusta Bay, one of the most polluted marine coastal areas of the Mediterranean Sea (Di Leonardo et al., 2014; Signa et al., 2017a; Sprovieri et al., 2011), showed an overall different chemical and biochemical response to pollutant exposure depending on the season of transplantation, as well as a clear partitioning of trace elements (TEs) among tissues. In particular, plants transplanted in summer bioaccumulated trace element to a larger extent than plants transplanted in winter, where, in some cases, biodilution of trace elements occurred. This result was particularly evident in leaves, although with some variability depending on the element analysed. Similarly, biochemical proxies for environmental stress showed a seasonal response to transplantation. More specifically, the higher leaf phenolic content and lower rhizome soluble carbohydrate content observed in the plants transplanted in the polluted sites in summer compared to those transplanted in the donor site (procedural control), were associated with As and THg accumulation, suggesting the occurrence of defence strategies to cope with pollution stress. As metal chelators, phenolic compounds are involved in the detoxification mechanisms of seagrasses, which respond to metal exposure with an increase in production and storage in leaves, as well as a decrease in sheaths and limited changes in rhizomes (Boumaza et al., 2022; Mannino and Micheli, 2020), in agreement with our findings. Similarly, soluble carbohydrate reserves in rhizomes decrease in response to disturbances, such as shading and sediment anoxia, due to the mobilisation of sucrose to meet respiratory and growth requirements (Gacia et al., 2012; Romero et al., 2007). Therefore, the observed decrease in carbohydrate content with increasing THg indicates a physiological response to pollution-induced stress.

The observed temporal pattern of trace element concentration, although apparently contrary to the literature where higher TE loads were observed in transplanted plants in winter than in summer by Capiomont et al. (2000) and in natural plants by Schlacher-Hoenlinger and Schlacher (1998), can also be explained by the seasonality of environmental variables and growth dynamics of *P. oceanica*. On the one hand, high temperatures and favourable light conditions experienced by transplanted plants in summer/early autumn may have favoured trace

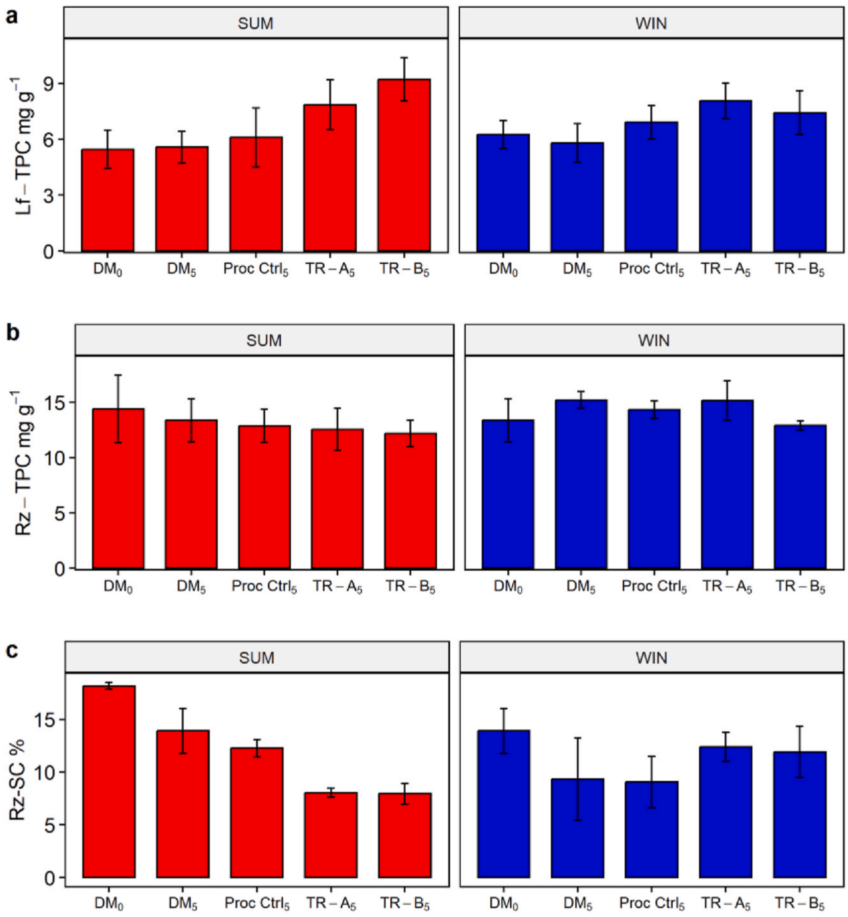

Fig. 5. Concentration of total phenolic compound (TPC) in leaves (a) and rhizomes (b) and soluble carbohydrates (SC) in rhizomes (C) of *Posidonia oceanica* in the donor meadow at the beginning (DM_0) and 5 months (DM_5) after transplantation, in the procedural control (Proc $Ctrl_5$) and in two transplantation sites (TR-A_5 and TR-B_5) in the two transplantation campaigns (summer SUM and winter WIN).

element uptake by increasing metabolic rates (Malea and Kevrekidis, 2013). In contrast, a "dilution" effect of trace element concentrations may have occurred in plants transplanted in winter, as the winter/spring period corresponds to the active growing season (Buia et al., 1992; Capiomont et al., 2000; Pergent-Martini and Pergent, 2000). Consistently, this general pattern has been more pronounced in leaves, which are the photosynthetically active tissues, than in perennial belowground organs (roots and rhizomes), which have lower productivity and metabolic rates (Buia et al., 1992; Schlacher-Hoenlinger and Schlacher, 1998). Similar patterns were also found by Oliveira et al. (2023) three months after transplanting *Z. noltei* in a mercury-contaminated lagoon area.

A clear trace element-specific partitioning between below- and above-ground tissues was also evident and only partially related to seasonality (Sanz-Làzaro et al., 2012). This provides insights into the uptake routes and physiological regulation mechanisms of transplanted plants and raises issues about the fate of trace elements in the environment following restoration activities in polluted sites, a currently neglected aspect of marine restoration. The greater tendency to accumulate As and Cr in roots than in leaves and rhizomes, and the positive relationship with their respective sediment concentrations in summer, suggest that *P. oceanica* acts as a sink, sequestering these two elements from the sediment and concentrating them mainly in roots, in line with the 'exclusion strategy' proposed by Bonanno and Borg (2018). In contrast, the observation that Cu and THg accumulated more in both leaves and roots than in rhizomes suggests the potential co-occurrence of the two main pathways for trace element uptake and accumulation in seagrasses: translocation from sediment interstitial water into roots to rhizomes and leaves *versus* translocation from surrounding seawater into leaves to rhizomes and roots (Ferrat et al., 2002; Malea et al., 2019). However, a positive relationship between trace element concentration in sediment and both leaves and roots of transplanted plants was found for only Cr and THg in both seasons. This result suggests that transplanted *P. oceanica* can increasingly sequester these two elements with increasing sediment contamination, and that some is transferred from roots to leaves, acting as both sink and potential source. The higher TE concentration in leaves may indeed reflect a 'removal strategy', according to which *P. oceanica* stores toxic elements in temporary aboveground organs, followed by their release during leaf senescence and decay, as already reported in *P. oceanica* for Hg (Cosio et al., 2014), and Ni and Zn (Bonanno and Di Martino, 2017). Therefore, *P. oceanica* transplanted in a polluted area showed a high biosequestration capacity for all the elements analysed, acting as a buffer against the effects of anthropogenic activities in coastal areas by reducing their bioavailability (Pergent-Martini and Pergent, 2000; Sanz-Làzaro et al., 2012; Serrano et al., 2020). At the same time, however, transplanted *P. oceanica* also showed the ability to remobilise and release some of the sedimentary contaminants into the environment through the accumulation in aboveground tissues and subsequent decay (Cosio et al., 2014; Lewis and Devereux, 2009). This is particularly relevant for THg, which biomagnifies along marine food webs and is particularly toxic to organisms, including humans (Bonsignore et al., 2013; Signa et al., 2017b). However, despite the general consensus on the occurrence of trace element transfer and Hg biomagnification in seagrass ecosystems, the assessment and quantification of the extent, pathways and flux rates between seagrasses and the ecosystem, including higher trophic levels, are, to our knowledge, relatively unknown. Only Sanz-Làzaro et al. (2012) through a mass balance analysis at the Mediterranean scale, showed that *P. oceanica* acts as a sink for several trace elements, including As and Cr, but as a source for others, including Cu, confirming the results of this study.

Having elucidated for the first time the chemical and biochemical response of *P. oceanica* transplanted to a polluted site, this study also attempted to further evaluate the potential ecological implications associated with the restoration of polluted areas in terms of the risk of contaminant mobilization and release to the environment by relating the averaged trace element concentration in leaves to the primary production of *P. oceanica* transplanted at a similar depth (1.4 g dw/shoot/year; Calvo et al., 2021) and the contribution of leaf blades to *P. oceanica* primary production (79%; Pergent-Martini et al., 2021). Based on these calculations, the amount of trace elements that accumulate in leaves in a year was rather low (0.0004, 0.003, 0.001 and 0.029 mg dw/shoot/year for THg, Cr, As and Cu respectively) under the specific conditions of the study area. Of this quantity, the amount potentially transferable from *P. oceanica* to the environment would further reduce, considering that only a part of the primary production in *P. oceanica* meadows in the Mediterranean is exported, as estimated by Pergent et al. (1997). According to these authors, about 40% is intended to be exported by hydrodynamic forcing, mainly in the form of leaf litter (33%) and less by herbivore consumption (7%) (Pergent et al., 1997). On the other hand, one third of the primary production (34%) is stored in the belowground *matte,* where roots and rhizomes mixed with sediment can persist for thousands of years with a very low rate of re-mineralisation (Mateo et al., 1997) and the remaining 26% is recycled *in situ* by decomposition, accounting for a total of 60% of the primary production remaining *in situ*. Although these are only estimates, they are consistent with the important role of *P. oceanica matte* as a biogeochemical sink, already documented in several coastal sites, including the study area (Augusta Bay, Apostolaki et al., 2022; Di Leonardo et al., 2017). High TE bioaccumulation in macroalgae growing on dead *matte* has also been previously recorded here, followed by TE transfer to primary consumers and Hg biomagnification to fish (Signa et al., 2017a, 2017b). The higher bioaccumulation potential of macroalgae than rooted macrophytes has already been documented, highlighting the risk of a 4- to 5-fold increase in Hg export to food webs in eutrophicated lagoons where macroalgae have replaced seagrasses (Coelho et al., 2009).

Seagrass transplantation appears to be a beneficial strategy even at contaminated sites, where, in addition to the general benefits expected (improved ecosystem functioning, stability and resilience, and enhanced provision of ecosystem services), it can improve environmental quality by sequestering trace elements in the *matte,* thus reducing their transfer into food webs. These benefits appear to outweigh the potential benefits of inaction over time, consistent with recent experimental results and modelled scenarios of restoration dynamics (e.g., Calvo et al., 2021; Oliveira et al., 2023; Orth et al., 2020; Sheaves et al., 2021).

5. Conclusions

This study aimed to understand and evaluate for the first time the dynamics and ecological implications associated with the transplantation of *P. oceanica* from a healthy donor site to a polluted site (Augusta Bay, Central Mediterranean). Transplanted plants responded to environmental pollution with element-specific biochemical mechanisms and transplantation season influenced trace element (TE) accumulation, with higher loads in plants transplanted in summer than in winter. Despite the accumulation patterns highlighted in transplanted plants, export of TEs to the environment and food webs appears to be low under the specific characteristics of the study area and certainly lower than TE storage in below-ground organs, consistently with the acknowledged role of the *P. oceanica matte* as a biogeochemical sink. Given the great ecological benefits and services provided by seagrass meadows, their high potential and increasing use for the restoration of degraded marine areas, but also the important role they play in TE cycling, further research of seagrass restoration at polluted sites is needed to improve current knowledge and to support efficient ecosystem-based coastal management.

CRediT authorship contribution statement

Geraldina Signa: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Agostino Tomasello:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Giovanna Cilluffo:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Cecilia Doriana Tramati:** Writing – review & editing, Investigation. **Antonio Mazzola:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Sebastiano Calvo:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition. **Salvatrice Vizzini:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.121008.

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