



Biochar as a sustainable amendment for mitigating mercury and methylmercury mobility in contaminated lagoon sediments: insights from incubation experiments

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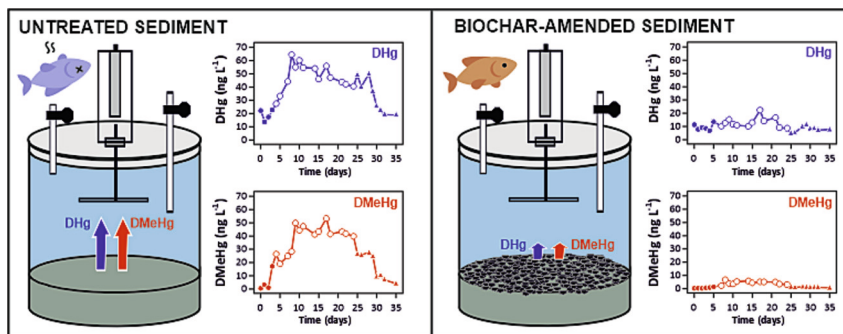
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HIGHLIGHTS

- Contaminated sediments showed Hg and MeHg release at the sediment-water interface.
- Biochar limited anoxic conditions in the water column and promoted re-oxygenation.
- Hg and MeHg effluxes were reduced by 75 and 90 % in the biochar-amended mesocosm.
- Biochar has proven effective in mitigating mobility and methylation potential of Hg.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) contaminated sediments may represent a secondary source of contamination, especially under anoxia which promotes the release of Hg, including its most toxic form methylmercury (MeHg), into the water column, posing a risk to marine life and human health. As such, sorbent amendments added to the sediment could be a sustainable approach to mitigate Hg methylation and mobility.

This study aimed to evaluate the effectiveness of biochar in reducing Hg and MeHg effluxes at the sediment-water interface (SWI) in a Hg-contaminated fish farm. Incubation experiments compared biochar-amended and untreated sediment using two benthic chambers, monitoring temporal changes in physico-chemical parameters (dissolved oxygen - DO, ORP), DHg, DMeHg, Fe, Mn, H₂S and nutrients (NO₃⁻, NO₂⁻, NH₄⁺, and PO₄³⁻) during oxic-anoxic transition and re-oxygenation.

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In the early stage, a faster DO consumption was observed, especially in the untreated system, alongside increases of NH_4^+ , PO_4^{3-} , Fe and Mn, due to the intense remineralisation of organic matter and reductive dissolution of oxy-hydroxides. In the untreated mesocosm, the highest DHg (64.3 ng L^{-1}) and DMeHg (53.1 ng L^{-1}) levels were observed under anoxia. Conversely, the biochar-amended chamber maintained lower DHg ($12.7 \pm 3.8 \text{ ng L}^{-1}$) and DMeHg ($4.26 \pm 1.26 \text{ ng L}^{-1}$) concentrations, with an average reduction of 75 and 90 %, respectively. Following re-oxygenation, oxic conditions were rapidly restored in the biochar-amended system whereas anoxia lasted for 6 days in the untreated chamber. Results suggest that biochar effectively mitigated Hg and MeHg fluxes at the SWI, reducing methylation potential, limiting the development of anoxia and promoting re-oxygenation.

1. Introduction

The occurrence, distribution and fate of mercury (Hg) in the environment is an issue of global concern due to its toxicity and the bioavailability of its organic and most toxic form, methylmercury (MeHg) (Ullrich et al., 2001; Clarkson et al., 2003; Regnell and Watras, 2019; Yang et al., 2021). Most of the Hg released into the environment via natural and anthropogenic sources is easily accumulated in sediments leading to a general worsening of the environmental quality in aquatic systems. Moreover, biogeochemical reactions occurring during early diagenesis may promote Hg methylation with the subsequent release of dissolved Hg (DHg) and MeHg (DMeHg) at the sediment-water interface (SWI), especially under anoxic conditions (Bloom et al., 1999; Fitzgerald et al., 2007; Merritt and Amirbahman, 2009; Beckers and Rinklebe, 2017) with subsequent potential bioaccumulation in the trophic chain (Bettoso et al., 2023), thus posing a risk to marine life, human health and often leading to expensive mitigation procedures. Hypoxic/anoxic conditions at the SWI may be common in confined aquatic systems and related to natural causes such as scarce water circulation, shallow water and intense degradation of sedimentary organic matter (OM) (Diaz and Rosenberg, 2008; Sánchez et al., 2018).

Remediation of Hg contaminated sites may be achieved through several approaches including the removal of Hg enriched matrices, their containment *via in situ* capping and the application of emerging technologies to reduce Hg and MeHg mobility and bioavailability (e.g. amendments, phytoremediation) (Eckley et al., 2020). Among these approaches, mass removal (e.g. dredging) and *in situ* capping are well-established and effective in reducing total Hg (THg) concentrations in sediments, especially in restricted contaminated areas. However, the excavation, removal and off-site storage of sediments enriched in Hg can be extremely expensive (Eckley et al., 2020) as well as cause resuspension events which can eventually promote the dispersion of both particulate and dissolved Hg species in the water column (Bloom and Lasorsa, 1999; Kim et al., 2006). On the other hand, although representing a low-cost alternative, *in situ* capping would cause a change in the bottom depth which is not feasible in the case of a lagoon environment characterised by shallow waters and sensitive sedimentary dynamics. Moreover, the general weak affinity between Hg and the capping material may promote the migration of dissolved Hg species through the capping layer and their subsequent dispersion (Liu et al., 2001; Hu et al., 2020; Sakhiya et al., 2020; Houssou et al., 2022; Qiu et al., 2022).

In recent decades, a low-cost and low-impact remediation approach has been provided by the application of sustainable sorbent amendments (such as activated carbon, biochar, organoclays and functionalised substrates) (Chen et al., 2019; Hu et al., 2020; Sakhiya et al., 2020; Houssou et al., 2022; Qiu et al., 2022). Among these materials, biochar is a high carbonaceous substance produced by thermal decomposition of organic material (biomass including wood, manure, leaves) without oxygen (pyrolysis) or under oxygen-limited conditions at relatively low temperatures ($<700^\circ\text{C}$) (Liu et al., 2015; Xiao et al., 2018). The biochar may be mixed with contaminated soils, sediments and capping materials or directly applied on capping layers or sediment/soil surfaces (Cornelissen et al., 2011; Gomez-Eyles et al., 2013; Patmont et al., 2015; Samuelsson et al., 2015). Although biochar is not designed to reduce or

remove THg concentrations from contaminated matrices, it enhances Hg and MeHg binding to solid particles reducing their mobility and bioavailability (Ghosh et al., 2011; Gilmour et al., 2013, 2018; Gomez-Eyles et al., 2013; Bussan et al., 2016; Liu et al., 2017; Wang et al., 2020; Yang et al., 2021). Indeed, it has been demonstrated that this material can reduce Hg and MeHg mobility in sediments by improving their binding to the solid phase through adsorption (Kong et al., 2011; Gilmour et al., 2013; Gomez-Eyles et al., 2013; Sørmo et al., 2022) due to its relatively high porosity, surface area and large number of adsorption sites and functional groups (Xiao et al., 2018).

Among the Mediterranean coastal areas, the Marano and Grado Lagoon (northern Adriatic Sea, Italy) is a transitional ecosystem where vital aquatic life coexists with economic/productive interests (fishing/fish farming) and anthropogenic activities (recreational and maritime). Sediments are known to be contaminated with Hg due to a double source of contamination: first the long-term mining activity (approximately 500 years) at the Idrija mine (Slovenia) along the Isonzo River drainage basin where the river is the main source of freshwater and suspended particles entering the Gulf of Trieste (Horvat et al., 1999; Covelli et al., 2001, 2007); second, the past direct Hg discharge from a now decommissioned chlor-alkali plant (CAP) in the central sector of the lagoon (Piani et al., 2005; Covelli et al., 2009; Pavoni et al., 2023).

This study aimed to evaluate the effects of biochar made up of the residue from pruning vineyard on the biogeochemical processes at the SWI as well as its ability in reducing Hg mobility from sediments to the overlying water column in one of the most productive fish farms of the Marano and Grado Lagoon. In order to achieve these objectives, a laboratory incubation experiment was performed comparing biochar-amended and untreated sediments. This approach allowed the observation of the effects of dissolved oxygen (DO) depletion on the biogeochemical processes regulating Hg and MeHg mobility at the SWI (Belias et al., 2007; Covelli et al., 2008; Emili et al., 2011, 2014; Koron and Faganelli, 2012). Although the biogeochemical cycling of Hg has been intensively investigated in this area (Piani et al., 2005; Covelli et al., 2009, 2012; Acquavita et al., 2012a, 2012b; Giani et al., 2012; Pellegrini et al., 2017; Petranich et al., 2017, 2018b; Bettoso et al., 2023), key information is lacking to understand if *in situ* sustainable remediation may be successfully employed to mitigate the risk of MeHg production and bioavailability.

2. Materials and methods

2.1. Environmental setting

The Marano and Grado Lagoon (northern Adriatic Sea, Italy) is one of the best conserved wetland systems in the Mediterranean Sea and extends for approximately 32 km with a maximum width of 5 km between the Tagliamento and Isonzo River deltas. The majority of freshwater inputs are restricted to the western sector (Marano Lagoon) with an overall average amount of approximately $70\text{--}84 \text{ m}^3 \text{ s}^{-1}$ mainly discharged by the Stella River (Sladonja et al., 2011). In addition, several drainage pumps primarily located near the coastal plain also contribute to water discharge from irrigation channels.

The majority of the edible fish species in the surrounding areas are produced by the few fish farms still in operation. Among those, Val

Noghera is one of the biggest (over 200 ha) and most productive fish farms in the lagoon (Fig. 1). The fish farm is characterised by a network of major and secondary channels that reach a maximum depth of 3 m and receives limited freshwater inputs from several artesian wells (with temperatures up to 33 °C), which allow for a decrease in salinity in summer and mitigate the low water temperatures during winter. The fish farm also benefits from controlled water exchange through sluice gates and rainwater. A semi-intensive farming strategy (Boatto and Signora, 1985) is employed in the fish farm. This approach allows fish to feed freely for a certain amount of time before gathering them in fish ponds and artificially feeding them until they reach commercial size. Seabream (*Sparus aurata*), seabass (*Dicentrarchus labrax*), and several mullet species (such as *Chelon labrosus*, *Liza salient* and *Mugil auratus*) make up the majority of the fish produced.

Previous research on the fish farm was focused on Hg and nutrient cycling at the SWI as well as on the Hg bioavailability for farmed fish species; results from these studies demonstrated that this environment represents a hot spot for Hg recycling and MeHg effluxes at the SWI, especially under anoxia (Petranich et al., 2018a, 2018b). Indeed, MeHg seemed to be produced and stored in the first 1–2 cm of hypoxic-anoxic sediments where sulphate-reduction mediated by bacteria occurs (Petranich et al., 2018b). Moreover, scarce water turbulence and mixing limit the amount of Hg lost by the system through volatilisation, thereby extending its persistence in the aquatic environment and its potential availability for methylation (Floreani et al., 2023).

2.2. Sampling for the incubation experiment

Field activities were performed at a selected site located in a shallow area (water depth 50 cm) in the eastern sector of the Val Noghera fish farm (site VN, Fig. 1) in September 2023. Surface sediments along with

the overlying water were manually collected using two cylindrical transparent benthic chambers made of Plexiglas ($h = 25$ cm, i.d. = 24 cm, wall thickness = 0.6 cm). For each chamber, a Plexiglas 10 cm long core a few millimeters in diameter lower than the chamber was collected, inserted into the chamber, and the chamber lid was sealed underwater leaving about 15 cm of bottom water above the sediment. Undisturbed short sediment cores ($h = 30$ cm, i.d. 16 cm) along with the supernatant water in direct contact with the sediment were also collected for both sediment and porewater characterisation before the incubation experiment.

2.3. Extrusion of the sediment cores and porewater extraction

The sediment cores were immediately transported to the laboratory and placed in a N₂-filled chamber to avoid variations in the original redox conditions. The redox potential (Eh) was promptly measured on the supernatant water, which was then collected, filtered (Millipore Millex HA 0.45 µm and Whatman GF/F filters 0.7 µm for nutrients) in different aliquots for the analysis of DO, hydrogen sulphide (H₂S), nutrients (nitrites NO₂⁻, nitrates NO₃⁻, ammonium NH₄⁺, phosphate PO₄³⁻), DHg, DMeHg, iron (Fe) and manganese (Mn) and then removed. The water aliquots for the analysis of nutrients were frozen, whereas those for H₂S, DHg, DMeHg, Fe and Mn were respectively acidified using Zn acetate (2 %), BrCl (0.5 % until the colour of the sample turned yellow according to the US-EPA Method 1631e (2002)), HCl (0.5 %) and HNO₃ (1 %), and stored in a dark refrigerator at 4 °C until analyses. Subsequently, the sediment cores were extruded, subsampled into 3 discrete levels (0–1, 1–2 and 2–3.5 cm), and Eh was measured in each sediment level prior to the subsample homogenisation. Each sediment subsample was then centrifuged (3600 rpm for 30 min) for the extraction of porewater which was filtered, collected into different aliquots and properly stored until

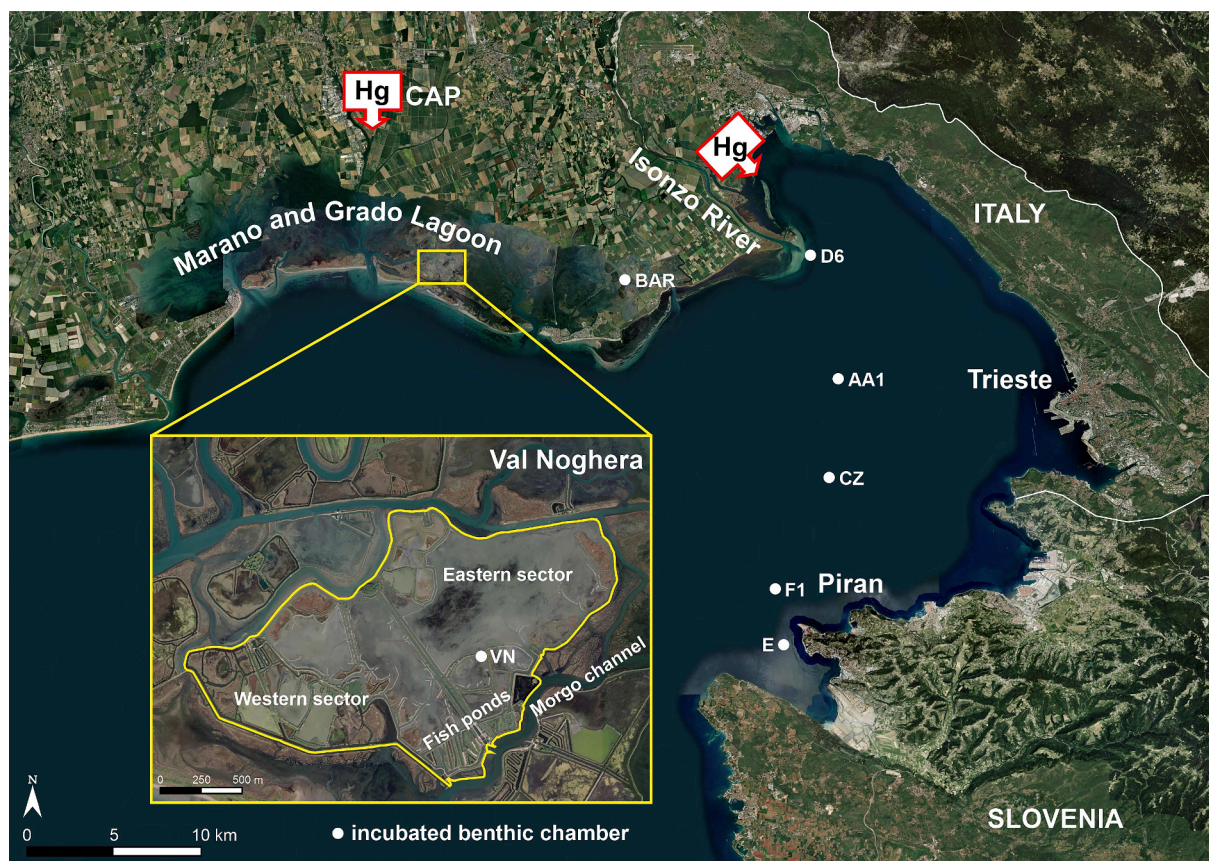


Fig. 1. Map of the study area with the location of the sampling sites for incubated benthic chamber experiments in the Val Noghera fish farm (VN, this study), in the Grado Lagoon (BAR, Covelli et al., 2008) and in the Gulf of Trieste (AA1, CZ, D6, E, F1, Emili et al., 2011, 2014; Koron and Faganeli, 2012).

analyses. The remaining sediment aliquots were frozen, freeze dried (CoolSafe 55-4 SCANAVC), ground and homogenised.

2.4. Incubation experiment

Following the careful transportation of the benthic chambers to the laboratory to minimise disturbance at the SWI, the overlying water was removed and carefully replaced with water collected at the same sampling site. Before water replacement, biochar (5 % biochar by dry weight of sediment, as typically performed in several other studies such as Gilmour et al., 2013, 2018; Bussan et al., 2016; Sørmo et al., 2022) was added to one of the chambers (benthic chamber B) and gently mixed on the sediment surface (1–2 cm), whereas the other chamber represented a control system (benthic chamber C) with untreated sediment. The biochar (supplied by the Italian company “Romagna Carbone” and named BRC) was leached in Milli-Q water overnight before the application and was produced by pyrolysis using material leftover from pruning vineyards at a temperature between 400 °C and 500 °C. This biochar was compliant with Italian legislation for soil improvement, and was listed as an approved soil amendment since 2015. The biochar characterisation, its aging in agricultural soils as well as the methodology employed for its production has been documented in previous studies (Baronti et al., 2014; Rombolà et al., 2015, 2023; Maienza et al., 2017). Physico-chemical parameters (pH, electrical conductivity (EC), total moisture, total solid (TS) and volatile solid (VS) percentages, loss of ignition (LOI), ashes, specific surface area (SSA), nitrogen (N_{tot}), carbon (C_{tot}), hydrogen (H) and sulphur (S)) previously determined on the BRC biochar are reported in Table S1.

Following the biochar application into the chamber B and the water replacement, both the chambers were incubated by sealing the top hole using lids and avoiding any oxygen supply from the atmosphere. The lids were equipped with two stopcocks allowing water collection during the experiment. The incubation was performed in a dark laboratory under stable conditions of air temperature and humidity (21.5 ± 1.1 °C and 73.5 ± 7.2 %) and lasted 36 days (Fig. 2).

According to the DO concentrations observed during experiment, the incubation was designed in 3 steps:

- Step (1) De-oxygenation: gradual transition from oxic to anoxic conditions with decreasing DO concentrations down to a value of 1 mg L^{-1}
- Step (2) Anoxia: stabilisation of DO at values below 1 mg L^{-1}
- Step (3) Re-oxygenation: by removing the upper lid of both chambers and leaving the water surface in direct contact with the atmosphere with the subsequent increase of in DO concentrations

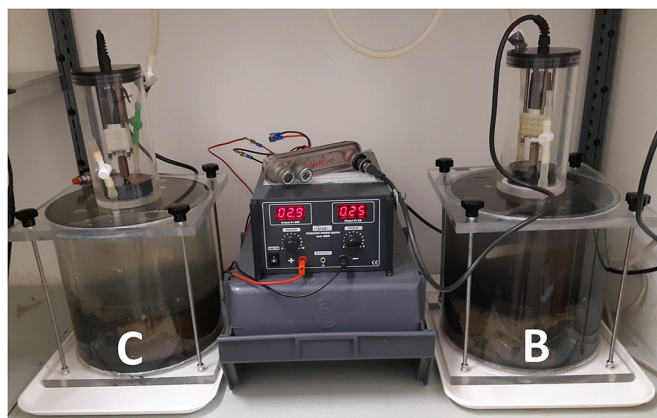


Fig. 2. Benthic chambers (control chamber C with untreated sediment and chamber B with sediment amended with biochar) used for the incubation experiment.

Following a gentle homogenisation of both the incubated mesocosms using a magnetic stirrer for 15 min, the overlying water inside the chambers was periodically collected by means of plastic syringes. At each sampling, the volume of water withdrawn was compensated for by adding an equal volume of water collected *in situ* and maintained in a closed circuit in an inert atmosphere for the duration of the experiment thus avoiding the introduction of oxygenated water. The sampled water was immediately filtered and split into different aliquots for the previously described analytical determinations. Moreover, the main physico-chemical parameters (pH, ORP, salinity, and temperature) were promptly measured using a multiprobe (HI98194, Hannah Instruments) every time samples were taken.

At the end of the incubation experiment, both the benthic chambers were placed in a N_2 -filled chamber as performed for the extrusion of the sediment cores. Following the removal of most of the water, the supernatant water in direct contact with the sediment was collected, filtered and subdivided in different aliquots for chemical analyses. Subsequently, a sediment sample was collected from the first cm of sediments from both chambers and properly stored for analyses after extracting porewater.

2.5. Mercury (Hg) and methylmercury (MeHg) analytical determinations

Total Hg (THg) concentrations in the sediment core levels were analysed by means of a Direct Mercury Analyser (DMA-80, Milestone) atomic absorption spectrophotometer according to US-EPA Method 7473 (1998a). Certified reference material (PACS-3 Marine Sediment Certified Reference Material, NRCC, Canada) was analysed in the same batch as the samples to evaluate the accuracy of the method and acceptable recoveries were obtained (95–106 %).

The analytical procedures for the determination of total and organic carbon (C_{tot} , C_{org}), total nitrogen (N_{tot}) as well as Fe and Mn in the sediment samples were reported in the Supplementary Material.

Total dissolved Hg (DHg) was quantified by means of Cold Vapor Atomic Fluorescence Spectroscopy coupled with a gold trap pre-concentration system (CV-AFS Mercur, Analytic Jena). According to US-EPA Method 1631e (2002), a pre-reduction was performed by adding $NH_2OH \cdot HCl$ (250 $\mu\text{L}/100 \text{ mL}$ sample until the yellow colour disappeared) followed by a reduction with $SnCl_2$ (2 % in HCl 2 %). The instrument calibration was carried out through the analysis of standard solutions ranging between 1 and 100 ng L^{-1} prepared by diluting a standard Hg solution (Hg standard solution, Merck Millipore). A certified reference material (ORMS-5, Canada) was analysed in the same batch as the sample for quality control and acceptable recovery was obtained (105 %). The limit of quantification (LOQ) was 1.44 ng L^{-1} and the precision of the analysis expressed as RSD% was <3 %.

Dissolved MeHg (DMeHg) was determined by sample distillation before Atomic Fluorescence Spectroscopy (AFS) analysis (US-EPA, 1998b; Horvat et al., 1993). Each sample was pretreated with 1 % H_2SO_4 8 M to avoid distillation interferences caused by excess chloride. Approximately 10 mL of each sample was distilled by adding 45 μL of Ammonium Pyrrolidine Dithiocarbamate (APDC) solution in sample teflon vials. Sample vials were connected to a flow of N_2 (60 mL/min) and maintained at 125 °C for approximately 90 min to allow distillation. The resulting distillate (between 80 and 85 % of the sample), was transferred into amber glass vials and diluted to a final volume of 40 mL with Milli-Q water. The pH was adjusted to 4.6 by adding 300 μL of acetate buffer and 50 μL of 1 % sodium tetraethylborate ($NaBET_4$) was added to ethylate MeHg. Subsequently, the amber glass vials were filled full with Milli-Q water and sealed with minimal headspace prior to analysis. The DMeHg content was determined using a Brooks Rand Merx AFS, Hg speciation GC and pyrolysis. The instrument calibration was performed using standard solutions ranging between 0.50 and 250 pg MeHg prepared through the dilution of a MeHg standard solution (1 mg L^{-1} , MeHg Brooks Rand) and the instrument detection limit was 5 pg MeHg/L. The accuracy of the analysis was evaluated by adding a known

amount of MeHg to the sample prior to distillation (a matrix spike ranging from 25 to 100 pg of MeHg depending on the sample). The matrix spike was performed on 25 % of the samples.

The analytical procedures for the determination of DO, H₂S, dissolved Fe and Mn as well as dissolved nutrients (NO₃⁻, NO₂⁻, NH₄⁺ and PO₄³⁻) in the water samples, including porewater, were reported in the Supplementary Material.

3. Results and discussion

3.1. Sediment and porewater chemistry before the incubation experiment

Reductive conditions were evident from the first centimetre of sediment (Table 1) suggesting that the remineralisation of OM through anaerobic metabolism was active at the sediment surface (Matijević et al., 2007). Total and organic carbon (C_{tot} and C_{org}) as well as N_{tot} occurred in a narrow range along the short sediment core (7.18 ± 0.28 , 4.69 ± 0.69 and 0.62 ± 0.13 for C_{tot}, C_{org} and N_{tot} respectively, Table 1) showing concentrations consistent with previous studies (Petranich et al., 2018a, 2018b), similar to those observed in another fish farm located in the Grado Lagoon (Valle Artalina, 2.70 ± 0.51 and 0.20 ± 0.07 % of C_{org} and N_{tot}, respectively; Covelli et al., 2008) and higher with respect to the sediments from the open lagoon (1.12 ± 0.03 and 0.15 ± 0.04 % of C_{org} and N_{tot}, respectively; Covelli et al., 2008). This is likely connected to the notable inputs of OM from activity related to the fish farm (Belias et al., 2003), enhancing the remineralisation of the labile organic detritus and the availability of N organic compounds (Hedges et al., 1997; Goñi et al., 2003).

Total Hg (THg) concentration in sediments ranged between 5.46 and 6.92 µg g⁻¹ (Table 1) notably exceeding the background level estimated for the nearby coastal sediments of the Gulf of Trieste (0.13 ± 0.04 µg g⁻¹, Covelli et al., 2006) as well as the local background for the western sector of the lagoon (0.02 µg g⁻¹, Covelli et al., 2017). Mercury (Hg) amounts of the same order of magnitude were observed by previous investigation in the fish farm (4.20 – 5.10 µg g⁻¹, Petranich et al., 2018b) as well as in the Marano (1.44 – 4.33 µg g⁻¹, Emili et al., 2012) and Grado (9.50 – 14.4 µg g⁻¹, Acquavita et al., 2012a) sectors of the lagoon. Comparably, Fe and Mn displayed quite constant concentrations in the short sediment core (3.28 ± 0.30 % of Fe and 327 ± 69 µg g⁻¹ of Mn, Table 1).

Dissolved Hg (DHg) increased from 15.1 ng L⁻¹ in the surtangent water to 46.8 ng L⁻¹ in porewater extracted from the first centimetre of sediment decreasing to approximately 20 ng L⁻¹ in the deepest core layers. Dissolved MeHg (DMeHg) was somewhat constant with increasing depth ranging between 3.16 ng L⁻¹ in the surtangent water and 5.16 ng L⁻¹ in the deepest core layer (Table 2). In the case of dissolved Fe and Mn, the trend was slightly similar, ranging from 447 and 911 µg L⁻¹ of Fe and Mn in the surtangent water to 1566 and 4204 µg L⁻¹ of Fe and Mn in first porewater level, then decreasing with increasing depth (Table 2). Although according to the expected early diagenetic sequence Fe reduction should occur below the Mn reduction zone (Froelich et al., 1979), the Fe and Mn reduction was observed in the first

2 cm of sediments as evidenced by the highest DHg, Fe and Mn concentrations in the uppermost porewater level. Indeed, the increase in DHg, Fe and Mn just below the SWI appears to be related to the desorption/dissolution of Fe and Mn oxy-hydroxides which are efficient scavengers under reducing conditions for several soluble metals including Hg (Gagnon et al., 1996; Bloom et al., 1999). The decrease in the concentration of dissolved Fe and Mn, as well as for DHg, in the deepest core levels may be associated with their precipitation in association with insoluble sulphides promoted by sulphate and Fe reduction (Petranich et al., 2018b and references therein). Nutrient concentrations in the surtangent and porewater samples were very low and often below LOQ. The only exception is represented by NH₄⁺ increasing from 75.0 µmol L⁻¹ in the surtangent water to 258 µmol L⁻¹ in the first porewater level and remained constant with increasing depth (Table 2).

3.2. Behaviour of DO, DHg, DMeHg, Fe, Mn and nutrients during the incubation experiment

The incubation experiment was performed under stable conditions of air temperature and humidity; similarly, notable variations of the main physico-chemical parameters such as temperature, salinity and pH were not observed in the biochar-amended mesocosm ($T = 20.3 \pm 1.2$ °C, salinity = 32.3 ± 0.3 PSU, pH = 7.60 ± 0.22) as well as in the untreated chamber ($T = 20.5 \pm 1.2$ °C, salinity = 32.5 ± 0.3 PSU, pH = 7.68 ± 0.37) during the 36 days of incubation. Variations of DO, ORP, H₂S, Fe, Mn, DHg, DMeHg, and nutrients during the incubation experiment in both benthic chambers are shown in Fig. 3 and discussed further on.

3.2.1. Step 1: de-oxygenation

During the de-oxygenation phase, the concentration of DO rapidly decreased from 7.20 to approximately 1 mg L⁻¹ in both the systems during the first days of incubation (Fig. 3) as the result of the DO consumption by heterotrophic bacteria in response to OM production (Rabalais et al., 2002; Eldridge and Morse, 2008). This phase lasted 4 and 6 days for the untreated and biochar-amended chamber, respectively, resulting in a slower DO consumption in the mesocosm amended with biochar, which seems to slow down the development of anoxia. The gradual DO consumption was associated with reductive conditions as testified by a general ORP decrease during this experimental step, especially in the untreated system. Indeed, ORP rapidly fell from 136 to -110 mV in the untreated chamber and from 168 to 17 mV in the biochar-amended system (Fig. 3). Under these conditions, the remineralisation of OM through anaerobic respiration is promoted resulting in the release of reduced species at the SWI such as NH₄⁺, dissolved Fe and Mn (Middelburg and Levin, 2009) whereas H₂S was mostly below LOD in both incubated systems. This suggested that the amount of DO at this early experimental stage, although relatively low, was enough to limit the activity of sulphate-reducing bacteria and the H₂S production. This is especially evident in the biochar-amended system whereas a certain amount of H₂S was observed in the untreated chamber during the last two days of de-oxygenation (1.59 and 6.23 µmol L⁻¹) and was most likely related to the faster transition from oxic to anoxic conditions. The gradual transition to anoxia also promoted the reduction of Fe and Mn oxy-hydroxides with subsequent release of dissolved Fe and Mn at the SWI (Kristiansen et al., 2002; Pakhomova et al., 2007; Lenstra et al., 2021) in both incubated mesocosms. Indeed, as DO gradually decreased, a clear increase in dissolved Fe (from values <LOD to 290 and 494 µg L⁻¹ in benthic chambers B and C, respectively) and Mn (from values <LOD to 287 and 453 µg L⁻¹ in benthic chambers B and C, respectively) was observed (Fig. 3). At this stage, the increasing trends of dissolved Fe and Mn were comparable between the two benthic chambers, suggesting a simultaneous dissolution and/or desorption of Fe and Mn oxy-hydroxides at the sediment surface (Burdige, 2006; Pakhomova et al., 2007; Homoky et al., 2011; Scholz et al., 2014).

Regarding DHg, quite constant concentrations were observed in each system (Fig. 3) although the untreated chamber showed values equal to

Table 1

Concentration of THg, Fe, Mn, C_{tot}, C_{org}, N_{tot}, percentage of C_{org} and C_{org}/N_{tot} molar ratio in the sediment core levels before the incubation experiment.

Parameter		VN 0–1 cm	VN 1–2 cm	VN 2–3.5 cm
Eh	mV	-179	-251	-390
THg	µg g ⁻¹	6.92	5.92	5.46
Fe	%	3.27	3.57	2.98
Mn	µg g ⁻¹	401	316	265
C _{tot}	%	7.22	7.44	6.89
C _{org}	%	4.33	5.48	4.24
% C _{org}	%	60.0	73.7	61.6
N _{tot}	%	0.63	0.75	0.49
C _{org} /N _{tot}		8.55	9.13	10.9

Table 2

Concentration of DHg, DMeHg, Fe, Mn, NO_2^- , NO_3^- , NH_4^+ and PO_4^{3-} along with the percentage of DMeHg/DHg in the surtutant water and porewater extracted from the sediment core levels before the incubation experiment (n.c.: not calculable).

Parameter		VN Surtant water	VN 0–1 cm	VN 1–2 cm	VN 2–3.5 cm
DHg	ng L ⁻¹	15.1	46.8	21.3	20.8
DMeHg	ng L ⁻¹	3.16	4.55	1.87	5.16
% DMeHg	%	20.9	9.72	8.76	24.8
Fe	μg L ⁻¹	447	1566	1354	331
Mn	μg L ⁻¹	911	4204	2998	796
NO_2^-	μmol L ⁻¹	0.65	0.32	<LOQ	<LOQ
NO_3^-	μmol L ⁻¹	882	n.c.	n.c.	n.c.
NH_4^+	μmol L ⁻¹	75.0	258	243	254
PO_4^{3-}	μmol L ⁻¹	0.06	0.49	0.06	0.05

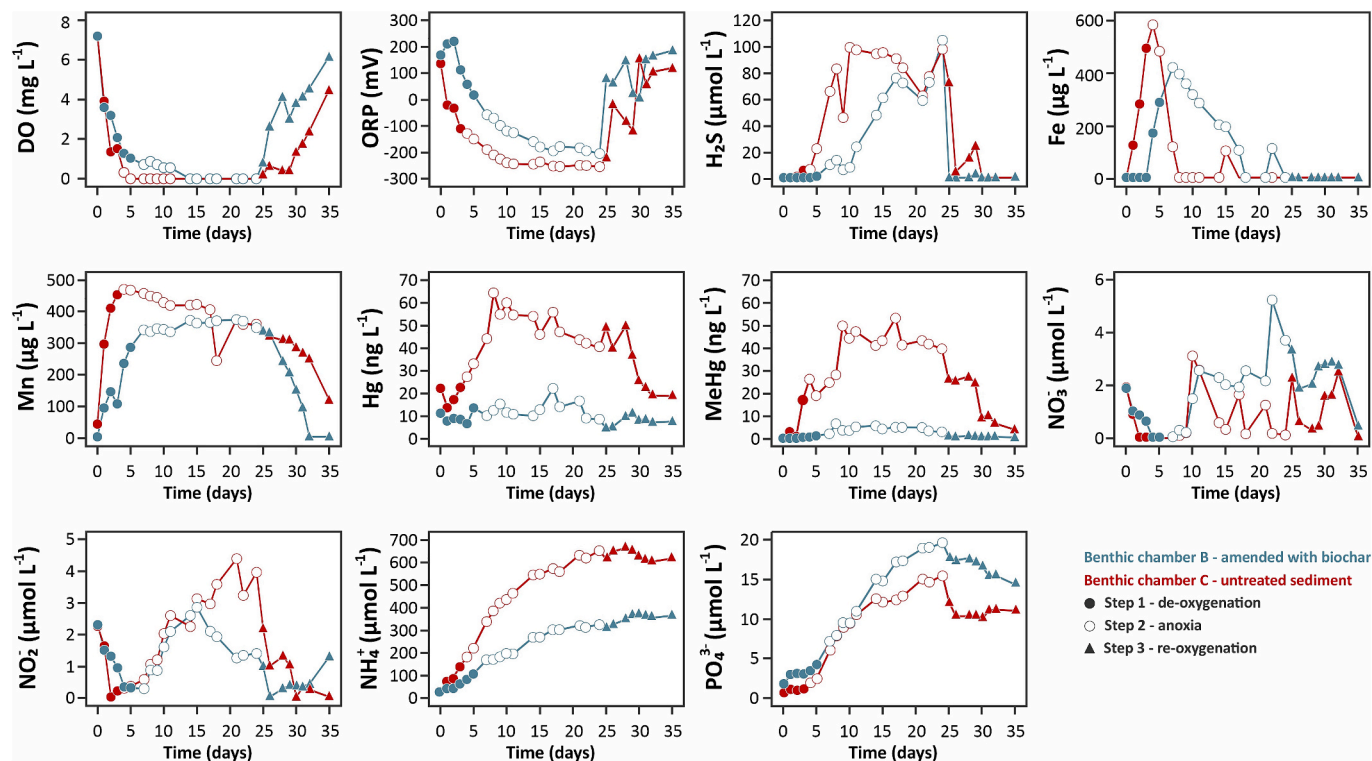


Fig. 3. Concentrations of DO, ORP, H_2S , Fe, Mn, DHg, DMeHg, and nutrients (NO_3^- , NO_2^- , NH_4^+ and PO_4^{3-}) in the benthic chambers (control chamber C containing untreated sediment and chamber B containing sediment amended with biochar) during the incubation experiment.

approximately double ($18.8 \pm 4.3 \text{ ng L}^{-1}$) with respect to the biochar-amended one ($9.29 \pm 2.53 \text{ ng L}^{-1}$). Since Hg may often be associated with Fe oxy-hydroxides in sediments, dissolution and/or desorption of these phases under hypoxia may promote the release of dissolved Hg species at the SWI (Mason et al., 2006). Comparable concentrations ($10\text{--}15 \text{ ng L}^{-1}$) were observed in the eastern sector of the Lagoon during the oxic-anoxic transition as part of a similar incubation experiment (Covelli et al., 2008). Accordingly, DMeHg increased (from 0.24 to 16.9 ng L^{-1}) in the untreated mesocosm (Fig. 3), whereas notably lower and quite constant concentrations were detected in the biochar-amended chamber (mostly $<1 \text{ ng L}^{-1}$ with a maximum value of 1.15 ng L^{-1}). At the end of the de-oxygenation phase, DMeHg accounted for 75 % and 9 % of the total DHg in the untreated and biochar-amended chamber, respectively, suggesting that biochar was effective in reducing Hg mobility and methylation, possibly due to its role as a sorbent for metals and other soluble components at the SWI.

Although occurring in very low concentrations during the entire experiment, NO_2^- and NO_3^- showed a decreasing trend in both incubated systems (Fig. 3) being the first electron acceptors used in the early diagenetic sequence during OM remineralisation (Froelich et al., 1979). On the contrary, the decrease in DO corresponded to a notable increase

in NH_4^+ which represented the dominant N species during the entire experiment in both incubated chambers (Fig. 3). During this first experimental step, the highest concentrations were observed in the untreated system where NH_4^+ raised from 30.6 to 139 μmol L^{-1} . Comparatively, lower values occurred in the biochar-amended chamber (from 27.8 to 106 μmol L^{-1}). This may be due to the lower efficiency of the nitrification process promoted under hypoxia in association with strong denitrification, NO_3^- reduction by bacteria and OM remineralisation with subsequent NH_4^+ effluxes (Belias et al., 2007; Middelburg and Levin, 2009). This evidence is further confirmed by the significant correlations (Fig. S1) between NO_3^- and NH_4^+ ($N = 4$, $r = -0.904$, $0.05 < P < 0.1$ and $N = 6$, $r = -0.914$, $0.01 < P < 0.05$ in chambers C and B, respectively), in both benthic chambers as well as between NO_2^- and NH_4^+ ($N = 4$, $r = -0.871$, $0.05 < P < 0.1$ and $N = 6$, $r = -0.929$, $0.001 < P < 0.01$ in chambers C and B, respectively), as also observed by Belias et al. (2007).

The geochemistry of P at the SWI is complex and regulated by several factors including the cycling of Fe and Mn as well as the DO conditions (Conley et al., 2002; Ingall et al., 2005) which controlled the P-exchange between sediment and water (Ghaisas et al., 2019). Similarly to NH_4^+ , the concentration of PO_4^{3-} gradually increased in both mesocosms (from

0.65 to 1.17 $\mu\text{mol L}^{-1}$ and from 1.74 to 4.35 $\mu\text{mol L}^{-1}$ in chambers C and B, respectively; Fig. 3) as a result of the combination of OM degradation and PO_4^{3-} release from Fe oxy-hydroxides representing the major drivers of P release at the SWI (Lehtoranta et al., 2009; Zilius et al., 2015; Ghaisas et al., 2019). Furthermore, the relatively fast kinetics of Fe oxidation-reduction (Davison and Seed, 1983; Millero et al., 1987; Kononov et al., 2004; Tribovillard et al., 2006; Dellwig et al., 2010) led to a quick PO_4^{3-} release at the SWI as DO levels dropped (Foster and Fulweiler, 2019). Indeed, DO consumption and the ongoing anoxic conditions led to Fe reduction promoting the transformation of insoluble FePO_4 into the more soluble $\text{Fe}_3(\text{PO}_4)_2$ with subsequent release of PO_4^{3-} in solution (Kemp et al., 1990; Ogrinc and Faganeli, 2006; Belias et al., 2007).

3.2.2. Step 2: anoxia

The phase of anoxia lasted 18 and 21 days for the biochar-amended and untreated system, respectively (Fig. 3). As a result of the higher rate of DO consumption, anoxic conditions (0.32 mg L^{-1} of DO) were reached after only 5 days from the beginning of the experiment in the untreated benthic chamber, whereas the biochar-amended system moved to anoxia (0.72 mg L^{-1} of DO) more slowly after one week. The concentrations of DO observed during this experimental phase were constantly below 1 mg L^{-1} indicating that there was no DO supply.

The predominant process occurring in the incubated mesocosms was the sulphate-reduction with subsequent H_2S production with an increasing trend in both chambers. In the untreated system, H_2S increased to a maximum value of 99.3 $\mu\text{mol L}^{-1}$ which was suddenly reached after 11 days from the beginning of the experiment and remained constant until the end of the anoxic phase. On the contrary, a more gradual increasing trend was observed in the biochar-amended chamber up to a value of 105 $\mu\text{mol L}^{-1}$ after 25 days of incubation (Fig. 3). Similarly, strongly reductive conditions were observed with ORP values decreasing from -129 to -254 mV and from -57.6 to -205 mV in the untreated and biochar-amended chamber, respectively.

As far as dissolved Fe is concerned, a strongly decreasing trend was observed, especially in the untreated chamber (Fig. 3), reaching values below LOD and persisting until the end of the experiment. This strong decrease in dissolved Fe may be related to intense sulphate-reduction activity (Middelburg and Levin, 2009; Lenstra et al., 2021) as suggested by the strong correlation observed between dissolved Fe, when detectable, and H_2S ($N = 4$, $r = -0.962$, $0.01 < P < 0.05$ and $N = 9$, $r = -0.952$, $P < 0.001$ in chambers C and B, respectively). Indeed, H_2S can react with Fe to form insoluble Fe sulphides, thus removing Fe from solution such as pyrite (FeS_2) (Dellwig et al., 2010; Jilbert and Slomp, 2013; Lenstra et al., 2021) which can also be seen as large framboids (up to 200 μm) in the sediments (Covelli et al., 2006).

A gradual decrease was also observed in the case of dissolved Mn, especially in the untreated chamber (Fig. 3), where Mn slightly decreased from 470 $\mu\text{g L}^{-1}$ at the beginning of anoxia to 360 $\mu\text{g L}^{-1}$ at the end of the anoxic phase. Similarly, dissolved Mn showed fairly constant concentrations in the biochar-amended chamber during the entire anoxic experimental step (avg. 355 ± 15 $\mu\text{g L}^{-1}$). In contrast to Fe, dissolved Mn remained in solution in both the mesocosms during anoxia. This different behaviour may be due to a higher mobility of Mn in sulphidic water compared to Fe (Burdige and Nealson, 1986; Yao and Millero, 1993; Lenstra et al., 2021) as well as to the varied kinetics of the reactions involving these elements (Kristiansen et al., 2002; Kristensen et al., 2003) and their different interaction with S. Indeed, Fe precipitation can be related to the formation of sulphides, whereas the precipitation of Mn sulphides is rather unusual leaving dissolved Mn in solution (Pakhomova et al., 2007).

Anoxic conditions also promoted DHg effluxes at the SWI, especially

in the untreated benthic chamber where a clear increase in DHg was observed ranging from 27.2 ng L^{-1} at an early stage of the anoxic phase to a maximum concentration of 64.3 ng L^{-1} after 5 days (Fig. 3). Dissolved MeHg (DMeHg) showed a comparable trend, gradually increasing from 26.2 ng L^{-1} at the beginning of anoxia to a maximum value of 53.1 ng L^{-1} after 14 days. Indeed, most of the DHg in solution in the untreated chamber was in the form of DMeHg (82 ± 18 % of Hg), confirming that Hg methylation and the subsequent release of DMeHg at the SWI were strongly promoted during anoxic events (Cabrol et al., 2023). Comparable results were observed in the framework of similar incubation experiments conducted in the eastern sector of the Lagoon (Covelli et al., 2008) and in the Gulf of Trieste (Emili et al., 2011).

The good correlation found between H_2S and both DHg and DMeHg suggests that sulphate reduction and methylation processes mediated by sulphate reducing bacteria play a key role in regulating DHg and DMeHg mobility at the SWI, as evidenced by the correlation with H_2S for both DHg ($N = 14$, $r = 0.669$, $0.001 < P < 0.01$) and DMeHg ($N = 14$, $r = 0.590$, $0.01 < P < 0.05$) (Compeau and Bartha, 1985; Gilmour and Henry, 1991; Gilmour et al., 2011; Rincón-Tomás et al., 2024). Moreover, changes in the redox conditions at the early stage of anoxia may also promote the dissolution of Fe and Mn oxy-hydroxides in the surface sediments (Mason et al., 2006) with the subsequent release of the associated Hg (Emili et al., 2011). Conversely, notable lower and quite constant DHg and DMeHg concentrations were observed in the biochar-amended chamber during the entire anoxic phase (12.7 ± 3.8 and 4.26 ± 1.26 ng L^{-1} of DHg and DMeHg, respectively). In detail, DHg and DMeHg concentrations were respectively reduced by 75 and 90 % under anoxia in the biochar-amended mesocosm. This suggested that biochar efficiently mitigated the mobility of Hg at the SWI and its availability for methylation primarily through adsorption and complexation processes occurring on the biochar particle surface, as also observed in other laboratory and *in situ* experiments (Gilmour et al., 2013, 2018; Yang et al., 2021).

No clear increasing or decreasing trend was observed in the case of NO_3^- , which remained quite constant during anoxia in both the mesocosms (0.74 ± 1.02 $\mu\text{mol L}^{-1}$ and 2.05 ± 1.49 $\mu\text{mol L}^{-1}$ in chambers C and B, respectively). The reduced NO_3^- concentrations were indicative of denitrification processes mediated by bacteria whose main products released in solution are NO_2^- and NH_4^+ (Froelich et al., 1979; Belias et al., 2007). Indeed, NO_2^- clearly increased at an early stage of anoxia in both chambers although occurring in relatively low concentrations (from 0.30 to 4.33 $\mu\text{mol L}^{-1}$ and from 0.29 to 2.82 $\mu\text{mol L}^{-1}$ in chambers C and B, respectively) and then decreased in the last days of anoxia (Fig. 3). Similarly, a strong increase in NH_4^+ was evident in both the incubated mesocosms (from 182 to 651 $\mu\text{mol L}^{-1}$ and from 168 to 325 $\mu\text{mol L}^{-1}$ in chambers C and B, respectively) due to the intense activity of denitrifying anaerobic bacteria (Belias et al., 2007; Middelburg and Levin, 2009). Ammonium (NH_4^+) concentration at the end of the anoxic phase was three orders of magnitude higher than NO_2^- and NO_3^- , thus suggesting that ammonification was only partially responsible for NH_4^+ production (Emili et al., 2011). The maximum NH_4^+ concentration observed in the biochar-amended chamber (325 $\mu\text{mol L}^{-1}$) at the end of this experimental phase was approximately half of the concentration in the untreated system (651 $\mu\text{mol L}^{-1}$) suggesting a potential limiting effect of biochar on the NH_4^+ production under conditions of DO depletion. A comparable increasing trend was also clearly evident in the case of PO_4^{3-} in both benthic chambers ranging from 1.99 to 15.5 $\mu\text{mol L}^{-1}$ in the untreated chamber and from 7.18 to 19.7 $\mu\text{mol L}^{-1}$ in the biochar-amended system (Fig. 3). The PO_4^{3-} release in association with the reduction of Fe oxy-hydroxides can be excluded since, as previously mentioned, persistent anoxic conditions promoted Fe precipitation as sulphides. Therefore, the increasing PO_4^{3-} concentrations may mostly

result from OM degradation (Kemp et al., 1990; Ogrinc and Faganeli, 2006; Anschutz et al., 2007; Middelburg and Levin, 2009; Emili et al., 2011) as suggested by the significant correlation between PO_4^{3-} and NH_4^+ (Fig. S1) both in the untreated ($N = 14$, $r = 0.997$, $P < 0.001$) and in the biochar-amended chambers ($N = 12$, $r = 0.993$, $P < 0.001$).

3.2.3. Step 3: re-oxygenation

The last phase of the incubation experiment consisted of the re-oxygenation of both systems. Starting on the 25th day after the beginning of the experiment, the upper lids of both incubated chambers were removed leaving the water surface in direct contact with the atmosphere and allowing oxygen exchanges.

The formation of a whitish, filamentous substance was suddenly observed on the sediment surface during the first few days of re-oxygenation, especially in the untreated chamber where the water also became “milky” (Fig. S2). This phenomenon may be related to the formation of Mn colloids following re-oxygenation (Li et al., 2019). However, this was not confirmed by the Mn concentrations which gradually decreased from $331 \mu\text{g L}^{-1}$ at the beginning of this phase to a final concentration of $121 \mu\text{g L}^{-1}$ (Fig. 3). Alternatively, this event may be due to microbial/bacterial activity such as the potential development of facultative anaerobic bacteria able to use both DO and NO_3^- as alternative electron acceptors. This is consistent with other similar observations already documented (Visser et al., 1995; Beutel, 2001; Jungo et al., 2001) and with the rapid consumption of DO which was available in solution during the first days of re-oxygenation. Indeed, DO concentrations remained very low ($0.42 \pm 0.16 \text{ mg L}^{-1}$) and anoxia persisted during the first 4 days of re-oxygenation as well as reductive conditions ($-110 \pm 84 \text{ mV}$) associated with an additional slight rise in H_2S concentration (Fig. 3). Indeed, re-oxygenation occurred more slowly in the untreated chamber. A gradual increase of DO concentrations, up to a value of 4.48 mg L^{-1} , was associated with a H_2S decrease to values below LOD at the end of the experiment. In contrast, a clear DO increasing trend was observed in the biochar-amended chamber (from 0.80 to 6.16 mg L^{-1}) as well as for ORP which increased from 80.0 to 186 mV . This suggested that re-oxygenation was more effective in the biochar-amended system where the DO concentration at the beginning of the experiment (7.20 mg L^{-1}) was promptly restored (6.16 mg L^{-1}). The re-oxygenation conditions inhibited the sulphate-reduction processes with a subsequent decrease in H_2S concentrations which fell to values below LOD during all this experimental step in the biochar-amended chamber (Fig. 3) most likely due to re-oxidation of the sulphides produced during the anoxic phase (Middelburg and Levin, 2009).

Dissolved Fe still remained mainly below LOD during the entire re-oxygenation step in both mesocosms since all the available Fe in solution was already scavenged as sulphide during anoxia. Conversely, the increasing DO concentrations during re-oxygenation led to the re-oxidation of dissolved Mn and precipitation as oxy-hydroxides (Froelich et al., 1979; Kristiansen et al., 2002; Kristensen et al., 2003) with a subsequent gradual decrease in dissolved Mn in both chambers (from 331 to $121 \mu\text{g L}^{-1}$ and from $338 \mu\text{g L}^{-1}$ to values below LOD in chambers C and B, respectively; Fig. 3).

In a re-oxygenated system, DHg decreased, as Hg remobilised under anoxic conditions can re-precipitate in the form of oxy-hydroxides or sulphides with subsequent removal of DHg and H_2S from solution (Covelli et al., 2008; Emili et al., 2014). Similarly, DMeHg decreased during re-oxygenation most likely due to co-precipitation with sulphides and potential demethylation processes (Hines et al., 2006). In this context, Fe and Mn oxy-hydroxides are excellent inorganic ligands for DHg under oxic conditions; consequently, the co-precipitation of DHg in association with Fe and Mn oxy-hydroxides may be a possible explanation for the decrease in DHg concentrations observed in both chambers (Emili et al., 2012). This was especially evident in the untreated benthic chamber where a reliable correlation was found between DHg and Mn ($N = 8$, $r = 0.721$, $0.01 < P < 0.05$) whereas notable variations in DHg and DMeHg concentrations were not observed in the biochar-

amended chamber, further confirming the positive role of biochar in reducing the mobility of Hg species at the SWI.

Regarding NO_3^- and NO_2^- , a fluctuation and subsequent further decreasing trend were observed in both mesocosms. On the contrary, NH_4^+ continued to be the dominant species of inorganic N under re-oxygenation since the change in the redox conditions appeared not to affect NH_4^+ production, as also observed during a similar incubation experiment (Emili et al., 2011); therefore, a progressive NH_4^+ increase was still observed reaching values of 617 and $364 \mu\text{mol L}^{-1}$ in the untreated and biochar-amended chamber, respectively, at the end of the experiment (Fig. 3). Indeed, the degradation processes of OM, under aerobic conditions, led to NH_4^+ production and effluxes through nitrification mediated by bacteria (Middelburg and Levin, 2009; Emili et al., 2011) as also demonstrated by the correlation between NO_2^- and NH_4^+ both in the control ($N = 7$, $r = 0.903$, $0.001 < P < 0.01$) and in the biochar-amended chamber ($N = 6$, $r = 0.873$, $0.01 < P < 0.05$).

The gradual increase in the concentration of DO corresponded to a slight decrease in PO_4^{3-} concentrations in both chambers. Indeed, oxidation and/or precipitation of Fe and Mn that sequestered the excess P by adsorption is promoted under oxic conditions (Ghaisas et al., 2019).

3.3. Mercury (Hg) and methylmercury (MeHg) benthic fluxes under anoxic conditions

Dissolved Hg and MeHg benthic fluxes for both the incubated mesocosms were calculated using the linear regression of DHg and DMeHg concentrations during the anoxic phase (Fig. 4, Covelli et al., 2008; Emili et al., 2011, 2014; Koron and Faganeli, 2012). The anoxic benthic fluxes of DHg and DMeHg were an order of magnitude higher in the untreated system (684 and $468 \text{ ng m}^{-2} \text{ d}^{-1}$ for DHg and DMeHg, respectively) compared to the biochar-amended chamber (68.6 and $71.7 \text{ ng m}^{-2} \text{ d}^{-1}$ for DHg and DMeHg, respectively) (Table 3). Moreover, both DHg and DMeHg anoxic fluxes in the untreated system were found to be consistent with results obtained from similar incubation experiments performed in the Gulf of Trieste (Emili et al., 2011, 2014; Koron and Faganeli, 2012) regardless of the THg concentration in the sediment. In the case of DMeHg, results were also consistent with anoxic benthic fluxes found in the eastern sector of the Lagoon (Covelli et al., 2008), whereas the DHg fluxes were notably higher despite the lower THg concentration in sediments (Table 3). However, the THg concentration in sediments cannot directly predict the DHg and DMeHg fluxes at the SWI during a transition from oxic to anoxic conditions.

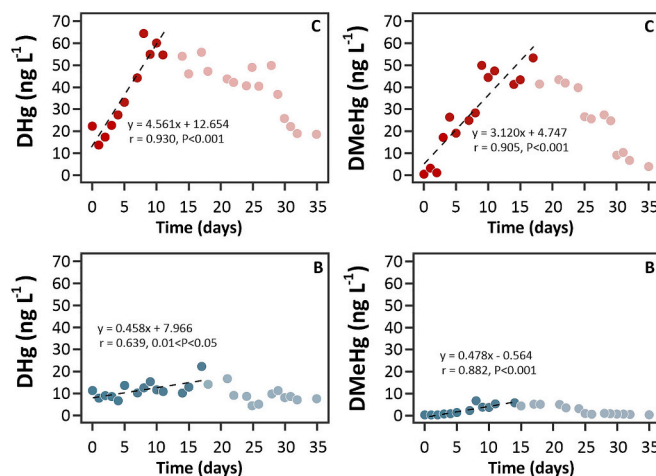


Fig. 4. Linear regression on DHg and DMeHg concentrations during the anoxic phase for the calculation of Hg and MeHg benthic fluxes (control chamber C containing untreated sediment and chamber B containing sediment amended with biochar).

Table 3

Total Hg (THg) concentration in sediments and benthic fluxes ($\text{ng m}^{-2} \text{d}^{-1}$) of Hg and MeHg calculated from incubation experiments in the untreated (C) and biochar-amended (B) mesocosms performed in this study compared to previous similar experiences in the Gulf of Trieste and the Lagoon (n.a.: not available; Fig. 1).

Site	Period	Depth (m)	THg ($\mu\text{g g}^{-1}$)	Anoxic fluxes ($\text{ng m}^{-2} \text{d}^{-1}$)			
				Hg		MeHg	
				C	B	C	B
This study	September 2023	0.50	6.10 ± 0.75	684	68.6	468	71.7
Grado Lagoon (BAR) ^a	September 2005	1.5	10.6–12.5	60		498	
Gulf of Trieste (D6) ^b	June 2010	3.50	12.6	665		550	
Gulf of Trieste (AA1) ^c	November 2006	22	2.80–4.02	n.a.		147	
Gulf of Trieste (CZ) ^c	November 2007	21	0.77–0.89 ^d	71		238	
Gulf of Trieste (E) ^e	May 2009	8	0.6 ^f	685		577	
Gulf of Trieste (F1) ^e	May 2010	22	0.2 ^f	646		361	

^aCovelli et al. (2008); ^bEmili et al. (2014); ^cEmili et al. (2011); ^dHines et al. (2000); ^eKoron and Faganelli (2012); ^fCovelli et al. (2001)

3.4. Sediment and porewater chemistry at the end of the experiment

At the end of the incubation experiment, the surface sediment (approximately 1 cm) of the two benthic chambers was analysed to evaluate differences between the untreated and biochar-amended mesocosm in both sediment and porewater. Indeed, porewater concentrations may be considered an index of the efficacy of the amendment since this phase is relevant for biotic uptake as well for Hg and MeHg effluxes at the SWI (Amirbahman et al., 2013; Gilmour et al., 2013; Ndungu et al., 2016; Sørmo et al., 2022).

A clear difference in C_{tot} and C_{org} was observed in the sediment collected in the biochar-amended chamber at the end of the experiment showing concentrations (15.4 and 12.3 % of C_{tot} and C_{org} , respectively) equal to about twice those observed in the sediment extruded from the untreated benthic chamber (6.83 and 3.50 % of C_{tot} and C_{org} , respectively) which was comparable to the C_{tot} content measured before incubation (Fig. S3). This marked difference was mostly represented by C_{org} accounting for 79.7 % of C_{tot} and due to the occurrence of biochar (Rombolà et al., 2023). Conversely, notable differences in the N_{tot} concentration were not observed. Total Fe, Mn and Hg in sediments collected at the end of the experiment displayed slightly lower concentrations for both the incubated chambers, suggesting that this difference may be related to a general heterogeneity in the concentration of those elements in sediments (Fig. S3). Furthermore, the sample collected from the biochar-amended chamber consisted of part sediment, part biochar; thus, the lower concentrations observed at the end of the experiment may be partially due to a dilution effect.

Regarding the dissolved phase, the concentrations of Fe and Mn in the supernatant water of both chambers were mostly comparable with each other and with the pre-incubation conditions, although slightly lower after the experiment (Fig. 5). Conversely, notably lower concentrations were observed in the first porewater level at the end of the

experiment both in the untreated system (243 and 338 $\mu\text{g L}^{-1}$ of Fe and Mn, respectively) and in the biochar-amended chamber (145 and 622 $\mu\text{g L}^{-1}$ of Fe and Mn, respectively) with respect to those observed before the experiment (1566 and 4204 $\mu\text{g L}^{-1}$ of Fe and Mn, respectively). This was mainly due to the intense effluxes of Fe and Mn to the overlying water column occurring during the hypoxic and early anoxic phase.

The water samples collected at the end of the experiment from the biochar-amended chamber showed DHg concentrations equal to about half (6.66 and 23.9 ng L^{-1} in the supernatant water and in the first porewater level, respectively) with respect to those observed before the incubation (15.1 and 46.8 ng L^{-1} in supernatant and in the first porewater level, respectively, Fig. 5). The same was true for DMeHg showing notably lower concentrations (0.46 and 1.07 ng L^{-1} in the supernatant water and in the first porewater level, respectively) compared to those found before the experiment (3.16 and 4.55 ng L^{-1} in supernatant and in the first porewater level, respectively). This DHg and DMeHg decrease in porewater may be due to adsorption processes on the biochar particle surface (Yang et al., 2021) further confirming the efficiency of the biochar application. Following the application of biochar, a notable reduction of the available MeHg in porewater was also observed by Sørmo et al. (2022) in the case of a long term *in situ* experiment in the Gunneklev Fjord, one of the most polluted fjord systems in Norway due to historic release of Hg-containing sludge from a chlor-alkali plant.

On the contrary, no notable variations were observed for the untreated chamber where the DHg concentrations in supernatant water and in the first porewater level were comparable to those that occurred before the incubation (Fig. 5). Conversely, DMeHg slightly increased in the supernatant water most likely due to DMeHg effluxes during the anoxic phase as also confirmed by the reduced DMeHg concentration in the first porewater level at the end of the experiment (Fig. 5). This evidence further suggested that the biochar employed in this research appears to be effective in reducing Hg methylation and mobility at the SWI,

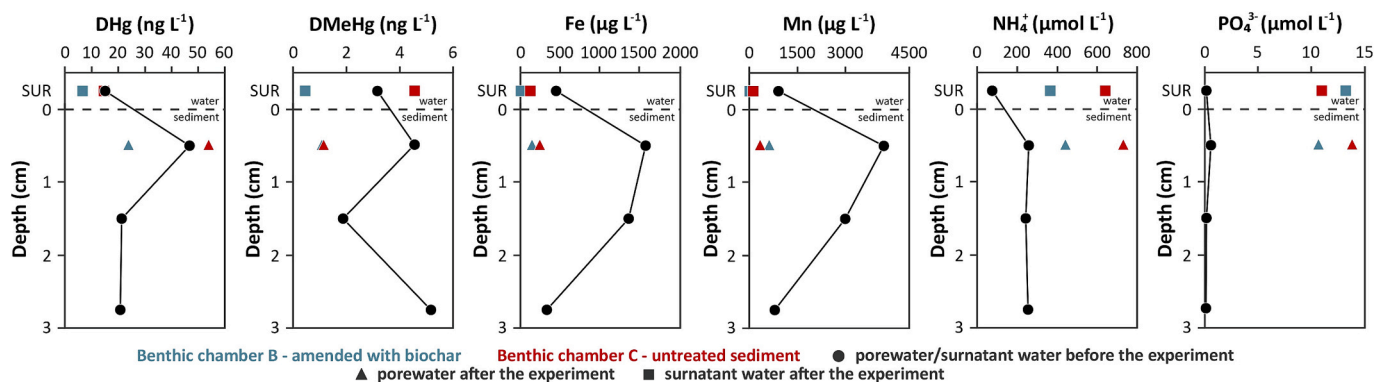


Fig. 5. Vertical profiles of DHg, DMeHg, Fe, Mn, NH_4^+ and PO_4^{3-} in the supernatant water and porewater extracted from the sediment core samples before the incubation experiment and their concentrations in the surface porewater layer (0–1 cm) extruded from both the incubated benthic chambers at the end of the experiment.

increasing partitioning to bulk sediment as also observed in the case of activated carbon during both laboratory and *in situ* experiments (Gilmour et al., 2013, 2018).

Ammonium (NH_4^+) concentrations in all the water samples collected at the end of the experiment were notably higher with respect to the pre-incubation conditions (75.0 and $258 \mu\text{mol L}^{-1}$ in surtatan water and in the first porewater level, respectively) both in the untreated (640 and $731 \mu\text{mol L}^{-1}$ in surtatan water and the first porewater level, respectively) and in the biochar-amended chamber (365 and $441 \mu\text{mol L}^{-1}$ in surtatan water and the first porewater level, respectively) (Fig. 5). Similarly to NH_4^+ , notably higher values of PO_4^{3-} were found in both the surtatan water (11.0 and $13.2 \mu\text{mol L}^{-1}$ in benthic chambers C and B, respectively) and in the first porewater layer (13.8 and $10.7 \mu\text{mol L}^{-1}$ in benthic chambers C and B, respectively) collected at the end of the experiment with respect to the pre-incubation level (0.06 and $0.49 \mu\text{mol L}^{-1}$ in surtatan water and the first porewater level, respectively). The sharp increase in NH_4^+ and PO_4^{3-} concentrations compared to the pre-incubation conditions was mainly due to the active OM degradation processes occurring during the anoxic phase, which was responsible for the release of nutrients such as P and N into solution. These processes were confirmed by the low concentrations of NO_3^- observed in the surtatan water of both the chambers at the end of the experiment (3.75 and $2.49 \mu\text{mol L}^{-1}$ in chambers C and B, respectively) which were notably lower with respect to that measured before the incubation ($882 \mu\text{mol L}^{-1}$). Notable differences in the concentration of NO_2^- were not observed in the dissolved phase collected before and after the incubation experiment.

4. Conclusions

Fishing, mussel collection, and fish-farming — all productive and economically relevant uses of the Grado and Marano Lagoon ecosystem — are in conflict with the high levels of Hg contamination found in the lagoon sediments. The bioaccumulation effects of Hg have been detected in the trophic chain of fish species of commercial relevance in the still-productive fish farms. The restricted water circulation in the fish farms appears to be a determining factor in accelerating the biogeochemical processes of the natural degradation of sedimentary OM under anaerobic rather than aerobic conditions, according to previous *in situ* studies performed over the last ten years. The lack of natural mixing and re-oxygenation of the water column causes a state of “distress” at the SWI due to oxygen depletion, which leads to the release of NH_4^+ , a toxin for the biotic compartment, and suitable conditions for Hg methylation.

In this study, the application of biochar as an amendment in Hg contaminated sediments has been evaluated as a promising remediation technique for reducing the risk associated with bioavailable Hg species. Results from the incubation experiment comparing untreated and biochar-amended sediment suggested that biochar can effectively lower the mobility and availability of inorganic Hg to be methylated, even while it cannot decrease the overall quantity of Hg in sediments.

At the early stage of the experiment, a faster consumption of DO was observed in the untreated system as well as a slower restoration of oxic conditions during the re-oxygenation step with respect to the biochar-amended chamber. Consequently, the untreated chamber showed stronger reductive conditions and a higher production of H_2S as a result of sulphate reduction processes at the sediment surface. A high reactivity of both Fe and Mn to changing redox conditions was observed in both incubated mesocosms as well as the effects of OM remineralisation processes during the anoxic phase with subsequent effluxes of NH_4^+ and PO_4^{3-} at the SWI.

Results from this research showed that hypoxic and anoxic conditions play a crucial role in the remobilisation of Hg and MeHg from contaminated sediments to the water column as the result of several processes such as diffusion from porewater, methylation and dissolution/desorption of Fe and Mn oxy-hydroxides at the SWI. This was especially evident in the untreated mesocosm where the maximum DHg

(64.3 ng L^{-1}) and DMeHg (53.1 ng L^{-1}) concentrations were reached during the anoxic phase, whereas DHg and DMeHg levels were generally low in the biochar-amended system ($12.7 \pm 3.8 \text{ ng L}^{-1}$ of DHg and $4.26 \pm 1.26 \text{ ng L}^{-1}$ during anoxia) suggesting that biochar was able to notably reduce DHg (by 75 %) and DMeHg (by 90 %) effluxes at the SWI. The difference between the two mesocosms suggested that the biochar obtained from pruning vineyards used in this study appeared to be effective in limiting the mobility of Hg and its availability to be methylated, reducing the development of anoxic conditions and promoting re-oxygenation at the end of the experiment.

The results obtained may represent a valuable starting point for further research activities focused on the use of biochar made up of the residue from pruning vineyards as a potential sustainable amendment to be directly applied *in situ* as an helpful solution to mitigate Hg and MeHg mobility and release from contaminated sediments, although its application reasonably seems more feasible in restricted areas of the lagoon environment such as fish ponds.

CRediT authorship contribution statement

Elena Pavoni: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Federico Floreani:** Visualization, Methodology, Investigation, Formal analysis. **Elisa Petranich:** Methodology, Formal analysis. **Daniela Berto:** Validation, Formal analysis. **Claudia Gion:** Validation, Formal analysis. **Stefano Fornasaro:** Validation, Formal analysis. **Nicolas Greggio:** Validation, Resources, Conceptualization. **Francesca Larese Filon:** Validation, Supervision, Resources, Funding acquisition. **Stefano Covelli:** Validation, Supervision, Resources, Methodology, Investigation, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2025.179883>.

Data availability

Data will be made available on request.

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