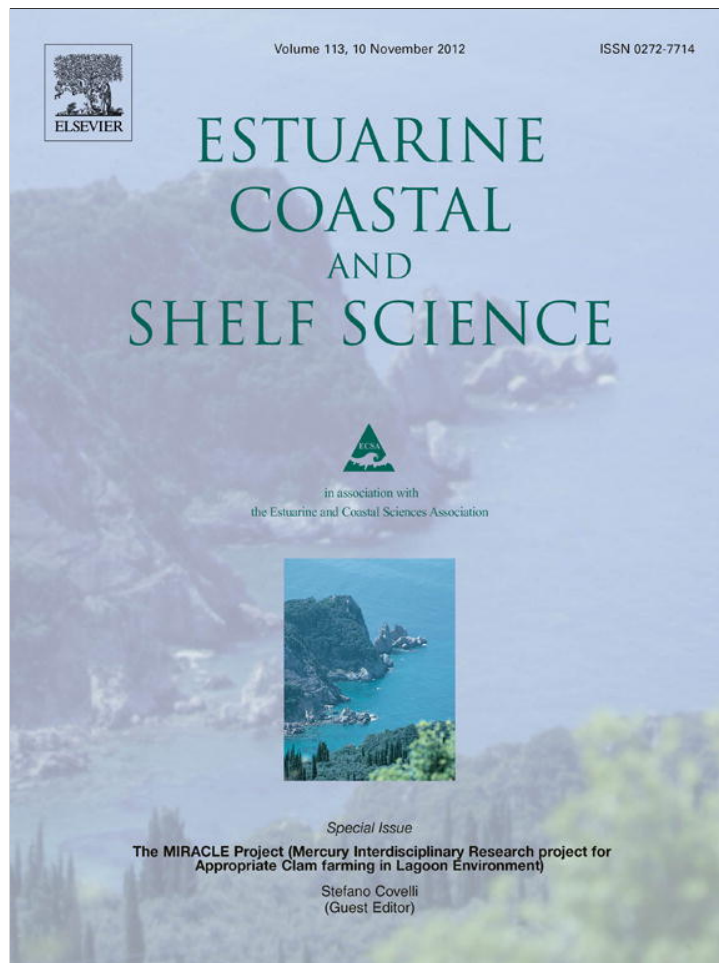


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The MIRACLE Project: An integrated approach to understanding biogeochemical cycling of mercury and its relationship with lagoon clam farming

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ABSTRACT

The “MIRACLE” Project was aimed at two specific issues: understanding Hg biogeochemical cycling in the Marano and Grado Lagoon and testing the coexistence of clam farming with Hg contamination in the sediments. Mercury contamination was measured in several matrices (water, sediment, biota) and its mobility was tested along with its speciation in relation to biogeochemical processes occurring in the lagoon environment, where bacterial communities have a primary role in converting Hg to its more toxic form, methylmercury (MeHg). Bioaccumulation of the Hg species was investigated on natural and seeded clams (*Ruditapes philippinarum*), the most important commercial bivalves in the Lagoon. The Editorial summarizes the main results obtained from this multidisciplinary study and reported in the Special Issue.

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1. Introduction and aim of the research

Coastal lagoon ecosystems are among the most ephemeral features on a geological time scale but they are also among the most productive ecosystems in the biosphere. These transitional environments experience significant short-term variations in physical and chemical properties and processes that control the biological community and its habitat. However, intense human development along the inland shorelines has often altered both fresh and salt-water inputs to many lagoons, as well as nutrient loading, sedimentation, and the input of anthropogenic contaminants. Due to their shallowness and, the limited water exchange with the open sea, lagoons tend to be efficient traps for sediments and, consequently, contaminants, from marine and fluvial origin. Contaminants accumulated in sediments may be undergo burial and/or biogeochemical processes, which affect their distribution, speciation and bioavailability to the lagoon biota. The high potential risk of contaminant remobilization from sediments into the water column and its subsequent bioaccumulation in the trophic chain are also enhanced by the abundance of animal and plant species in this ecosystem. The ultimate risk is the potential uptake of contaminants by human residents since fishing and clam and mussel collection are usually common in coastal lagoons.

In the Mediterranean Sea, some of the major coastal lagoons are located along the northern Adriatic coastline. The Marano and Grado Lagoon is the second largest wetland in the area while the Lagoon of Venice is the most relevant and well known example of these ecosystems, where natural and human induced modifications coexist. The lagoon includes some historical sites designed to protect wildlife migration submitted to the Ramsar Convention in 1971. Following the implementation of the Habitats Directive (92/43/EEC), concerning the protection of biodiversity, the entire basin has been identified in the “Natura 2000” state-sponsored survey as a site to be included among the sites of Community importance (SCIs – IT3320037). In addition, fishing, collection of clams and mussels, and fish and clam farming in the lagoon are important economically for the inhabitants.

Due to human activities, this natural environment has suffered apparent transformations from the morphological point of view, which are coupled with the negative sediment budget presently occurring in this ecosystem (Fontolan et al., 2012). In addition, the Marano and Grado Lagoon has experienced significant contamination by heavy metals, polychlorinated dibenzodioxins (PCDDs) and furans (PCDFs), polycyclic aromatic hydrocarbons (PAHs) and aromatic hydrocarbons related to the Torviscosa chemical complex, the most important industrial site in the region (Ramieri et al., 2011). Contamination affected soils, groundwaters as well as fluvial and lagoon sediments. Among contaminants, mercury (Hg) was deliberately discharged in effluents from the chloralkali plant (CAP) operative since 1949. This source contributes to increased

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metal concentrations in the Aussa-Corno river system and lagoon sediments (Piani et al., 2005) already extensively altered by Idriza mercury mine activities during the last centuries (Covelli et al., 2001). Although direct discharge of Hg from the CAP no longer exists, the metal is still released from the source area into freshwaters flowing into the lagoon (Covelli et al., 2009). The ecosystem has been severely impacted as evidenced by elevated Hg contents in aquatic plants and organisms (Brambati, 2001). This evidence also increased the concern about human health risk linked to Hg exposure through fish and mussel consumption. Fish farms located in embankment areas enclosed by sluice gates cover about 1800 ha of the whole lagoon, with 1400 ha in the eastern (Grado) sector alone (Fontolan et al., 2012). Fish farms are devoted to gilt-head bream (*Sparus auratus*), bass (*Dicentrarchus labrax*) and grey mullet (*Mugil cephalus*).

Due to the environmental and socio-economic problems caused by contamination, in May 2002 the Italian Prime Minister declared a state of emergency for the Marano and Grado Lagoon (Decree of President 3rd May 2002). Part of the lagoon and of the nearby mainland has been included in the Contaminated Sites of National Relevance (SIN) list since 2003 (Fig. 1). A special Deputy Commissioner – *Commissario Delegato* of the lagoon was appointed by the national government in order to deal with and to solve the following main issues: environmental remediation of contaminated soil and groundwaters; re-establishment of safe navigation conditions in the lagoon channels, involving sediment dredging and management of dredged material; improvement of the lagoon ecosystem, especially with respect to water and sediment quality; protection of high-value lagoon habitats; morphological restoration of the lagoon; reduction of contaminant loading from the catchment area; environmental monitoring.

Within these activities, the MIRACLE Project (*Mercury Interdisciplinary Research project for Appropriate Clam farming in Lagoon Environment*) was carried out in the period 2008–2009. The main aim was to test the coexistence of clam farming with Hg

contaminated sediments and to evaluate the possible extension of the existing commercial rearing activities in those areas of the Lagoon where the risk of Hg bioaccumulation in clams would appear minimal. The MIRACLE project was set up as an interdisciplinary study bringing together researchers involved in several fields such as geochemistry, chemistry, sedimentology, microbiology and marine biology. Mercury occurrence was investigated in several compartments (water, sediment, bacteria, and bivalves) and its mobility was explored along with its speciation in relation to biogeochemical processes occurring at the sediment–water interface in this coastal environment, where bacterial communities have a primary role in converting inorganic Hg to its more toxic form, methylmercury (MeHg). Bioaccumulation of the Hg species was investigated on natural and seeded clams of the *Ruditapes philippinarum* species, the most important commercial bivalve of this Lagoon. After its introduction in the lagoon 25 years ago, the total area devoted to clam farming, which is located in the western sector (Marano), amounts to about 800 ha of which 130 are in full regime of production (Sladonja et al., 2011).

2. Study area and sampling strategy

The Marano and Grado Lagoon covers a total area of 160 km², between the Tagliamento and Isonzo River deltas, in the northernmost sector of the Adriatic Sea. The lagoon basin is characterized by semi-diurnal tidal fluxes (65 cm and 105 cm mean and spring tidal range, respectively). Small rivers flow into the lagoon, which drain waters coming from the spring line. Particulate matter from these streams is of secondary importance, restricted to areas surrounding the spring river mouths. Conversely, the primary source of suspended sediments enters from the sea through the six tidal inlets, as contributions from river deltas and from erosion of the barrier islands. Dispersion of sediments into the lagoon is controlled by tidal fluxes. The same fluxes control salinity, which ranges from very low values (2–7 PSU) near the mouths of the

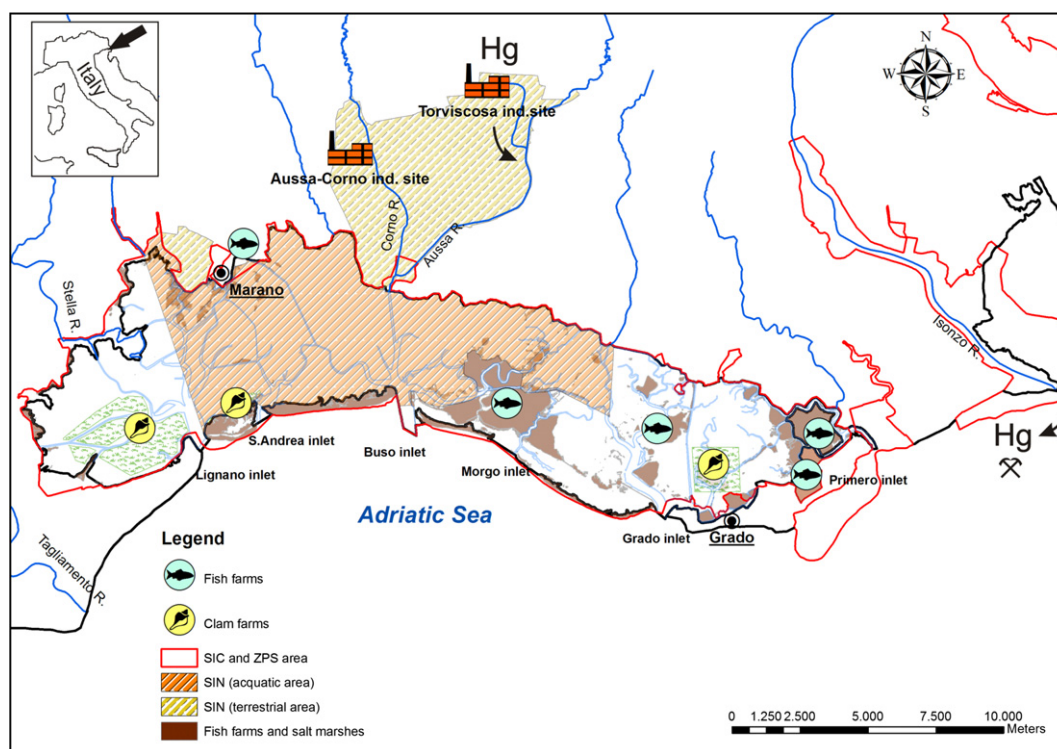


Fig. 1. Main geographical features of the Marano and Grado Lagoon. The two sources of mercury are indicated.

tributaries to values between 24 and 36 PSU close to the tidal inlets (Covelli et al., 2009). However, freshwater inputs are scarce and mainly limited to the western sector (Marano) where salinity is, on average, lower than that recorded in the Grado sector.

Sampling sites were selected by evaluating previous information on Hg distribution in sediments and biota (Brambati, 2001; Piani et al., 2005; Covelli et al., 2008) and according to the specific objectives of each subtask of the project (Fig. 2). Sediment cores for the characterization of lagoon sediments and the evaluation of the historical flux of Hg were sampled to be representative, at large scale, of the lagoon bottom variability. In addition, surface sediment samples were also recovered from the Aussa River, the main source of Hg from industrial origin. Natural populations of clams were collected considering the spatial variability in the lagoon and following the useful suggestions provided by local fishermen on the most promising harvesting areas. The four seeding sites for clams were chosen considering that site MA is located close to the commercial clam farming and site MB is near the Stella River mouth, characterized by nutrient-rich freshwater inputs, and it was included in a candidate area for the extension of clam rearing activities. Site MC was selected close to the Aussa-Corno river mouth, where clam collection is currently forbidden since it is a declared contaminated area (SIN). The fourth site (st. GD), in the eastern sector (Grado), is in an area reserved for clam farming, which is currently not in use. Mercury methylation, demethylation and sulfate reduction in sediments were measured at the same four sites using radiotracer techniques performed seasonally. In addition, the direct role of Hg-resistant bacteria in detoxifying Hg was also explored taking into account the abundance of Hg and MeHg, the amount and quality of the organic matter along with those above-mentioned processes mediated by bacterial activity. Two of these experimental sites (st. MB and MC) were considered for understanding the basic biogeochemical processes involving carbon and nutrient recycling at the sediment-water interface. These processes are also related to trace metal cycling such as Hg, whose mobilization and sequestration in this specific compartment were the main objective of the *in situ* benthic chamber experiments carried out in three campaigns. The effects of sediment resuspension on the fate of Hg species, using a short-term small-reactor-based experimental system, were finally conducted on samples collected from the Aussa River (st. A3) and from a lagoon channel (st. BAR) that is periodically dredged for shipping purposes.

3. Summary of major results

3.1. Sediment geochemistry and historical accumulation of mercury

The sedimentary records of Hg document very well the history of the metal contamination in this coastal environment (Covelli et al., 2012). For most of the sediment cores, natural background levels of Hg have been observed between 50 and 100 cm depth whereas the lowest interval (20–30 cm) are reached only in the western sector (Marano). The increasing trend of Hg content in core profiles are mainly related to the primary source, the Idrija mercury mine. On the basis of ^{210}Pb and ^{137}Cs sedimentation rate assessments, geochronological reconstruction of the historical Hg contamination confirms that the depositional flux of Hg has been influenced by anthropogenic inputs beginning around 1800, when a sharp increase of mining activities at Idrija is documented. After 1950, when the CAP was operative, the occurrence of Hg in the uppermost part of the sedimentary sequence may be due to both sources, mining and industrial. However, their complementary contribution was effective only in the central–western sector of the lagoon. The impact of the CAP seems to be restricted to the sediments of the Aussa River and the lagoon area near its mouth. At the moment, the two superimposed contributions cannot be discriminated although speciation studies demonstrated that the detritic Hg (microcrystalline cinnabar, HgS) component is virtually present in the entire lagoon (Acquavita et al., 2012; Piani et al., 2005) and being predominant in the eastern sector (Grado). The long-term source of contamination is responsible for an east–west decreasing Hg concentration gradient in the superficial sediments. The present situation compared to a comprehensive historical dataset (since 1991), confirms that despite the mine closure 15 years ago, there is no apparent evidence of a consequent decline of the Hg flux. A short-term drastic reduction of Hg input from the Isonzo River is not foreseeable since a large quantity of Hg is still stored in the drainage basin on the basis of the most recent estimates (Zibret and Gosar, 2006; Gosar and Zibret, 2011).

Total Hg concentrations in the lagoon sediments are primarily associated with fine-grained sediments ($<16\ \mu\text{m}$), transported and dispersed in suspension by littoral and tidal currents (Acquavita et al., 2012). However, in the Grado basin, the sandy component appears significantly enriched in Hg as sulfide. MeHg content in the sediment is very low, comparable to similar coastal environments and not strictly related to total Hg abundance. Higher MeHg

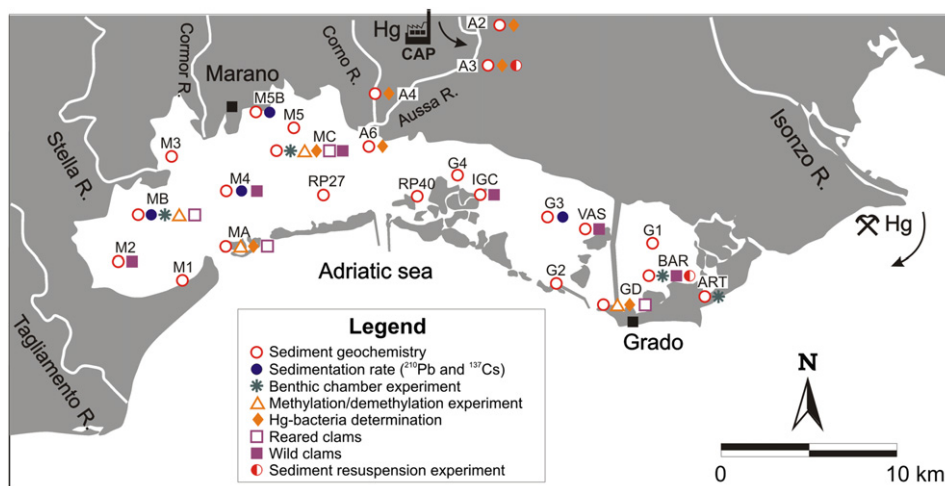


Fig. 2. Sampling site and location of the stations in the Marano and Grado Lagoon. Symbols indicate the activities arranged within the MIRACLE Project and illustrated in the papers.

concentrations in low to intermediate Hg contaminated sediments, and where Hg is mainly not present as sulphide, indicate that the total metal content is not a limiting factor for MeHg occurrence, thus suggesting a major role played by environmental conditions and/or speciation. In addition, MeHg seems not to be dependent on the overall amount of organic matter (OM), whereas its quality appears of great importance. The interaction occurring at the sediment–water interface between humic acids (HAs) and MeHg could favor MeHg accumulation, especially where HAs are of marine origin, structurally less refractory and aromatic than the fluvial ones, and then more inclined in MeHg binding and/or production.

3.2. Effects of physical processes on Hg contaminated sediment redistribution

Regarding the effects of dredging operations on the lagoon channels, Hg mobility, as a consequence of sediment resuspension, was tested in the laboratory (Acquavita et al., 2012). After sediment resuspension, Hg concentration in the water column was similar to the starting conditions and the “natural background” in the Marano and Grado Lagoon. This evidence is in good agreement with the literature, that is a resuspension event, limited in time, has poor impact on the water column, due to dilution of the Hg species. In our tests, Hg released during sediment resuspension was rather low, suggesting that porewaters play a minor role in contributing Hg to the water column. However, it would be useful to couple laboratory tests with field experiments, at the same time as dredging operations, to better check the effects of resuspension on thicker portions of the sediment, for a longer timeframe after the disturbance event. Such field experiments should also take into account the geochemical and grain-size variability of the lagoon sediment. These results appear particularly interesting if related to the pressing need of conservation and management of this lagoon environment. Saltmarshes act as a temporary repository for sediments and associated contaminants. However, their rapid erosion due to global sea level rise is significantly enhanced by human activities such as periodic dredging of the main lagoon channels and land reclamation. The spatial analyses of changes in saltmarsh area over about a 50-year period revealed the reduction (16%) of the original saltmarshes and the primary role played by direct human activities (Fontolan et al., 2012). If the direct human impact is not considered, the system is able to react and to counterbalance erosion with sediment deposition according to the different local and short-term forcing factors which vary in each lagoon sub-basin. Mercury in association with sediments is then subject to a complex recycling. Understanding the fate of the element when dredged sediments are used for saltmarshes reclamation could be, for instance, a future challenge in research.

3.3. Early diagenetic processes and remobilization of mercury at the sediment–water interface

Biogeochemical processes involving oxygen, nutrients (C, N, P) and redox-sensitive elements (Fe, Mn) control degradation and regeneration of the sedimentary organic matter mediated by microbial activity and may also affect recycling of contaminants at the sediment–water interface. Early diagenetic processes are usually studied following different approaches from the estimation of diffusive fluxes to the measurements of benthic fluxes by deploying *in situ* benthic chambers or based on sediment incubation experiments in the laboratory (Berelson et al., 2003). The first two techniques were applied to investigate benthic metabolism and nutrient fluxes in two experimental sites of the Marano Lagoon and their seasonal variations. The fate of carbon and nutrients, depicted by constructing tentative annual budgets for each site

assuming steady-state conditions, was compared to the result obtained from two other sites previously investigated in the Grado Lagoon, a subtidal zone (st. BAR) and a fishfarm (st. ART) (De Vittor et al., 2012) (Fig. 2). It appeared that, due to the observed site-dependent variability, the calculated benthic nutrient budgets should be considered as representative of specific sites and not extended on the whole ecosystem. This is already evident from the content of the organic carbon (C_{org}) that is higher in those sites where riverine inputs (st. MC) and aquaculture (st. ART) occur. The same parameter along with total N, P, organic P and biogenic Si decrease along the sedimentary sequence due to degradation of the labile organic matter. There was an increasing trend with depth of the porewater concentrations of DIC, DOC, NO_3^- , NH_4^+ , PO_4^{3-} and SiO_4^{4-} and high diffusive fluxes were obtained in the warmer periods at all sites, particularly at MC and ART. Bioturbation and biogeochemical processes at the sediment–water interface are probably responsible for the great difference observed between the diffusive and *in situ* benthic fluxes, which exhibited significant seasonal variations. The fishfarm site (st. ART) only showed O_2 consumption whereas O_2 production was mostly present in both lagoon basins. Sediments appeared heterotrophic except at BAR which seems in trophic balance or weakly heterotrophic on the basis of the O_2 and DIC metabolism. Phosphorous appeared to be the limiting factor for primary production in the lagoon as suggested by the low PO_4^{3-} fluxes affected by redox reactions. The tentative annual budget for P indicates that 50% is buried in the sediments. Conversely, C, N and Si are intensively recycled. NO_3^- and SiO_4^{4-} exhibited influxes due to intense microphytobenthic assimilation, mostly by diatoms, and denitrification, confirmed by dark NO_3^- influxes. NH_4^+ appeared to be actively assimilated.

Where Hg contents are far above the background level, sediments are not only repositories for the metal, but also are key role of secondary source in relation to the important biogeochemical processes occurring at the sediment–water interface. In this context, benthic chamber experiments were designed to help in quantifying *in situ* Hg recycling (Emili et al., 2012). The highest benthic fluxes occurred generally in summer when bacterial degradation of organic matter releases greater amount of Hg into pore waters. Its bioavailable fraction can be methylated, leading to higher MeHg fluxes. This behavior is common in the whole lagoon although MeHg fluxes are up to three orders of magnitude higher in the eastern sector (Grado) where the total Hg content is the highest and in spite of its origin being associated with the mostly insoluble cinnabar. The estimated annual Hg budget indicates that MeHg is highly recycled (89–99%) into the water column whereas the buried fraction is negligible. On the other hand, about 30% is the Hg fraction mainly recycled to the water column. It was interesting to note that Hg reduction, which drives the formation of dissolved gaseous mercury (DGM), was more prevalent than Hg methylation, probably due to the presence of Hg-resistant bacteria (Baldi et al., 2012). This would also explain the DGM production observed in the absence of light. Overall, the Grado lagoon sector appears to be the most critical in terms of Hg transfer from sediments to the water column, in particular in the fish-farming site. Although, clams are filter feeding, it cannot be excluded a possible link between fluxes of dissolved Hg species and benthic organisms. Benthic fluxes measurements should not be then neglected in evaluating those areas more favorable for clam farming.

3.3.1. Mercury transformation processes mediated by bacterial activity

MeHg accumulation in coastal environments results from a balance between MeHg production and degradation, which are processes almost entirely mediated by microbial activity and controlled by seasonal changes affected by the sulfur cycle and the

availability of dissolved inorganic Hg. Radiotracer experiments conducted in lagoon sediments confirmed active Hg methylation by sulfate-reducing bacteria (SRB), under anaerobic conditions, which was most rapid in summer (Hines et al., 2012). On the other hand, bacteria are also responsible for the demethylation of MeHg via either reductive process producing Hg^0 and CH_4 , or by an oxidative process where Hg^{2+} and CO_2 are the final products. This second pathway usually occurred in the lagoon driven by anaerobic bacteria, primarily SRB, except in winter when the reductive pathway became important in surface sediments. In winter, when sulfate reduction decreased, iron-reducing bacteria seemed to become significant contributors to methylation in surface sediments. It is interesting to note that, when porewater Hg concentrations are considered to calculate net rates of MeHg production, these rates are similar to MeHg benthic fluxes (Emili et al., 2012), supporting the opinion that MeHg is produced and recycled *in situ* to the water column.

Demethylation rate constants in these shallow waters were generally much higher than rates reported in other areas of the region (i.e. Gulf of Trieste, Isonzo/Soča river system; Hines et al., 2000, 2006) and were higher than methylation rates. Both rates decreased with sediment depth suggesting that demethylation in lagoon sediments may naturally help in limiting the MeHg accumulation both in sediments and in biota such as clams (Giani et al., 2012). In the Lagoon, as a consequence of high Hg content in the sediment, a microbial community of Hg-resistant and Hg-tolerant bacteria was able to develop (Baldi et al., 2012). However, the highest number of aerobic heterotrophic Hg-resistant bacteria were isolated from the Aussa River sediments, downstream from the CAP, suggesting even a greater bioavailability of Hg than in the lagoon. Several bacterial strains were capable of reducing Hg^{2+} and volatilizing $\text{Hg}(0)$, thus contributing to Hg detoxification by removing Hg from sediments (Baldi et al., 2012).

3.4. Mercury bioaccumulation in bivalves

Although the lagoon is significantly contaminated by Hg, selecting one site over another for clam farming and/or the extension of rearing activities cannot be decided *a priori* on the basis of metal concentrations in sediments (Acquavita et al., 2012). The evidence obtained from the other research lines of the project needed to be accomplished by testing real bioaccumulation rates on seeded and natural populations of *Ruditapes philippinarum* and by assessing the potential risk for human consumption (Giani et al., 2012). The 18 months long monitoring conducted in selected sites indicated that Hg content in reared bivalves was always lower than the $0.5 \mu\text{g g}^{-1}$ w.w., which is the European Community (EC) limit for commercialization. At the same time, as clams grew in size, their Hg and MeHg concentration decreased, and it was lower than in the starting seeded pool. During growth, it is possible that a dilution of Hg species occurs in clams. In addition, during microbiology experiments conducted on the bacterial population (Baldi et al., 2012), several Hg-resistant bacterial strains, which are capable of active detoxification, were isolated from within the clam's soft tissues (siphons, in particular). These sites appeared to be remarkably suitable for clam farming in spite of Hg contents in bottom sediments. Conversely, wild clams highlighted the increased bioaccumulation of total Hg but not of MeHg with size, showing the highest concentrations in the eastern sector (Grado). In this area, the EC threshold was exceeded for individual clam sizes larger than 35 mm, thus suggesting that the collection of large size natural Manila clams in the Grado sector be avoided. However, potential risk assessment for human health based on clam consumption indicates that no harmful effects are expected even in the worst case scenario.

4. Conclusions

Factors controlling Hg cycling in lagoon environments are normally poorly defined, but critical to understanding the links between sources and higher trophic levels, that are ultimately vectors of human exposure. Although each lagoon system experiences differences in sources of Hg and details of how Hg is transformed and transported, Hg in each system is strongly affected by biogeochemical transformations, which makes it difficult to obtain a general coherent picture of its behavior (Faganeli et al., 2012). The Miracle Research Project represents an informed knowledge base for the drafting of best practice guidelines for clam farming management in a highly Hg contaminated environment, which is not reclaimable, such as the Marano and Grado Lagoon. The total Hg “entrapped” in the lagoon sediments and the complexity of the lagoon ecosystem, suggest that an *in toto* reclamation of the sediments is not feasible. A partial removal of contaminated sediments could be conducted in restricted areas (fish farms), but this kind of intervention is economically and environmentally unthinkable on the whole scale of the lagoon. As a consequence, the presence of Hg in the lagoon environment must be considered a permanent issue and the biogeochemical behavior of this metal in the sediment–water system must be carefully considered in the future whenever a new use for the lagoon is contemplated. From the experimental evidence, it clearly appears that despite very high Hg contamination levels, seeded clams are able to grow and their Hg accumulation does not exceed the concentration limits for commercialization. For such reasons, other areas of the Lagoon, particularly in the western Marano sector, which are currently unused or prohibited for clam farming, could be suitable for such activities, thus representing an economic and occupational resource for the resident population. Even so, the high environmental variability of the area, common to all lagoons, suggests that multidisciplinary studies should be performed on the aforementioned areas, in order to verify their suitability for rearing activities, taking into account Hg and MeHg contents and the potential health risks associated with dietary habits of the resident population.

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