Science of the Total Environment 708 (2020) 134837

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Marine sediment toxicity: A focus on micro- and mesocosms towards remediation

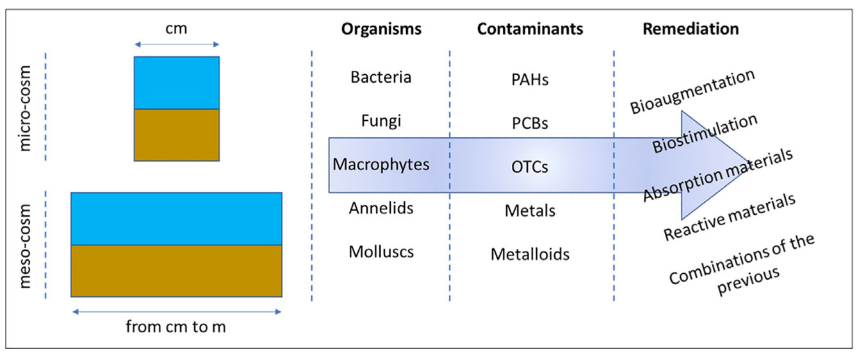
Luisa Albarano [a,b], Maria Costantini [a,*], Valerio Zupo [a], Giusy Lofrano [c], Marco Guida [b], Giovanni Libralato [a,b]

[a] Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy
[b] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126, Napoli, Italy
[c] Centro Servizi Metrologici e Tecnologici Avanzati (CeSMA), Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126, Napoli, Italy

HIGHLIGHTS

- M-cosms provide a link between observational field studies and natural environment.
- Remediation of contaminated marine sediments could be supported by m-cosms studies.
- Case-studies exist on m-cosms and remediation about PAHs, PCBs and heavy metals.
- Main limitations are on m-cosm's structure and components, and model species.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 July 2019
Received in revised form 24 September 2019
Accepted 3 October 2019
Available online 18 November 2019

Editor: Damia Barcelo

Keywords:
Marine sediment
Microcosm
Mesocosm
Polychlorinated biphenyls
Polycyclic aromatic hydrocarbons
Remediation

ABSTRACT

Micro- and/or mesocosms are experimental tools bringing ecologically relevant components of the natural environment under controlled conditions closest to the real world, without losing the advantage of reliable reference conditions and replications, providing a link between laboratory studies and filed studies in natural environments. Here, for the first time, a formal comparison of different types of mesocosm applied to the study of marine contaminants is offered, considering that pollution of coastal areas represented a major concern in the last decades because of the abundance of discharged toxic substances. In particular, the structural characteristics of micro- and mesocosms (m-cosms) used to study marine contaminated sediments were reviewed, focusing on their advantages/disadvantages. Their potentiality to investigate sediment remediation have been discussed, offering new perspective on how the use of m-cosms can be useful for the development of practical application in the development of solutions for contaminated sediment management in the contaminated marine environment.

Contents

* Corresponding author.
E-mail address: maria.costantini@szn.it (M. Costantini).

https://doi.org/10.1016/j.scitotenv.2019.134837
0048-9697/

1. Contaminated marine sediment and EU directives

Environmental concerns arose in most European countries in the mid-1960s, and national governments started to be more active in their attempts to monitor and control environmental pollution. Major focus was devoted to developing and implementing environmental quality guidelines in policies and regulations (Van Wensem, 1989). Special attention was dedicated to the disposal of sediment, resulting from dredging activities of port channels and seaways to maintain maritime navigation. Marine sediments are fundamental and integrated parts of water bodies and are composed of soluble and insoluble matter, primarily rock and soil particles transported from land areas to the oceans by some natural processes, mainly rivers, glaciers and ice, windblown dust, coastal erosion (Brils, 2008). Recent studies showed that Mediterranean coastal sediment may be heavily contaminated, due to industrial, shipping and other anthropogenic activities (Pougnet et al., 2014; Lofrano et al., 2017). Sediment pollution is associated with potential economic, social and environmental problems (Eggleton and Thomas, 2004; Arizzi Novelli et al., 2006; Libralato et al., 2008; Lofrano et al., 2016).

Marine pollution in coastal areas is a major concern, due to the large number of toxic substances discharged and accumulated in sediment, which act as sink and source of pollution (Arizzi Novelli et al., 2006). Especially in harbours and marinas, where exchanges of water masses with the open sea is limited, the accumulation of toxic substances can pose major concerns for human and environmental health such as in presence of recreational waters and maricultural activities (Mamindy-Pajany et al., 2010). Frequently, contaminants occur as mixtures showing combined effects, which are still largely unknown. Dredging activities in industrial and commercial ports tend to remobilize sediment, as well as the associated pollution through the washing out of both short- and long-term contaminant loadings (Arizzi Novelli et al., 2006; Libralato et al., 2008). An effective assessment of sediment quality is compulsory for the management of marine environment as required by the Water Framework Directive (2000/60/EC) (WFD) and the Marine Strategy Framework Directive (2008/56/EC) (MSFD) that considered sediment as one of the key issues for the proper management of surface water bodies. Sediment toxicity proved to be essential in monitoring studies to characterize the state-of-the-art of aquatic environments and to take decisions about contaminated areas according to the TRIAD approach (Losso and Volpi Ghirardini, 2010; Libralato et al., 2008; Hurel et al., 2017). Polluted sediment represents an important issue for fresh, brackish and marine ecosystems, especially coastal ones, due to the high human pressures (i.e. commercial and industrial port activities, human settlements and tourism) and sedimentation rates caused by solid discharges from catchment basins (Nikolaou et al., 2009a; Lofrano et al., 2016). Sediment drains and temporarily segregates pollution with potential direct acute and chronic effects for benthic communities. Natural (i.e., bioturbation) or artificial (i.e., dredging) perturbative events can release the accumulated contamination causing acute concerns to water column populations and the re-allocation of contaminants within the same aquatic environment. Thus, contamination can be scattered in vaster areas or sometimes exported outside from confined aquatic ecosystem (e.g., lake or lagoon) due to flooding events or tides (Arizzi Novelli et al., 2006; Nikolaou et al., 2009b; Mamindy-Pajany et al., 2010). This activity must be carried out in a highly efficient and environmentally safe manner to reduce and keep impacts. Dredged sediment can be treated *ex situ* "on-site" or "off-site" and, finally, transported to its destination (e.g., landfill) or second life (e.g. construction materials). Sometimes the problem of polluted sediment can be tackled without dredging, keeping them in place especially when it must not be removed for assuring drafting ships and if physical dynamics of sites (i.e., currents and wave actions) are not of concern. Dredging and disposal of dredged material are constantly identified as top priority concern by European seaports since 1996 and they are a top priority independently of the port's size. As recently estimated, up to 200 million m^3 of dredged sediment are produced yearly at EU (SedNET, 2004; http://www.sednet.org/download/Sednet_booklet_final.pdf). No clear indications on how to manage them, besides landfill disposal, are available.

Anthropogenic activities can change the natural compositions, the rate, the deposition and the transport of sediment causing a radical shift of biological responses (Burton and Johnston, 2010). Since sediment represents the habitat for benthic community, they could negatively affect the marine flora and fauna (Yi et al., 2011). Several biological communities can react differently to contaminant exposure: i) tolerant species will respond differently with respect to sensitive species; ii) the overall community tolerance is often induced by the constant contaminants' exposure (Clements et al., 2009). The distribution of contaminants within sediment and their affinity with sediment particles may change following receipt to sediment disturbance that stimulates the mobilization of contaminants. Desorption, partitioning, bacterial degradation and the oxidation of organic compounds can accelerate changes of the redox potential and pH (Eggleton and Thomas, 2004). Anthropogenic activities, such as maintenance, capital dredging and the disposal of historically contaminated sediment are also able to induce the remobilization of pollutants in the water column (Eggleton and Thomas, 2004). This resuspension can negatively influence the marine environment and human health, because pollutants can accumulate in bottom feeders and magnify through marine food webs, reaching humans through fishery products (Zupo et al., 2019). For these reasons, the European Water Framework Directive 2000/60/EC (WFD) was developed and constantly updated. This directive provided a new approach for preventing the qualitative and quantitative deterioration of water, to improve the state of water and to assure a sustainable use (Borja et al., 2004). This directive does not specifically address sediment, although sediments are essential for the aquatic environmental.

Since the sediment management plays an important role in preservation of good marine water status, the European Marine Strategy Framework Directive 2008/56/EC (MSFD) includes 11 descriptors including sediment as well (Rice et al., 2012). Both Directives indicated what a "good marine environmental status" is, but the WFD classified ecosystems at different levels by comparing their features until combining them; whereas the MSFD focuses on the 11 descriptors, which together represent the function of the entire system. Thus, the WFD could be combined with the MSFD (Borja et al., 2010). Considering the potential great impact of contaminated sediment to the marine environment, the first step to deal with this problem could be the control of the contaminant source and/or its elimination of both *in situ* and *ex situ* treatments (Gomes et al., 2013).

To the best of our knowledge, scattered studies exist on the use of micro- and mesocosms (m-cosms) to investigate the effects of polluted marine sediment and remediation activities. For these reasons the main aim of this review is to assess m-cosms studies applied to contaminated sediment. In particular, the present study focuses on i) the problem of contaminated marine sediment and the EU directives, ii) structural properties of micro- and mesocoms applied to aquatic studies to detect sediment toxicity; iii) advantages and disadvantages of m-cosms for sediment quality assessment; iv) sediment remediation assessment through m-cosms, focusing on sediment contaminated with polycyclic aromatic hydrocarbons, polychlorinated biphenyls and heavy metals.

1.1. Sediment assessment: From micro- to mesocoms

M-cosms are experimental systems that enable controlling ecologically-relevant components of the natural environment. These controlled conditions are close to the real world and present the advantage of reliable referencing of conditions and replication (Watts and Bigg, 2001). M-cosms provide a link between observational field studies that take place in natural environments, evaluating how organisms or communities might react to environmental changes or exposure to pollutants. They can represent a good compromise between the ecosystem complexity and the highly artificial settings of the laboratory experiments. The debate about m-cosms' definition is still open as well as about their advantages or disadvantages compared to the one-species toxicity testing (Cairns, 1983; Tarradellas et al., 1996). M-cosms can be defined in terms of the size and complexity of the experimental system rather than the number of species they support.

A mesocosm was defined by Odum (1984) as "a bounded and partially enclosed outdoor experimental unit, which simulates the natural environment, particularly the aquatic environment". This type of system provides a bridge between the real environment and the laboratory, particularly useful to study the biological effects of chemicals in ecotoxicology (Crossland and La Point, 1992).

In such experiments the microcosms consisted of polyethylene tubes, glass bottles and beakers starting from few liters to a maximum volume of 500 L. The differences between microcosms and mesocosms were only a matter of scale in terms of volumes as well as of parameters to be analyzed. The physical size of mesocosm systems ranged from 1 m depth (Petersen et al., 1997) to 18 m or roughly 1–400 m^3 in volume (Harada et al., 1996). Mesocosms (Teuben, 1992) were mainly used in field experiments to fill the gap between experimental laboratory work and natural conditions. Some examples are reported in Fig. 1.

M-cosms are small-scale experimental systems which inform about ecological processes that are applicable at larger scales (Carbonell and Tarazona, 2014; Vidican and Sandor, 2015). More recently, the use of these systems played a role in exploring the consequences of seemingly insurmountable global issues, such as the effect of climate change on species distributions (Petchey et al., 1999), the impacts of pollution and fisheries on ecosystems (Micheli, 1999) or harvesting on population dynamics (Cameron and Benton, 2004). Micro- and mesocosms are basically small ecosystems, able to improve the understanding of natural processes by simplifying the complexities of natural environments. This review assesses some micro- and mesocosm structural properties to strengthen the knowledge about their role in environmental studies applied to marine scenarios.

Micro- and mesocosms can be closed or open systems. However, for studying soil ecosystem processes, the best results were reached using the open flow-through experimental systems.

At the beginning, the microcosms were used by French et al. (1989) to evaluate the effects of different nutrient and chemical additions on water quality. The effects of organic additions on nitrification and denitrification were examined in sediment microcosms (Caffrey et al., 1993). Laboratory-based microcosms were used to determine the persistence of the faecal indicator organism *Escherichia coli* in recreational coastal water (Craig et al., 2004). Results of this microcosm demonstrated the prolonged survival of *E. coli* in coastal sediment compared with overlying water, implying an increased risk of exposure because of the possible resuspension of pathogenic micro-organisms during natural turbulence or human recreational activity.

Anthropogenic inputs of crude and refined petroleum hydrocarbons into the sea required knowledge of the effects of these contaminants on the receiving of assemblages of organisms. A microcosm experiment was carried out to study the influence of diesel on a free-living nematode community of a Tunisian lagoon (Mahmoudi et al., 2005). Sediments were contaminated by diesel and the ecological effects were examined. Dose-dependent changes on the community structure were revealed, as well as the responses of nematodes to the diesel treatments that varied according on a species-by-species basis. Duan et al. (2009) used closed microcosms with glass beakers containing sediment and seawater to define the effects of microbial activity and carbon source on arsenic and iron mobilization. More recently, Santos et al. (2018) tested the effects of exposure to sewage-impacted pore water simultaneously at the community level using meiofauna microcosms and at the individual level of the copepod *Nitokra* sp. by using standard fecundity tests. Considering the importance of meiofauna for benthic ecosystems, the microcosm approach using natural meiobenthic communities provided highly relevant ecological information on the toxicity of contaminated sediments. More in detail, the toxicity of pore waters was tested from three sites according to a contamination gradient. Both approaches revealed differences in toxicity between sites at various levels of contamination.

1.2. Microcosm and sediment toxicity

The first applications of mesocosms in aquatic studies referred to the patterns of productivity and respiration during eutrophication in lower Narragansett Bay (Rhode Island, USA; Oviatt et al., 1986; see Fig. 1A). Another study was applied to detect the effects of chemicals in freshwater environment, varying in size, design, construction and costs according to the purpose (Beklioglu and Moss, 1996). Mesocosm experiments were used to study the interaction of sediment influence, fish predation and aquatic plants with the structure of phytoplankton and zooplankton communities (Becklioglu and Moss 1996).

The Multiscale Experimental Ecosystem Research Center (MEERC) conducted several experiments in mesocosms with the aim of quantifying the effects of scale in terms of time, depth, radius, exchange rate and ecological complexity, on biogeochemical processes and trophic dynamics in different coastal habitats

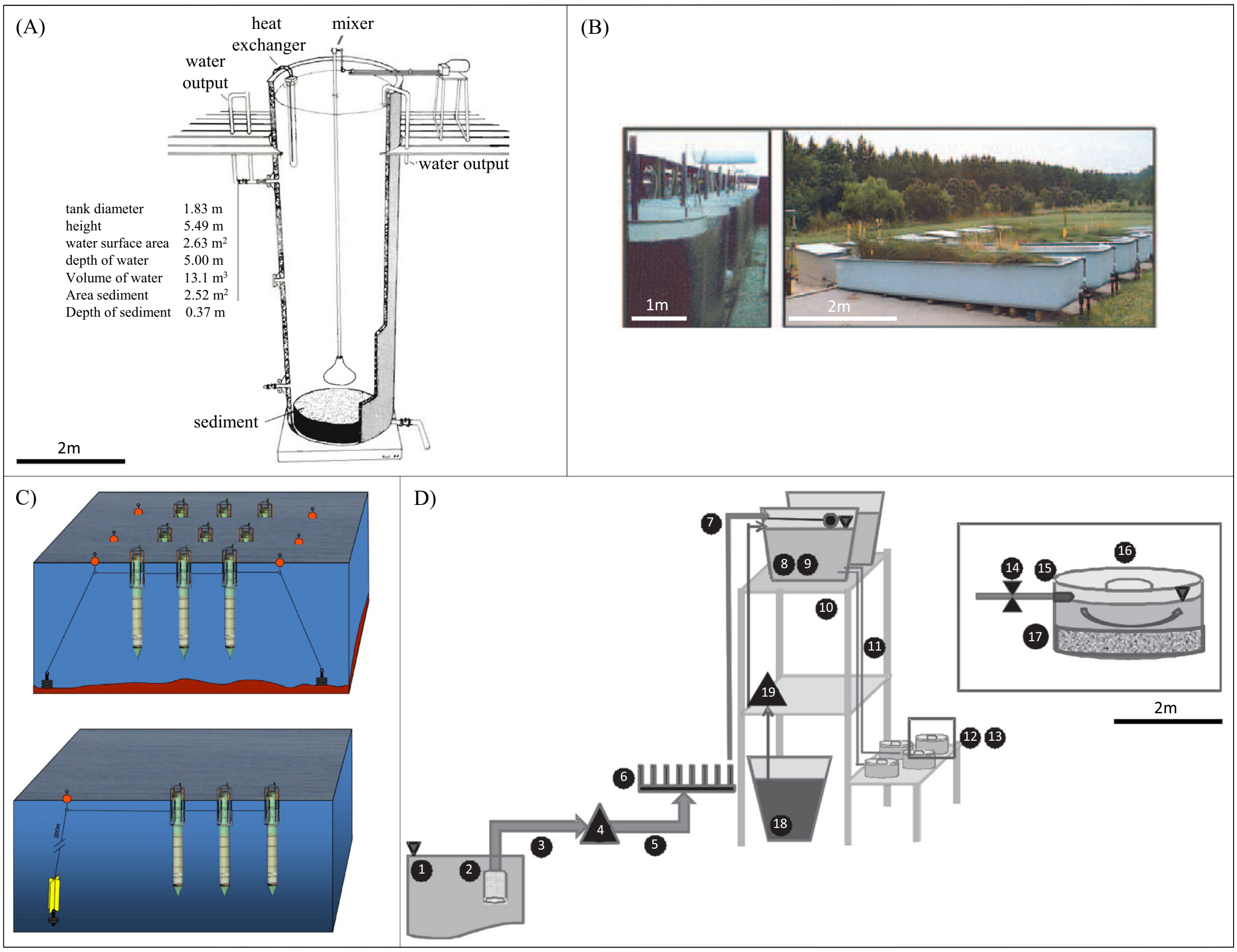


Fig. 1. Mesocosms. (A) Cross section of a mesocosm, showing input and output pipes, mixer, sediment container and heat exchanger. The tanks are constructed of fiberglass reinforced resin with interior walls to maximize the reflection of sunlight. Seawater is fed in a pulsed flow of 10 1iters per minutes for 12 min period every six hours. Temperature control is accomplished with glass heat exchangers able to heat and cool (modified from Oviatt et al., 1986). (B) Multiscale, multihabitat experimental ecosystems for planktonic–benthic systems (left panel), marsh (right panel) (modified from Peterson et al., 2003). (C) Schematic representation of the two modes of mesocosm operation: mesocosms in moored mode in packs of three with anchor weight at each end (upper panel); mesocosms in free-floating mode connected to a weighted drogue hanging from a buoy at 150 m water depth (lower panel) (modified from Biebesell et al., 2013). (D) Experimental set-up. (1) Kauru River, (2) pump inflow covered with 4.5 mm mesh, (3) 20 m of 50-mm polythene piping, (4) two centrifugal pumps, (5) 80 m of 50-mm polythene piping, (6) eightfold manifold, used to split water flow into eight pipes, (7) 8 9 10 m of 25-mm polythene piping, (8) eight header tanks (volume 135 L), each one feeding a batch of 16 mesocosms, (9) water level in header tanks regulated by ball-cock, (10) two-level scaffold, 4.1 m high and 20 m long (11), 128 9 4 m of 13-mm polythene piping supplying individual mesocosms with water, (12) 1-m-high and 1.2-m-wide wooden bench running along scaffold, (13) 128 circular flow-through stream mesocosms, (14) tap regulator, (15) inflow jet, (16) mesocosm water outflow, (17) base layer of substratum, (18) seven barrels (volume 300 L) containing highly concentrated $NaNO_3$ and KH_2PO_4 solution for nutrient treatment levels 2–8, level one received ambient nutrient concentrations, (19) seven fluid metering pumps used to drip highly concentrated nutrient solutions into seven header tanks (mod. from Wagenhoff et al., 2013).

(Petersen et al., 2003; see Fig. 1B). To this end, a series of experiments were performed, in which time, space and complexity in a variety of estuarine habitat types were systematically manipulated. Firstly, the effects of mesocosm size and shape on the dynamics of planktonic-benthic ecosystems were assessed. Fifteen cylindrical mesocosms were constructed according to five sizes, three volumes and three replicates per size and they were organized into three series: one with a constant depth (1.0 m), one with a constant shape (radius-to-depth ratio = 0.57) and one with constant volume (10 m^3). Mesocosms received artificial light on a 12:12 light–dark cycle and an exchange of filtered estuarine water at a rate of 10% per day, mixed to mimic turbulence in a tidal environment. Because tidal estuarine marshes play an important role in processing nutrients and organic matter at the land-sea interface, the effects of species diversity on this function was evaluated. Six marsh mesocosms were constructed using fiberglass tanks (6 m long, 1 m wide, 1 m deep) angled at a 20:1 slope. In this system pumps were used to simulate a 12.5-h tidal exchange with filtered water from an adjacent estuary. The experiment was run for five years to evaluate long-term dynamics of three common plant species found in Chesapeake marshes (*Spartina alterniflora*, *Spartina patens* and *Distichlis spicata*) and no macrofauna were added. While the plant species diversity over the temporal extent of the experiment was preserved, unsuccessful maintenance of the additional animals introduced in the high diversity treatment was recorded. More in general, the results indicated that the effects might be categorized in fundamental (evident in both natural and experimental conditions) and artefacts of enclosure (attributable to the artificial environment in mesocosms).

A mesocosm study was also applied to detect the impact of resuspension on the fate and bioaccumulation of mercury and methylmercury in shallow estuarine environment (Kim et al., 2006). Two experiments of four weeks were performed in spring and fall in cylindrical tanks (1 m^2 of surface area and 1 m deep), demonstrating that the interplay between mercury and methylmercury degradation determined the overall methylmercury pool in sediments. In turn, their resuspension lead to changes in the association to mercury binding phases, influencing its methylation.

Wagenhoff et al. (2012) studied macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients in a stream mesocosm. Most responses were additive multiple-stressor patterns. Later these authors also studied the composition of benthic algae and cyanobacteria using the same experimental approach (Wagenhoff et al., 2013; see Fig. 1D).

To perform mesocosm experiments in different hydrographic conditions and in areas considered most sensitive to ocean change, Riebesell et al. (2013) developed a mobile sea-going mesocosm facility, the Kiel Off-Shore Mesocosms for Future Ocean Simulations (KOSMOS). The KOSMOS platform, transported and deployed by mid-sized research vessels, was designed for operation in moored and free-floating mode under low to moderate wave conditions (up to 2.5 m wave heights; see Fig. 1C). It consisted of a water column 2 m in diameter and 15–25 m deep (about 50–75 m^3) without disrupting the vertical structure of the planktonic community. This system minimized the differences in starting conditions between mesocosms, extended experimental duration, determined the mesocosm volume and air–sea gas exchange.

Open system mesocosms were mainly used to simulate the ocean acidification and it has been demonstrated that this approach is low-cost and allows for pioneering long-term experiment with continuous intake of seawater ensuring the maintenance of quasi-natural environmental conditions (Jokiel et al., 2014). This approach is based on a low-cost method applicable to small or very large experimental systems and consisted of a gravity-feed seawater supply system with a peristaltic pump to regulate CO_2 injection rates and a power head, cavitating the injected CO_2 into microscopic bubbles that are dissolved immediately. The system permitted long-term experiments under full sunlight with rapid seawater turnover rate with realistic environmental conditions in the experimental chambers.

Moreover, a modular, small size (60 L) and indoor mesocosm was used to detect the environmental risk of engineered nanomaterials (Auffan et al., 2014). Initially, they waited for the settlement of suspended particles, then the stabilization of the physical parameters (O_2, redox potential and pH) and the growth of organisms were measured. This experimental system allowed the simultaneous monitoring of several parameters (e.g. aggregation, biotransformation, oxidative stress, microbial diversity) under environmentally meaningful conditions. This experimental design can accommodate several types of ecosystems such as lotic, lentic, estuarine, or lagoon environments, without the necessity of expensive infrastructures.

Pansch et al. (2016) used open systems to study the global change of temperature, ocean acidification, rising sea level and eutrophication. The system consisted in tanks of 170 $\times$ 85 cm connected to a measurement system used to assess different parameters such as temperature, dissolved oxygen and pH. Closed micro- and mesocosm systems are self-sustaining once built-up without intake of nutrients or more water. These systems were mainly used in toxicity experiments because they avoided the environmental dispersion of contaminants. Another interesting study was recently conducted by Lehto et al. (2017) usinga closed mesocosm of 15 $\times$ 14 $\times$ 8 cm to explore possible effects of deposition of particulate organic matter and the release of metals from sediment. The examples showed the high versatility of these systems to different conditions, suggesting their challenging applications for contaminated sediment assessment.

2. Pros and cons of m-cosms for sediment quality assessment

At the beginning, the expectancy on the use of microcosms were very high (Draggan, 1976), but three years later Gillett and Witt (1979) concluded that they needed substantial improvements in the accuracy, sensibility and reliability to fulfil these expectations. Later, Giesy (1980), Hammonds (1981) and Van Vorris et al. (1983) reported about the improvements and discussed about advantages and disadvantages of the use of these systems. One of the major points concerned technical questions on the physical, chemical, biotic structure as well as the duration of experimental micro- and mesocosm (Table 1). Moreover, another critical point referred to the relationship of the results with a natural ecosystem, considering if m-cosms were analogous to natural ecosystems and/or are model ecosystems. Ausmus and O'Neill (1978) suggested some criteria for the extrapolation of m-cosms experiments to the field, considering if they reflected a natural ecosystem.

As reported, concerning the different types of m-cosm systems (Cole et al., 1978; Coleman et al., 1978; Anderson et al., 1979; Parkinson et al., 1979; Clarholm et al., 1981; Hassall et al., 1986) the best results for a valuable comparison can be expected from open systems (Huhta et al., 1988; Setälä et al., 1988; Taylor and Parkinson, 1988, 1989; Williams and Griffiths, 1989). Some authors reported the results of laboratory microcosm experiments (Teuben and Roelofsma, 1990) and field experiments with mesocosms (Teuben, 1991a, b) comparing them with measurements of process variables from the field, to test the relevance of these micro- and mesocosm studies. These results concluded that m-cosms were reliable to unravel the intrinsic soil ecosystem interactions, but mesocosms in the field were referred to laboratory microcosms. At the beginning microcosm tests are more expensive and time consuming, than other types of traditional tests for environmental issues (Gorsuch et al., 1993).

In addition, it is important to consider that the prevention of the ecological impacts of global environmental changes require scientific evidences, but the temporal and spatial scales of these environmental problems are too extensive and become difficult to gain only by using the traditional experimental manipulation (McCann, 2007). M-cosms might help, even if a 'credibility gap' can arise from within the scientific community, in understanding if insights from these experimental systems could be relevant to study larger scale processes. Benton et al. (2007) considered the key role of m-cosms in the attempt to understand large-scale processes. having two important roles: i) providing ideal systems on which the statistical approaches can be tested; ii) managing recommendations on systems with limited knowledge of the ecological processes are involved.

Despite their distinct contribution to our understanding of ecology and the environment, however, microcosm approaches have been often criticized for being irrelevant for policy-relevant issues (Carpenter, 1996; Srivastava and Vellend, 2005). An opposite view-

Table 1
Advantages and disadvantages in the use of micro- and mesocosms.

Advantages	Disadvantages
More sensitive than laboratory experiments	Low number of replicates
More reproducibile data and easier to conduct experiments	Small size and short experiment time
Use of more species for defining the interaction beetween them	Inaplicable to understanding less simple parts of nature
Fills a gap beetween laboratory and field studies	Not realistic

point was presented by Cadotte et al. (2005), claiming that laboratory models were necessary for investigating complex ecological communities. This may have been pushed by the urgency to explore the fundamental processes of ecology rather than with the aim to use model systems to address real world and global-scale problems.

Ecologists tried to build projects around microcosm experiments, because, in addition to the basic-science insights, *m*-cosms have pragmatic advantages important in the competition between environmental sciences and molecular biology (Carpenter, 1996). They can provide i) results to be rapidly published; ii) modest costs. Ecology is now considered a significant applied science with a responsibility versus the society in contributing to solve environmental problems by using appropriate scaled field studies. (Likens, 1992; Shrader-Frechette and McCoy, 1993), using all available tools to advance the analysis of ecosystems and their communities at the scales of natural processes, management and societal concern.

More recently, Drake and Kramer (2012) evidenced that m-cosms are ecologically simplistic, mainly the subset of species included, such as the exclusion of large-bodied species (Schindler, 1998), meaning that the reduction of complexity decreases the capacity of an experiment to provide reliable predictions about the ecosystem. M-cosms did not consider natural spatial and temporal variation, being small in size and short in duration and so limiting physical environmental heterogeneity and excluding processes at large spatial extents (Diamond, 1986; Ricklefs, 2004). The use of mesocosms, essentially larger than microcosms, often includes at least partially these sources of variation (Relyea, 2005), giving rise to potential artefacts.

The use of m-cosms enables to study the stress response of various species and the effects on the communities at different levels (Crossland and La Point, 1992; Beyers and Odum, 2012). Microcosm studies were more sensitive to detect the toxic impacts when compared to laboratory experiments. Santos et al. (2018) studied the toxicity of pore water of three different sites using two approaches: i) experiments in microcosms, ii) and in laboratory. In both cases, different toxicity between three sites has been established. Microcosm experiments are easy to conduct and give more reproducible data than field experiments. Chen et al. (2001) tested the effects of three different fungicides using a plastic tube as a microcosm, obtaining good results and reproducing the basic features of natural environmental at a small scale.

One of the advantages of using these systems is the great number of organisms that can be used (Steele, 2001). The use of mesocosms offered a solution to fill a gap between laboratory and field experiments, as in the case of Riebesell et al. (2013), where they made analyses about the effects of ocean challenges on pelagic community.

Hose et al. (2004) compared the species-sensitive distribution (SSD) curves of laboratory with local experiments in mesocosms and field data to evaluate the water quality in Australia. The mesocosm species were less sensitive than the species in laboratory tests, suggesting that the laboratory data can be used to determine the concentrations of mesocosms.

Several studies showed that the combination of the field, mesocosm and *in vitro* results were interesting to denife toxicant effects. Culp et al. (2000) evaluated the regional assessment of rivers by using these combined studies establishing the main effects of the discharges and the community responses to these stressors.

Grenni et al. (2012) tested the effects of microbial activity for ecological bioremediation of contaminated ecosystems in mesocosm systems. Using these systems, they assessed the degradation property of microbial activity under different natural conditions and under more stressor factors by providing realistic results. In any case, careful attention must be paid in generalizing the conclusion to natural ecosystems (Petersen 2003).

3. Sediment remediation assessment and m-cosms

Marine sediment is a fundamental and integrated part of water bodies accumulating biological, physical and chemical contaminants (Brils, 2008). Organic pollutants in sediment represent a worldwide problem, considering that sediment acts as a sink of hydrophobic, recalcitrant and hazardous compounds (Perelo, 2010). According to the biogeochemical processes, these contaminants are available to benthic organisms as well as to organisms present in the water column and toxic and carcinogenic, entering in the food chain and accumulating in the biological tissue. Among these compounds polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals represent the most abundant pollutants present in some marine sediments (Mamindy-Pajany et al., 2010), being released during perturbation events, absorbed by suspended particles and therefore settle at the bottom with a high impact on marine environment and consequently on human health (Geffard et al., 2001; Akcil et al., 2014).

Because of the potential toxic effects of these compounds on human health, researchers explored methods to detoxify/remove them from natural marine environments with both *in situ* and *ex situ* treatments, using different chemical, biological and thermal methods (Bamforth and Singleton, 2005; Gomes et al., 2013). These processes were classified as forms of "bioremediation", which refer to a process through which toxic compounds are biologically degraded and/or transformed to an innocuous state (Mueller et al., 1997). This process consists in the removing of pollutants from the natural environment converting them to a less harmful product (Bamforth and Singleton, 2005). In this review, we focused on sediment restoration contaminated with PAHs, PCBs and heavy metals, mainly using m-cosms.

3.1. PAH case studies

Polycyclic aromatic hydrocarbons (PAHs) consist of a group of over 100 different organic compounds, formed by two or more fused benzene rings and pentacyclic molecules differing in their physical and chemical properties. Their toxicity depends on the number of benzene rings (Perelo, 2010) and can be generated by: i) natural events such as volcanic eruptions, fossil fuels or incomplete combustion of organic materials; ii) anthropogenic activities such as industrial processes, oil and diesel spills and waste incineration. Anthropogenically produced substances that are considered the most impacting inputs into the natural environment (Bamforth and Singleton, 2005). These compounds tend to persist in the environment because of their low water solubility and hydrophobicity that permit them to be adsorbed and accumulated in sediment, where their degradation is very slow. The persistence of PAHs in the environment can also be controlled by such environmental factors such as soil type, pH, temperature, oxygen levels (Sutherland et al., 1995; Bamforth and Singleton, 2005). Sixteen PAH compounds were classified as priority pollutants by the United States Environmental Protection Agency (US-EPA; Perelo, 2010). Some studies have demonstrated that these organic molecules can have toxic effects for human health with many PAHs showing carcinogenic, teratogenic and mutagenic properties (Ferrarese et al., 2008). For these reasons, several remediation techniques were used for PAHs removal from contaminated sediments (Table 2). Some researchers suggested bioremediation techniques using the microbial metabolism to remove pollutants from contaminated sediments. Three fundamental methods exist: a) bacterial degrada-

Table 2
Strategy, site, operating conditions, efficiency of treatment for bioremediation by PAHs.

Strategy	Site	Operating conditions	Efficiency of treatment	References
Biological	Italian harbor in Mediterranean sea	Mixtures of inorganic nutrients and sand amendaments	40% PAHs	Beolchini et al., 2010
	Ancona port	Mixtures of inorganic nutrients and switch of temperature, in aerobic and anaerobic conditions	80% PAHs	Dell'Anno et al., 2012
	Carteau cove	Combination of marine polychaete *Nereis diversicolor* and oil contaminated sediments	Increase of bacteria communities to reduce contaminations	Cuny et al., 2007
	Dredged sediments from DeGray Reservoir	Inoculation of *Mycobacterium* sp.	25% 2-methylnaphthalene, 74% phenanthrene, 34% pyrene, 5% benzo-α-pyrene	Heiktamp et al., 1989
	Van-veen grab in Nyborg port	Combination of marine polychaete *Capitella* sp. I and oil contaminated sediments	No effects on PAHs decrease	Holmer et al., 1997
	Stanford le Hope, Essex, UK	Mixtures of inorganic nutrients and three hydrocarbon-degradation bacteria	78% PAHs with *Alcanivorax*, 70% *Thalassolituus*, 70% all bacteria	McKew et al.,2007
	Kamaishi (Japan)	Inoculation of *Cycloclasticus* sp.	38% PAHs	Miyasaka et al., 2006
	Gulf of Fos (France)	Natural develop of hydrocarbon-degradation bacteria	35% PAHs	Syakti et al., 2006
	Yiu Lian Floating Dock (France)	PAHs-addition	Increase PAHs-degradation bacteria, 50–98% PAHs	Wang and Tam 2011
Chemical/ physical	Oxford	AC-addition	80% PAHs	Bussan et al., 2016
	River Tyne	AC-addition	94% PAHs	Hale et al., 2010
	Oslo harbor	Capping	93% PAHs retained	Eek et al., 2008
	Norwey	AC-Capping	93% PAHs retained	Samuelsson et al., 2015
	Rivers Tyne and Wear (UK)	MAC-addiction	98% PAHs retained	Han et al., 2015

tion, b) lignolytic and c) non-lignolytic fungal degradation, which all together on benzene rings (Bamforth and Singleton, 2005). The bacterial degradation may take place aerobically, anoxically, or anaerobically, but PAH-restoring are usually associated with anoxic/aerobic biodegradation because most of the contaminated sediments are in these conditions (Lu et al., 2011).

The main mechanism for aerobic bacterial metabolism of PAHs is the initial oxidation of the benzene ring by the activity of dioxygenase enzymes, which can then be further metabolised via catechol to carbon dioxide and water. The metabolic pathways and enzymatic reactions involved in the microbial degradation of naphthalene have been studied in detail (Cerniglia, 1992). Several bacteria can oxidize naphthalene through dioxygenase enzymes, including organisms from the genus *Pseudomonas* and *Rhodococcus* (Cerniglia, 1992; Mueller et al., 1997; Revelet et al., 2000).

PAH-restoring also took place thanks to the action of other microorganisms, as cyanobacteria, green algae and diatoms, which can transform naphthalene into different metabolites (Haritash et al., 2009). Several studies under controlled conditions were performed using the miniaturized ecosystems micro- and mesocosms (Grenni et al., 2012). The first study was performed by Heitkamp et al. (1989), using sediment and water collected with a Peterson dredge from DeGray Reservoir (a manmade impoundment which receives no significant exposure to chemical compounds), near Arkadelphia (Arkansas, USA). A microcosm (consisting of a flow-through system, 0.5 L glass tanks containing 20 g of sediment and 180 mL of lake water) was used to conduct biodegradation tests to monitor mineralization and recovery of chemicals. *Mycobacterium* sp. was added to sediment and water from this pristine ecosystem. Microcosms inoculated with the *Mycobacterium* sp. showed enhanced mineralization, alone and as components of a mixture of 2-methylnaphthalene, phenanthrene, pyrene, and benzo[*a*]pyrene. In (Miyasaka et al., 2006), the efficiency of bacteria in PAHs marine sediment restoring has been confirmed. In fact, they investigated three different bioremediation methods using microcosms: biostimulation with fertilized, bioaugmentation with *Cycloclasticus* sp. and mixtures of fertilized and bacteria. All microcosm experiments resulted efficiently for PAHs restoring after 90 days of exposure, having effects on bacterial abundance.

Considering the important role of the microorganisms in contaminated sediment bioremediation, several studies were performed on the effect of nutrient additions (e.g., nitrates) on the microbial communities of contaminated sediment with PAHs. The structural properties and the recycling processes of these microorganisms were highly enriched after inorganic materials addiction, suggesting that this bioremediation *in situ* can be stimulated by the contaminated sediments for bioremediation were collected from an Italian harbour in the Mediterranean Sea, using microcosms with 20 g of wet sediment samples and 100 mL of pre-filter seawater. Inorganic materials were added to all tanks (nitrogen and phosphorus) and to sand amendments and after thirty days the growth of prokaryotic organisms was detected, and the PAHs decreased of 40%. Dell'Anno et al. (2012) showed that bacterial abundance and biodiversity changed and increased at high temperature in both aerobic and anaerobic conditions. Bioremediation experiments were performed with sediment samples from two sites of the port of Ancona (Italy) characterized by two different redox potentials, using microcosms in anaerobic and aerobic conditions. The nutrients were added together with the increasing of temperature from 20 °C to 35 °C, observing major changes in the bacterial biodiversity and abundance at higher temperature in both conditions (aerobic and anaerobic), with a subsequent decrease of PAHs. No significant effects of nutrient addictions were evidenced. A part from the important role of hydrocarbon-degradation bacteria in restoring sediments, little information is available on their different strategy of bioremediation. McKew et al. (2007) analysed the degradation efficiency of three hydrocarbon-degradation bacteria (*Alcanivorax*, *Thalassolituus*, *Cycloclasticus*) adding inorganic nutrients (i.e. N and P) and using microcosm and seawater collected from a site (Essex, UK) neighbouring the Shell-Haven and Coryton BP oil refineries. Different microcosms were built under several conditions: oil-only microcosm, *Alcanivorax* + N-P microcosm, *Thalassolituus* + N-P microcosm and *Cycloclasticus* + N-P microcosm, all bacteria + N-P + microcosm. They observed an increase of all bacteria after one month, mainly of *Alcanivorax*. However, in all microcosms with nutrients, they recorded a decrease of PAHs levels, showing a significant correlation between availability of nutrients and bacteria abundance.

Furthermore, it has been demonstrated that bacterial biodiversity and abundance were correlated to contamination. Syakti et al. (2006) recorded an increase of hydrocarbon-degradation and heterotrophic bacteria in the oil contaminated sediments. Specifically, microcosms were used with/without oil-contaminated sediments to assess PAHs-biodegradation and phospholipids fatty acid (PLFA) composition. An increase of hydrocarbon-degradation bacteria was observed after 21 days, due to a composition change in PLFA and consequently the decrease of PAHs. Wang and Tam (2011) demonstrated an increase of PAHs-degradation bacteria by adding PAHs to hydrocarbon-contaminated sediment. This experiment was performed in a microcosm (consisting of 12 conical flasks of 250 mL, each containing 150 mL sediment slurries prepared by mixing 800 g fresh marine sediments and 1600 mL sterilized) with/without added PAHs to contaminated sediment. A clear enhancement of degradation bacteria was recorded after 60 days, and a reduction in species diversity in all treatments. This change was coupled by a significant depletion of PAHs (50–98%; see also Table 2).

Moreover, Cuny et al. (2007) observed that petroleum contaminated sediments inhabited by a marine polychaete *Hediste diversicolor*, allowed the growth of specific bacteria, such as *Psychroserpens burtonensis*, known to degrade PAHs. Then, they investigated on the possible influence of these polychaetes on the development of different bacterial species using microcosms, consisting of twelve 2.5 L aquariums. They established that the presence of *H. diversicolor* in contaminated sediments promoted the degradation of oil and the development of bacterial communities able to reduce pollution after one month of exposure. However, the possible role of some polychaetes on PAHs-degradation was already evaluated in previous studies. Holmer et al. (1997) conducted a study on the polychaete *Capitella* sp. influence in the biogeochemistry of oil-contaminated sediments. Specifically, they used microcosms inoculating the PAH fluoranthene and worms, and established that the initial rate of PAHs did not decrease but these compounds were transported to deeper sediment layers where microbial degradation activity was less efficient.

Several studies were conducted to assess the potential role of chemical oxidation in PAHs degradation in contaminated sediments. There are various reactive liquids (such as modified Fenton's reagent, hydrogen peroxide, potassium permanganate, activated sodium persulfate) and he residual concentration of PAHs after each type of chemical oxidation has been followed to assess the efficiency of treatments. The best results were achieved with the use of modified Fenton's reagent, hydrogen peroxide and potassium permanganate (Ferrarese et al., 2008; Flotron et al., 2005).

Another method of chemical remediation is the addition of activated carbon (AC) to contaminated sediment, that can be defined as all substances with high carbon content and porosity enabling to absorb different compounds (Rakowska et al., 2012). PAHs-concentrations in sediment decreased of >50% after AC treatment (Hilber et al., 2010). The efficiency of this amendment depends on the quantity of AC added to the sediment per unit of volume/weight. The ratio, then, is influenced by the physical–chemical properties of sediment. Recently Bussan et al. (2016) investigated the role of AC in PAHs bioremediation of polluted sediments using marine microcosm systems before any treatment with AC. Then they set up different experimental designs: i) only sediments without amendments, ii) autoclaved sediments, iii) sediments + bio char; iv) sediments + AC. After two weeks, they observed a considerable mercury-depletion in all treatments. Hale et al. (2010) compared bioremediation and AC amendment for PAHs contaminated sediment from River Tyne. The efficiency of different amendments was followed using microcosm, including: i) an unamended system of 7 g sediment and 40 mL of River Tyne water and two amended systems with 7 g sediment, ii) 40 mL nutrient solution and iii) 40 mL nutrient solution inoculated with *Pseudomonas putida* (i.e. a strain that can degrade naphthalene and phenanthrene). These three systems showed a significant decrease in total sediment PAH concentrations over one month. Polyethylene passive (PE) samplers were embedded for 21 days in these sediment microcosms in order to measure the available portion of PAHs and accumulated from the unamended, biostimulated, and bioaugmented microcosms, respectively. Higher PAH uptake by PE samplers in biostimulated and bioaugmented microcosms coincided with slower degradation of spiked phenanthrene in sediment-free filtrates from these microcosms compared to filtrates from the unamended microcosms. Microbial community analyses revealed changes in the bacterial community directly following the addition of nutrients, but the added *P. putida* community failed to establish itself.

This amendment was usually associated to another *in situ* treatment, the capping, which is a physical cover of contaminated sediment, made of several materials, such as sand, clay or mixtures of different components. The combination between capping and AC is successful and do not lead to massive AC resuspension (Cornelissen et al., 2011). Eek et al. (2008) tested a mineral cap (crushed limestone) to limit the PAHs spread into the water column using microcosms. During 410 days of treatment, they assessed that the flux of contaminants from capped sediment was low respect the flux from uncapped sediment. Samuelsson et al. (2015) evaluated three different *in situ* AC-capping (AC-only, AC + clay and AC + sand) to assess the cap efficiency. After one year of capping, they collected samples to understand the best method using microcosms, using the worm *H. diversicolor* and the clam *Abra nitida* as test organisms. They measured the PAHs-bioaccumulation of two organisms, after one month, demonstrating that the AC-capping + clay was most efficient. Nevertheless, the AC addiction is an efficient method of bioremediation, and it has been demonstrated that it causes adverse effects on some organisms, especially on benthic organisms. In fact, one part of the AC added can diffuse in the water column causing negative impacts on various organisms. Another option can be the use of iron-based amendments into "magnetic AC" (MAC) because it has been demonstrated that it has remediation features as also AC, but it enables their magnetic retrieval (Libralato et al., 2018). Han et al. (2015) tested the MAC remediation using microcosm experiments to evaluate the remediation capacity and possible negative or positive effects on microorganisms. After six months, they showed that the MAC use efficiently reduced PAHs concentrations. They recorded the 77% of MAC and the remainder of MAC had fewer negative effects respect to AC on *Lumbriculus variegatus* while maintaining PAH availability low.

3.2. PCB case studies

Polychlorinated biphenyls (PCB) are persistent organic compounds and are considered among the most impacting contaminants because of their toxic and carcinogenic properties, vast distribution and high permanence in environmental systems (Meagher, 2000; Scragg, 2005; Perelo, 2010). These compounds mainly derive from lubricants, plasticizers and adhesives, as well as from the production of, hydraulic and dielectric fluids. The accumulation of PCB-contaminated sediment by biota represents a great problem because they can be introduced into the food webs, constituting a high risk for human health (Gomes et al., 2013). Several studies have been conducted to assess the best method of PCB-

Table 3
Strategy, site, operating conditions, efficiency of treatment for bioremediation by PCBs.

Strategy	Site	Experimental design	Operating conditions	Treatment efficiency	References
Biological	Strážsky (Slovakia)	Microcosm	Addiction of 15 different bacterial strains	10–20% PCB 153 and 118, 25% PCB 138, 40–45% PCB 101, 30–80% PCB 52, 55–80% PCB 28	Dudášová et al., 2012
	Busan coast (South Korea)	Microcosm	Addiction of 2 bacterial strains and plant terpenes	60–70% PCB	Kwon et al., 2008
	Lake Hartwell, New Bedford harbor, Roxana Marsh	Microcosm	Addiction of iron to produce hydrogen	Enhancement of PCB-degradation	Srinivasa Varadhan et al., 2011
	Brentella canal (Venice, Italy)	Microcosm	Addiction of iron to produce hydrogen	Enhancement of PCB-degradation	Zanaroli et al., 2012
	Anacostia River	Microcosm	Mixtures of electron donors, alternate halogenated electron acceptors, tetrachlorobenzene, pentachloronitrobenzene and bacteria	Enhancement of PCB-degradation	Krumins et al., 2009
	Brentella canal (Venice, Italy)	Microcosm	Addiction of PCBs	90% PCB	Zanaroli et al., 2010
	Mar Piccolo (Taranto, Italy)	Microcosm	Addiction of PCB-degradation bacteria	Important decrease of PCB	Matturro et al., 2015
	Grasse River (Massena, NY)	Microcosm	Addiction of PCB-degradation bacteria	83% PCB	Kaya et al., 2017
	Grasse River (NY), Fox River (WI), Baltimore harbor (MD)	Microcosm	Addiction of PCB-degradation bacteria	51–88% in Grasse River, 83–88% in Fox River and 70% in Baltimore harbor	Kaya et al., 2018
Chemical/ physical	Grasse River (Massena, NY)	Microcosm	AC-addition	70–90% PCB	Sun et al., 2007
	Grasse River (Massena, NY), Hunters Point Naval Shipyard (San Francisco Bay, CA)	Microcosm	AC-addition	70–90% PCB	Mcleod et al., 2008
	Lac du Bourget (Savoie, France)	Microcosm	GAC + biofilm	Efficiently PCBs adsorption	Mercier et al., 2013
	Lac du Bourget (Savoie, France)	Microcosm	Three different granular AC-addition	98.3–100% PCBs	Mercier et al., 2014
	Baltimore harbor (USA)	Mesocosm	Bioaugmentation with anaerobic halorespiring *Dehalobium chlorocoerci* and aerobic *Burkholdeira xenovorans,* and GAC addition	>80% PCBs	Payne et al., 2013
	Oslo harbor	Microcosm	Capping	93–98% PCBs	Eek et al., 2008

contaminated sediment remediation (Table 3). The use of bacteria to reduce PCBs concentration in marine contaminated sediment was reported. Kwon et al. (2008) demonstrated that the best rate of bacterial degradation can be obtained by adding plant terpenes. To date, data on the application of plant terpenes in marine sediments contaminated with PCBs remained limited, being mainly used in soil systems. Various microcosms were built only sediment, sediment + plant terpenes and sediment + plant terpenes + bacteria. In this case, the experimental setup was prepared into a 250 mL Erlenmeyer flask. A significant PCBs decrease has been observed after one month in microcosms that contained both plant terpenes and bacteria with respect to other two conditions. Krumins et al. (2009) used microcosm experiments and demonstrated that the enhancement of PCBs-degradation can be obtained with biostimulation and bioaugumention. Microcosms were constructed, using 200 mL site sediment in 250 mL stock bottles with homogenized sediment recovered from the Anacostia River (USA) capping site control plot. Various microcosm conditions were used as the following: only sediment (control), sediment + bacteria, sediment + mixture of electron donors, alternate halogenate acceptors, tetrachlorobenzene, pentachloronitrobenzene and sediment + bacteria + bioaugumentation. After 400 days, they assessed a decrease of PCB-concentrations in microcosms with bioaugumentation and biostimolation. Moreover, the PCB-degradation bacteria, as PAHs-degradation microorganisms, can be isolated from contaminated sediments and can be stimulated by adding pollutants (PCBs). In fact, Zanaroli et al. (2010) demonstrated that the degradation of PCBs was enhanced by the addition of contaminants, because it promoted the development of PCBs-dichlorination bacteria. They showed an important decrease of PCBs after 30 months, caused by the growth of bacteria after the addition of the contaminants. Kaya et al. (2017) conducted similar experiments using microcosms with addition of PCBs. After 30 days of incubation, they assessed a crucial reduction of PCBs in marine sediments with a corresponding increase of dichlorination bacteria. One year later, Kaya et al. (2018) evaluated the depletion of PCB after the addition of pollutants, using three different sediment microcosm samples. After 30 days, they measured a decrease of PCB concentrations, which were recorded at different percentages, suggesting that there were different dichlorination activities. Certainly, it is not related to different contamination history of three sediments because the sites with the highest percentages of dichlorination had also the lowest initial bacterial concentrations. Probably, individual congeners of PCBs may affect the activity of individual PCBs-degradation bacteria. Dudášová et al. (2012) performed microcosm experiments to assess the bacteria role in PCB-restoring using 15 different bacterial strains, isolated from PCBs-contaminated sediments. After 21 days, five of the 15 bacterial strains were suitable for restoring PCBs-contaminated sediments, because these microorganisms demonstrated high biodegradation ability compared to others.

Srinivasa Varadhan et al. (2011), enhanced the PCBs-degradation by dehalorespiring microorganisms in contaminated sediments by providing hydrogen produced in anaerobic corrosion of iron added to sediments. In that case, three different microcosm sediments were used, consisting of 125-mL borosilicate serum bottles, in which they added different iron concentrations. After 18 months, they observed an improved performance of bacterial degradation, at low levels of iron, suggesting that the bacteria preferred low concentrations of hydrogen. Zanaroli et al. (2012) conducted a similar study using microcosm experiments in which they added iron to stimulate PCBs microbial dichlorination. After 36 weeks, an inhibitor effect of iron on sediment indigenous microbial communities was observed, favoring the growth of PCB microbial dichlorination and their biodegradation activity. The iron, probably, reduced the lag phase of PCB-dichlorination by blocking the sulfate reducing bacteria and by promoting hydrogen release. Matturro et al. (2015) studied the PCBs-degradation in marine sediments (consisting in sterile 250-mL serum), using different microcosm experiments with sediments and sediment with/without addition of *Dehalococcoides mccartyi*, microorganism known able to degrade PCBs. After 320 days, they observed an increase of the PCBs-degradation community and a decrease of PCBs in microcosms with *D. mccartyi.*

Furthermore, different studies were conducted on possible roles of AC in PCBs-degradation. In fact, as reported in Sun et al. (2007) the use of little AC addition in PCBs-contaminated sediments reduced the bioavailability of these compounds for the oligochaete *Lumbriculus variegatus*. Setting-up different microcosms (only sediment, sediment + AC and sediment + AC + worms), after one month of exposure a decrease of PCB-concentrations was registered together with a reduction of PCB bioavailability in worm's chemical activity. McLeod et al. (2004) performed microcosm experiments with AC additions to reduce the PCBs bioavailability in contaminated sediments from two different sites using the clams *Corbicula fluminea* and *Macoma balthica* as bioindicators. A considerable reduction of PCBs in microcosms was assessed after 28 days of exposure, and after the addition of AC. In addition, several studies have been conducted on the PCBs absorption of granular activated carbon (GAC) and the biofilms that grow on these particles. Mercier at al. (2013) evaluated the efficiency absorption of three different GAC conditions using different microcosm experiments: raw GAC, GAC particles with and without biofilms. The adsorption capacity was excellent for all conditions after one month, even in the case of GAC particles plus biofilms where the PCBs removal was not hampered by biofilm development. Afterwards Mercier et al. (2014) evaluated the PCBs removal efficiency of three different GACs in contaminated sediments. After 8 months of treatment in microcosms, all granular particles were able to efficiently absorb the PCBs in marine sediments and the bacterial adhesion depended on physical and chemical properties of particles. Later, Payne et al. (2013) demonstrated that the use of GAC with simultaneous application of aerobic and anaerobic microorganisms was able to better reduce the PCB concentrations by about 80%. Different mesocosm conditions were used: only GAC, GAC + sodium lactate, GAC + zerovalent iron, GAC + anaerobic *Dehalobium chlorocoercia* (DF1), GAC + DF1 + sodium lactate, GAC + DF1 + zerovalent iron, GAC + DF1 + aerobic *Burkholderia xenovorans* (LB400), GAC + DF1 + LB400 + sodium lactate and GAC + DF1 + LB400 + zerovalent iron. The results demonstrated that i) the best effects were obtained with bioaugmentation using both organisms after one year, and ii) a treatment employing both aerobic and anaerobic microorganisms could be considered a suitable strategy to remove PCBs in contaminated aquatic sediments.

Another strategy used to remove PCBs in contaminated marine environment is the capping, also used for PAHs (see above). Eek et al. (2008) tested a mineral cap (crushed limestone) to prevent PCBs diffusion and to limit bioavailability of these compounds in the marine environment. After 13 months of treatment in microcosm, they showed that the use of capping significantly limited PCBs concentrations respect to uncapped sediment microcosm.

3.3. Heavy metals case studies

Heavy metals (zinc, copper, manganese, silver, iron, cadmium, and so on) often dispersed in marine environments derive from anthropogenic activities and represent an issue for marine environments and consequently for human health. These elements can diffuse and accumulate in sediment where they influence physical and chemical factors (Peng et al., 2009). Several studies have been conducted to investigate their removal strategies (Table 4). As for PAHs and PCBs, the most common applied strategies concerned biological and chemical activities. The potential role of bacteria versus heavy metals degradation was tested in several studies. Beolchini et al. (2009) tested the potential role of different bacteria strains in bioremediation of dredged sediments contaminated by heavy metals using microcosms. Three conditions were set up: i) microcosms with autotrophic bacteria, ii) microcosms with heterotrophic bacteria, iii) and microcosms with mixed culture. In presence of both bacterial types, the efficiency of heavy metal extractions increased after 15 days, suggesting a potential synergistic action of these microorganisms. Fonti et al. (2013) studied three contaminated sediment microcosms in which they added autotrophic Fe/S oxidizing and heterotrophic Fe-reducing bacteria. The presence of autotrophic Fe/S oxidizing bacteria enhanced after 14 days of treatment, to maintain the acid and oxidative condi-

Table 4
Strategy, site, operating conditions, efficiency of treatment for bioremediation by heavy metals.

Strategy	Site	Operating conditions	Treatment efficiency	References
Biological/ Chemical	Site of US	Injection of ethanol	Increase of bacteria communities able to reduce contaminations	Cardenas et al., 2008
	Ancona harbour (Mediterranean Sea)	Mixture of autotrophic and heterotrophic bacteria	>90% Cu, Cd, Hg and Zn	Beolchini et al., 2009
	Oak Ridge (Tennessee, USA)	Addition of the electron donors	5–7% with ethanol, 49% with glucose, 93% with metthanol	Madden et al., 2009
	Three sites (New Caledonia)	Addition of glucose in oxic and anoxic conditions	Enhancement of nickel removal	Pringault et al., 2010
	Ancona harbour (Mediterranean Sea)	Addition of the electron donors	Change of metal partitioning	Rocchetti et al., 2012
	Three sites in New Caledonia	Addition of glucose in oxic and anoxic conditions	Enhancement of zinc removal	Pringault et al., 2012
	Ports of Livorno, Piombino and Ancona (Mediterranean Sea)	Mixture of autotrophic and heterotrophic bacteria	40–76% Zn, 0–7% Pb, 13–39% Cd, 1–8% Cr, 20–23% As, 35% Ni	Fonti et al., 2013
	Port of Piombino (Mediterranean Sea)	Addition of the electron donors	32–40% Zn, 14–40% Pb, 26% Cd, 15% Cr, 2–20% As	Fonti et al., 2015
	Contaminated sediment	Addition of microalgae	Enhancement of copper and zinc removal with RED LEDs	Kwon et al., 2017
	Port of Ancona (Mediterranean Sea)	Mixture of different chemical leaching agents and autotrophic and heterotrophic bacteria	30% Zn, 30% Cr, 40% Ni, 35–58% As	Beolchini et al., 2013

tions, so influencing metal solubilization in the marine environment. Pringault et al. (2010) evaluated the bacteria role in Ni removal. Different experimental designs were built up: microcosm without metals (control), microcosm with Ni and formaldehyde (to sterilize sediment and seawater), and microcosm with nickel and glucose (to stimulate the bacterial growth). Nickel removal was always significantly higher in non-sterilized microcosms after 8 days, indicating the important role of bacteria. Pringault et al. (2012) evaluated the possible effects of Zn on bacterial structures and if these microorganisms could play a role in adsorption of this metal. They set up oxic and anoxic microcosm experiments using different conditions: one microcosm without Zn (control), one microcosm with Zn, one microcosm with metal and formaldehyde or glucose. The microbial structure changed after 8 days, more in microcosms with glucose than in microcosms with metal. Probably, these different effects were due to the strong adsorption of Zn in presence of bacteria (uptake).

Cardenas et al. (2008) stimulated the bacterial growth in uranium-contaminated sediments injecting ethanol *in situ* microcosm experiments. After two years of treatments with ethanol addition, they showed that the denitrifying bacteria (especially *Desulfovibrio* spp.) increased with the reduction of U (VI) to U (IV). Madden et al. (2009) added different electron donors (ethanol, methanol, glucose and methanol with humic acids) to improve the bacterial performance in uranium reduction. Microcosm experiments were performed to different exposure time, depending on the type of electron donors: 77–153 days for ethanol, 53 days for glucose and 90 days for methanol. All electron donors stimulated the U-reduction with no difference with humic acids addition. The enhancement of U-reduction was probably due to encouragement of bacterial activities with electron donors' additions. In all microcosms they found different bacteria, known to reducing U. Rocchetti et al. (2012) added different electron donors, lactose and sodium acetate, to stimulate the prokaryotic development. They performed anaerobic microcosm experiments at either 20 °C or 35 °C, to investigate the effects of biostimulation activities on sediments contaminated with hydrocarbons and metals. After 60 days of incubation, they determined a significant change of metal partitioning and hydrocarbon concentrations in microcosms with lactose and sodium acetate at 20 °C. These results showed that additions of electron donors and the temperature influenced the prokaryotic metabolism, determining a decrease of PAHs concentrations and a change also on metal partitioning. In fact, the bioremediation strategies on contaminated sediments can influence the mobility and bioavailability of metals found in sediments. As reported in Fonti et al. (2015) during the restoring activities, the use of bacteria and the addition of electron donors to enhance the microbial growth were associated to metal decreases, suggesting a potential role of prokaryotes in metal assembly processes. Specifically, they used microcosms in which lactose and sodium acetate were added, and after 60 days the metal partitioning and the abundance and diversity of bacteria were measured. A considerable increase of abundance and diversity of bacteria was recorded in all microcosms and a decrease of metal concentration, confirming that the bacterial activities play an important role in metal mobility in the sediments. The heavy metal restoration involving microorganisms and algae was not an exception. Kwon et al. (2017) used microcosm experiments with addition of microalgae demonstrating that the use of these microorganisms could be efficient in metals removal. They studied the effects of monochrome and mixed wavelength on absorption of copper and zinc by microalgae *Phaeodactylum tricornutum*, *Nitzschia* sp., *Skeletonema* sp. and *Chlorella vulgaris*, showing in the last one the highest copper and zinc removal.

Another strategy used to remove heavy metals in contaminated marine environment is the chemical treatments. Beolchini et al. (2013) performed microcosm experiments with addition of different chemical leaching agents, oxalic, citric and sulfuric acids, and autotrophic and heterotrophic bacteria to evaluate extraction of toxic metals from contaminated sediments. After 14 days, they assessed that citric and sulfuric acids were the most appropriate to remove the toxic metals. They also measured similar effects in presence of ferrous iron showing that the role of autotrophic bacteria was the most important in the metal extraction and the heterotrophic bacteria were used as support for the autotrophic bacterial activities.

4. Conclusions and future perspectives

This review highlighted that how small-scale experiments in microcosms and/or mesocosms could represent an potential useful method to assess contaminated sediment along with their remediation process. Such kind of approach could strengthen the knowledge about potential side effects of remediation activities (e.g. adverse effects on benthic communities), suggesting the most viable treatment to be administered as well as support technology advancements to reduce the impact of old and new treatment technologies especially those related to the addition of adsorbents (e.g. AC), reactive compounds or capping. Currently, the use of m-cosms present some limitations that must be overcome in the near future about: i) exact definition of m-cosm's structure and components in time and space; ii) identification of common standards to be shared at international level (i.e. standardization process); iii) model species batteries. The matter of costs is of course important and are generally country based, but standardization and economy of scale may increase savings.

Author contribution

L.A., M.C., V.Z., G.L., the general content of the review; L.A. performed most bibliographic research; L.A., M.C., G.L. wrote the original draft; all authors contributed to paper writing.

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.

Acknowledgments

Luisa Albarano was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship co-funded by the Stazione Zoologica Anton Dohrn and University of Naples Federico II. We thank Mrs Rosanna Messina for English language editing.

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