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Review

The potential of microorganisms as biomonitoring and bioremediation tools for mercury-contaminated soils

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ABSTRACT

Mercury (Hg) pollution is a global issue due to the high toxicity and wide dispersion of Hg around the world. Whether due to anthropogenic activities or natural processes, Hg emissions are steadily increasing, with very high levels in some regions, directly threatening human and ecosystem health. However, bacteria and fungi have evolved and adapted in response to Hg-induced stress and have developed tolerance mechanisms, notably based on the *mer* operon system that is involved in Hg uptake and biovolatilization via Hg reduction reactions. Other processes, such as bioaccumulation or extracellular sequestration, are involved in Hg resistance, and the study of contaminated soils has allowed the isolation of a number of microorganisms capable of these mechanisms, with strong potential for the implementation of bioremediation approaches. In addition to playing an important role in determining the fate of Hg in the biogeochemical cycle, these microorganisms can indeed be applied to reduce Hg concentrations or at least stabilize Hg for the remediation of polluted soils. Moreover, thanks to the development of biotechnological tools, bioremediation based on Hg-tolerant microorganisms can be optimized. Finally, these microorganisms are relevant candidates for biomonitoring, for example, through the engineering of biosensors, because the detection of Hg is a major issue in preserving the health of living beings.

1. Introduction

Mercury (Hg) is part of the metals and is the 66th most abundant element in the Earth's crust. For a long time, Hg has fascinated people since it is the only metal that exists in liquid form at Earth's temperature and pressure. Formerly called hydrargyrum, which is why its chemical symbol is Hg, mercury has been associated with a spiritual dimension: it was used, for instance, to bless houses, and alchemists were convinced that Hg could transmute other metals into gold (Natasha et al., 2020). Hg was also used for centuries in medicine and cosmetics at a time when its great toxicity was not yet known (Natasha et al., 2020). To date, Hg still has no identified biological role in organismic life and is considered nonessential, in contrast to other metals (Fe, Cu, Zn, Se, etc.), which have roles in many metabolic pathways (Durand et al., 2015). In contrast, according to the World Health Organization (WHO), Hg is among the 10 compounds of greatest concern for human health (WHO, 2017). In addition, the Agency for Toxic Substances and Disease Registry (ATSDR) ranked Hg in the third position in the priority list of hazardous substances (ATSDR, 2017).

At the organism scale, Hg can cause hemorrhagic gastritis and colitis and has genotoxic activities leading to the development of benign tumors (WHO, 2017); the main target of inorganic Hg is the kidneys. In the short term, during acute Hg exposure, the main symptoms are skin problems, causing dermatitis, discolouration of nails, corrosion of mucous membranes, and corrosive burns, whereas chronic exposure to Hg pollution can cause acrodynia, anorexia, fatigue, irritability, apathy, photophobia, polydipsia, and other hypersensitivity-related reactions (Risher et al., 2003). At a cellular level, the presence of Hg induces changes in cell membrane permeability and alterations in macromolecular structures. It also causes mitochondrial dysfunction and increases the formation of radical oxygen species (ROS), which can cause irreversible oxidative damage (Rice et al., 2014). Finally, regarding molecular mechanisms, the toxicity of Hg is due to its high affinity for SH groups, notably carried by cysteines: this leads to the disruption of protein structures, thus affecting protein functions (Safari et al., 2019).

Among all forms of Hg, methylated forms of Hg (MeHg) are the most toxic form for organisms: they can cause severe neurological damage because it is able to cross blood—brain and placental barriers (Clarkson

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et al., 2003; Gworek et al., 2020). Usually, methylmercury refers to monomethylmercury but the dimethylated form can also exist and is even more toxic, causing death in the majority of reported cases of exposure (Rice et al., 2014; Siegler et al., 1999). MeHg constitutes only 2% of the total amount of Hg in soils, but this form is favored during biomagnification, i.e., the increase in harmful substance concentrations in organisms at each level of the food chain (Xu et al., 2015). As human beings are at the top of the food chain, they are the most exposed to the biomagnification of Hg in successive trophic levels, and food is the main source of Hg intake for humans (Priyadarshane et al., 2022; WHO, 2017). The guidelines of the WHO advise a maximal inorganic Hg concentration of 6 µg/L in drinking water and set a tolerable daily inorganic Hg intake of 2 µg/kg of body weight (WHO, 2017). Dietary Hg intake varies across populations, and some are particularly exposed, for instance, Arctic populations that consume fish and marine mammals as a significant component of their diet, tropical riverine communities, coastal and small-island communities that consume large amounts of seafood, and individuals who either work or live near gold mining sites (UNEP 2013). However, all populations and ecosystems can be affected by Hg contamination: there is therefore a need to better monitor the evolution of Hg concentration in soils and to reduce it through the implementation of bioremediation systems to limit damage caused by Hg toxicity.

Indeed, a major threat linked to Hg is its persistence, particularly in the atmosphere, which gives it the potential to be distributed globally: its quasi-ubiquity on the Earth's surface is therefore an environmental and public health issue. Hg pollution has accelerated since the industrial era, as many anthropogenic sources of Hg have been added to natural sources of Hg emissions including volcanic and geothermal activity (Gworek et al., 2020). In 2013, the Minamata Convention on Mercury was created to tackle the global concerns related to Hg: this reflects the magnitude of the problems associated with Hg pollution (Bank, 2020; UN Environment 2013).

Unlike other contaminants, Hg does not undergo degradation processes of chemical or microbial origin (Wuana and Okiyeimen, 2011). However, some microorganisms have evolved and adapted or acclimated to Hg-contaminated environments by developing tolerance mechanisms (Osborn et al., 1997). Among these mechanisms, the mercuric reduction (*mer*)-mediated pathway is widespread in bacteria (Christakis et al., 2021): *mer*-mediated Hg resistant mechanisms appeared in Archaea and thermophilic bacteria from hydrothermal habitats enriched in Hg, and the genes involved were harbored by plasmids, allowing potential vertical and horizontal gene transfer with the involvement of transposons through evolution (Barkay et al., 2010; Osborn et al., 1997). The *mer* operon is a cluster of genes involved in Hg transport across the cell membrane and in Hg reduction into the volatile form Hg⁰, with regulatory mechanisms of the operon directly related to cellular Hg levels (Priyadarshane et al., 2022), which is investigated later in this review. Fungi are also able to cope with Hg toxicity via several mechanisms: they present a great capacity to accumulate significant amounts of Hg in intracellular compartments and by complexation with thiol compounds (Gutensohn et al., 2023; Kavčič et al., 2019). In a similar way to bacterial mechanisms, biovolatilization of Hg has been observed in fungal species as well (Durand et al., 2020). The use of these Hg-resistant microbes and their molecular pathways therefore seems to be an interesting alternative for lowering Hg concentrations to an environmentally safe level via bioremediation.

Bioremediation relies on the use of living or dead organisms (usually bacteria, microalgae, fungi, and plants) to remove or convert hazardous substances into less toxic or non-hazardous substances from contaminated environments (Borthakur et al., 2022). Most of the time, biological systems used in bioremediation are naturally capable of accumulating or degrading contaminants, and they constitute a relevant alternative to classical physicochemical treatments because they are more sustainable, less expensive and environmentally friendly. The implementation of bioremediation methods consists mainly of stimulating processes

already in place in ecosystems and promoting the development of certain native species (Megharaj and Naidu, 2017).

Biomonitoring is based on the use of organisms to qualitatively or quantitatively measure changes in the environment and monitor their evolution. It can be conducted at various levels of biological organization (molecular, cellular, tissue, ecosystem) to detect pollutants and chemicals within different environments. Organisms can also be genetically engineered in order to obtain biosensors that are specifically designed to detect certain compounds.

Over the last few years, numerous bioremediation and biomonitoring strategies have been thriving to mitigate the impact of Hg on the environment and the health of living beings. For all the reasons mentioned above, Hg represents a major issue that justifies the ongoing efforts to date to develop new methods for controlling its fluxes using biological systems.

Based on the latest available literature, this review summarizes the current state of knowledge on the fate and behavior of Hg in ecosystems and its impact on health. The genetic engineering of Hg-tolerant microorganisms and the development of biosensors for biomonitoring purposes are described in a nonexhaustive manner. Finally, several perspectives on bioremediation based on microbial pathways associated with Hg tolerance are presented. This review focuses on Hg-contaminated soils and provides an overview of different bioremediation approaches targeting the use of microorganisms rather than plants, although they also have great potential for phytoremediation.

2. Hg place in the environment

2.1. Speciation of Hg

In nature, many isotopes of Hg can be found in different proportions: the most prevalent isotope is ²⁰²Hg. In addition, Hg can take several forms in the environment depending on physicochemical parameters that increase the diversity of Hg compounds, including elemental (Hg⁰), inorganic (HgS, referred to as cinnabar, and HgCl₂) and organic (mostly MeHg forms) (Natasha et al., 2020).

The speciation of Hg depends on its interactions with soil particles and directly influences its bioavailability: like any charged element, metal ions can interact in the soil with any other charged organic or mineral particle. The balance between the free and complexed forms of the ion depends on its bioavailability, which is directly linked to its toxicity, as a metal is only toxic for living organisms if it is in a bioavailable form (Adriano, 2001). However, it is sometimes difficult to define and therefore assess the bioavailability of Hg: the terms labile, soluble, and exchangeable may be mentioned when the bioavailability of Hg is discussed, which creates confusion around this term. Huang et al. (2020) defined it as the fraction of soil Hg with the highest impact on the surrounding environment and human health, which depends on the soil nature, anthropogenic activity, surrounding living organisms and several other factors. The most bioavailable forms are Hg²⁺ and MeHg (Natasha et al., 2020). Mercurial species exist in 5 different states in the soil matrix: (i) dissolved (free ion or soluble complex), (ii) nonspecifically adsorbed (binding mainly as a result of electrostatic forces), (iii) specifically adsorbed (strong binding owing to covalent or coordinative forces), (iv) chelated (bound to organic substances) or (v) precipitated (as a sulfide, carbonate, hydroxide, phosphate, etc.) (Schuster, 1991).

In acidic soils, Hg mobility is mainly controlled by organic matter because it presents a high affinity for Hg, especially humic acids, which increases Hg transfer into solutions. Conversely, in neutral and alkaline soils, mineral components are the dominant factors that influence Hg solubility (Xu et al., 2015). Moreover, Hg mobilization is also increased by chloride compounds that are highly complexing agents (Kabata-Pendias, 2010). Ozone and OH radicals promote the oxidation of Hg⁰ to Hg²⁺ in the atmosphere, and this oxidized form of Hg is deposited primarily on land unless conditions are reducing, in which case Hg and

sulfur compounds are present in the majority. In addition, the methylation of Hg^{2+} , which is favored by high-temperature and dark conditions, lower Hg fluxes, which increases its toxicity (Gworek et al., 2020; Priyadarshane et al., 2022). In general, the following environmental parameters influence the Hg cycle from least to most impacting: temperature, relative humidity, Hg concentration in the atmosphere, light, and soil moisture (Ericksen et al., 2006).

2.2. Hg sources and fluxes

In its elemental form Hg^0 , Hg is very volatile: with a residence time of a few days to a few weeks in the atmosphere, Hg can be transported over a few thousand kilometers (Lindqvist and Rodhe, 1985). The main Hg fluxes in the biogeochemical cycle are described in Fig. 1, which highlights that in general, deposition rates are higher than emission rates for Hg, leading to Hg enrichment in ecosystems. This is especially true for certain ecosystems, such as deserts, where Hg turnover is very slow, with high deposition rates and no uptake from biomass (Gworek et al., 2020).

Indeed, biomass plays a key role in the Hg cycle: for example, vegetation lowers solar radiation and temperature, and the leaves concentrate 80% of Hg in aboveground biomass, as they constitute the major uptake surface for Hg deposition (Ericksen et al., 2003). In fact, Hg uptake by the leaves of forest canopies is one of the predominant Hg sources in soil via litter deposition (Wright et al., 2016). After forest fires, burned soils are depleted in Hg, especially in MeHg, and as a result, Hg is released into the atmosphere, contributing to the re-emission of past Hg deposits from natural or anthropogenic sources (Gworek et al., 2020). Other organisms, such as bacteria and fungi, are also involved in Hg speciation, whose roles are detailed below.

Naturally, the average Hg concentration is approximately 1.1 mg/kg in soil: soils constitute the largest reservoir in the Hg cycle. Each year, 80–600 tons of Hg are emitted in the atmosphere from natural sources. These emissions are due to natural processes, such as volcanic or geothermal activity and the weathering of Hg-containing rocks in the Earth's crust (Xu et al., 2015).

However, for several decades, the natural carbon cycle has been strongly disrupted by anthropogenic activities: today, the global atmospheric deposition rate of Hg is approximately three times higher than that in preindustrial times (Gworek et al., 2020). Anthropogenic Hg emissions can be classified into four categories: (i) area sources, which include dental amalgamation, landfills and laboratory usage; (ii) combustion processes, including municipal and medical waste incinerators and coal-fired power generation; (iii) the manufacturing of alkali, metals and cement; and (iv) other industrial/agricultural activities (Natasha

et al., 2020). Indeed, the uses of Hg are very diverse, including the electrolytic production of chlorine, gold mining, chlor-alkali production, ferrous and nonferrous metal production, and the incineration of thermometers, batteries, or fluorescent lamps, and the levels of Hg have locally and globally increased due to all these human activities (WHO, 2017; Xu et al., 2015). The most important source of Hg remains artisanal and small-scale gold mining, which was responsible for 838 tons of Hg emission in 2015, representing 37.7% of the total emissions. Between 2010 and 2015, Hg emissions increased by 20%, which can be explained by a global increase in cement and steel production and coal consumption, especially in developing Asian countries (AMAP, 2019). Anthropogenic activities have therefore heavily impacted the natural cycle of Hg and will continue to do so in the near future, especially because of global warming, which leads to the re-emission of natural deposits in permafrost in the Arctic; as a result, Hg levels are inevitably increasing in the Northern Hemisphere (Schuster et al., 2018).

3. Role of microorganisms in the Hg cycle and their tolerance mechanisms

The study of numerous Hg-contaminated sites has highlighted the ability of microorganisms to cope with Hg pollution (González et al., 2022; Jafari et al., 2014; Mariano et al., 2020; Saranya et al., 2017; Urík et al., 2014; Zappellini et al., 2015). Indeed, bacteria and fungi are able to tolerate high concentrations of Hg in their environment, and some Hg transformations can be driven by biotic processes of microorganisms (Durand et al., 2020; Priyadarshane et al., 2022). They therefore have a significant role in the Hg cycle, which is the result of the development of tolerance mechanisms through years of evolution.

The first way to cope with Hg toxicity is to reduce the uptake of Hg from the contaminated environment by cells: at present, there are no known efflux-mediated mechanisms for maintaining Hg homeostasis, although there are other heavy metals in microbial processes (Das et al., 2016). However, the co-occurrence of the *mer* operon responsible for Hg tolerance traits with advantageous capacities, such as biofilm formation or antibiotic resistance, which includes efflux-mediated mechanisms, can increase the ability of microbes to resist Hg (Chenia and Jacobs, 2017; Kis et al., 2017): quorum sensing is an avenue to be explored, since the associated mechanisms involve interactions with metals such as iron for the production of siderophores and can induce the production of biofilm allowing a better tolerance to heavy metals (Boyd, 2010).

Biosorption is another mechanism that enables the extracellular sequestration of Hg on bacterial cell membranes. This phenomenon relies on the presence of sulfide and organosulfur compounds on the

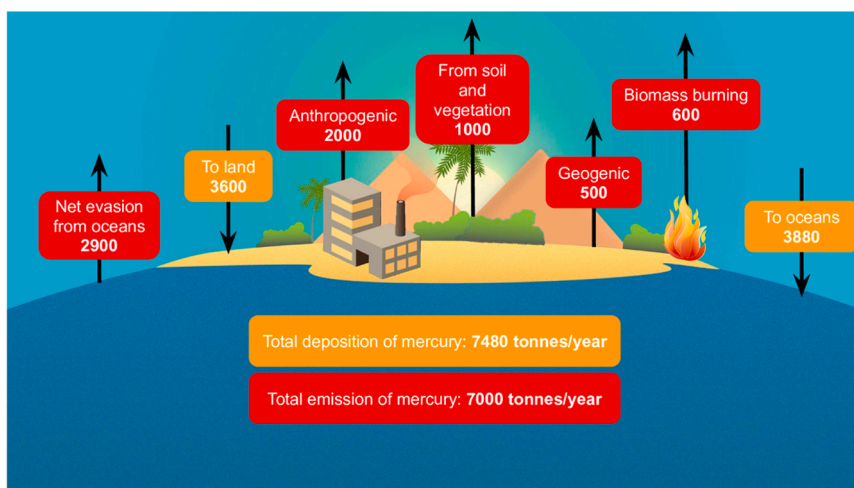


Fig. 1. Biogeochemical cycle of Hg and the global budget for each flux (in tons/year). Numerical data from Gworek et al. (2020).

surface of the outer membrane, for which Hg has a strong affinity. In addition, some microorganisms are able to produce extracellular polymeric substances (EPS) that can sequester Hg (François et al., 2012; Pal and Paul, 2008). Finally, some intracellular bioaccumulation mechanisms can also occur with the presence of metal-binding peptides such as metallothioneins and phytochelatins, to which Hg can bind (Aschner et al., 2006). Similar mechanisms exist in fungi that are also based on extracellular or intracellular chelation. Hg uptake in the cytosol can be reduced by the presence of sulfide compounds and cell wall components for which Hg has affinity, such as chitin, chitosan, and hydrophobin (Amin and Latif, 2011; Durand et al., 2020; Puglisi et al., 2012). Inside fungal cells, metallothioneins and phytochelatins involved in bioaccumulation are also present (Khullar and Reddy, 2018). In addition, fungal metal tolerance relies on Hg sequestration in intracellular compartments such as vacuoles (Guedry et al., 2003). Recent studies also showed that fungal necromass presents a great biosorption potential which could favor the immobilisation of Hg in the soil rather than its

biovolatilization (Maillard et al., 2023).

Hg resistance mechanisms help microbes cope with the toxicity of Hg but also to convert it into less harmful species. Many microorganisms possess an enzymatic system that allows them to convert Hg from one form to another, which directly involves them in the global Hg cycle. As mentioned above, the *mer* operon encodes a set of 9 genes involved in Hg tolerance mechanisms, which are not necessarily all present and can have various positions in microbial genomes, including plasmids, transposons or bacterial chromosomes (Marathe et al., 2022; Morgado and Vicente, 2021). Although there is little discussion in the literature, phages could also be vectors of Hg resistance genes since similar phenomena exist for the spreading of antibiotic resistance genes (Brown-Jaque et al., 2015). The operator of the *mer* operon is regulated by MerR, which represses the expression of the operon in the absence of Hg and loses its affinity for the operator in the presence of Hg by binding to Hg^{2+} ; thus, transcription of the operon is allowed, as depicted in Fig. 2A. A second gene, *merD*, is involved in regulating the expression of the

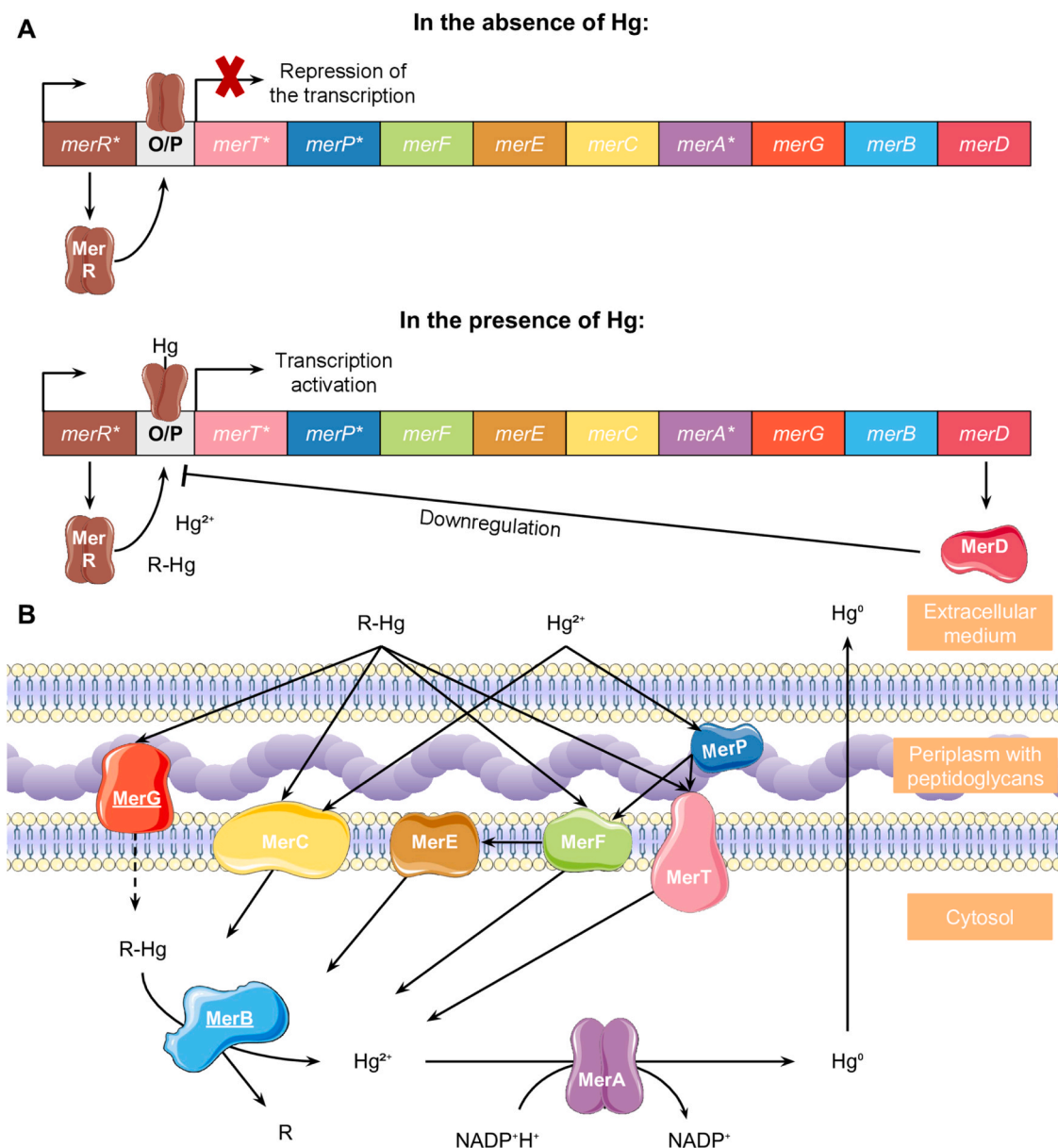


Fig. 2. Operation of the *mer* operon and associated cellular mechanisms. (A) Regulation of the expression of the *mer* operon by Hg. In the absence of Hg, the expression of the operon is repressed by MerR, and in the presence of Hg, transcription is activated. The asterisks indicate the genes composing the generic structure of the operon that are always present. (B) Transport and enzymatic processes encoded by the *mer* operon and involved in Hg resistance in the case of Gram- bacteria. Proteins with underlined names are involved in the broad-spectrum pathway.

operon because the protein it encodes is an antagonist of MerR. The resistance mechanisms related to the *mer* operon can be divided into two pathways: narrow-spectrum mechanisms, which confer resistance to only inorganic Hg, and broad-spectrum mechanisms, which confer resistance to organic Hg (MeHg and phenyl Hg) and inorganic Hg compounds (Das et al., 2016; Dash and Das, 2012). The *merT*, *merP*, *merF*, *merE*, *merG* and *merC* genes encode proteins responsible for Hg uptake and transportation (Hamlett et al., 1992; Sone et al., 2013; Zheng et al., 2020). The last two genes of the operon are involved in Hg transformation: *merA* and *merB*, coding for MerA and MerB enzymes, respectively. MerA is a Hg reductase that is involved in the narrow-spectrum mechanisms of the *mer* operon; this homodimeric protein catalyses the reduction of Hg^{2+} to Hg^0 by oxidizing NADPH-H^+ to NADP^+ . MerB is an organomercurial lyase that takes part in broad-spectrum mechanisms and enables the demethylation of organic species such as MeHg to obtain Hg^{2+} , which can then be transformed into elemental Hg by MerA (Barkay et al., 2003; Das et al., 2016). All the processes in which the Mer proteins described above are involved are presented in Fig. 2B for the case of Gram- bacteria. The *merA* gene is commonly used as a molecular marker of Hg resistance for some strains (Allen et al., 2012; de de Luca Rebello et al., 2013; Yang et al., 2022), but although environmental pressure related to Hg stress results in the selection of genes of the *mer* operon, it seems that other mechanisms and markers need to be investigated to detect the Hg resistance of microbes (Trojańska et al., 2022).

In general, in bacteria, the methylation of Hg occurs as a stress response. For instance, sulfate-reducing bacteria are able to methylate several forms of Hg (including HgS) via the methyltransferase-mediated transfer of a methyl group from methylcobalamin via the acetyl-CoA pathway. Iron-reducing bacteria could also be involved in MeHg production (Barkay et al., 2003). At the gene level, Hg methylation is associated with a common pathway relying on the *hgcAB* gene cluster expression which has been identified in a great diversity of organisms (Parks et al., 2013). The *hgcAB* cluster encodes a methyl carrier, HgcA, and an electron donor, HgcB, and can be used a molecular marker to characterize methylators (Peterson et al., 2020). Hg methylators are ubiquitous and mostly anaerobes (Lin et al., 2021): aerobic bi-methylation exists but seems to rely on passive stress response and remains inefficient in comparison to the anaerobic pathway (Cao et al., 2021). Hg methylation can also be driven by abiotic processes, in particular in the presence of certain organic compounds such as humic and fulvic acids, carboxylic acids, and alkylated compounds (Barkay et al., 2003). However, microbial processes remain mainly responsible for Hg methylation, predominantly in aquatic ecosystems. Indeed, in water, MeHg can interact with inorganic ions such as Cl^- and HS^- or organic ligands including reduced sulfur groups to form stable complexes (Priyadarshane et al., 2022).

As MeHg is known to be the most toxic for organisms, demethylation therefore remains an important step for lowering Hg toxicity and Hg methylation reactions are often coupled to reverse processes in bacteria (Cao et al., 2021). Aerobes and facultative aerobes have demethylation capacities based on a reducing process producing Hg^0 and CH_4 that are mostly related to MerB activity (Priyadarshane et al., 2022). Anaerobes that lack the *mer* operon (such as methanogens and sulfate-reducing bacteria) are still capable of oxidative demethylation (Bystrom, 2008; Lin et al., 2021) by producing Hg^{2+} and CO_2 such as bacteria belonging to the *Desulfomicrobium*, *Desulfovibrio*, *Desulfatibacillum*, and *Desulfobulbus* genera (Priyadarshane et al., 2022). Beyond demethylation, the export of MeHg outside the cell has been observed in iron- and sulfate-reducing bacteria and may constitute a method for coping with Hg toxicity (Pedrero et al., 2012; Schaefer et al., 2011).

Microorganisms are also involved in the oxidation processes of the Hg cycle: Hg^0 is converted into Hg^{2+} by aerobic and phototrophic bacteria, such as *Escherichia coli*, *Bacillus* or *Streptomyces* genera (Smith et al., 1998). These mechanisms allow Hg to be obtained in a more reactive form, which can then be reduced by Hg reductase enzymes,

such as MerA. Other reducing mechanisms exist, especially because a relatively high concentration of Hg is required to activate the *mer* operon. For instance, the dissimilatory metal-reducing bacterium *Shewanella oneidensis* MR-1 is able to reduce Hg^{2+} to Hg^0 by an activity not related to MerA (Wiatrowski et al., 2006). In every case, Hg^0 bioavailability is significantly increased by microbes that volatilize Hg via reducing processes in the atmosphere (Priyadarshane et al., 2022). Biovolatilization has also been observed in fungi as a key mechanism to increase the Hg efflux from the cytosol to the extracellular medium (Durand et al., 2020; Urík et al., 2014). For instance, *Penicillium* spp. DC-F11, a fungal strain isolated from a mining area, showed a potential for bioremediation in polluted soil via volatilization and for the first time, a study reported that this process is controlled by the *mer*-mediated detoxification system in collaboration with other mechanisms (thiol compound metabolism, oxidative stress defense and damage repair metabolism). It therefore gives a better understanding of the fungal resistance mechanisms (Chang et al., 2020a). Another Hg-volatilising fungus, *Lecytophora* sp. DC- F1, has been analysed with transcriptomics approaches. The results suggest that Hg stress response relies on multisystem collaborative pathways with three major transcriptional levels to Hg stress: a *mer*-mediated detoxification system, a thiol compound metabolism, and a ROS stress response system (Chang et al., 2020b). These findings strengthen the interest the role of fungi in the mercury cycle and in the bioremediation of contaminated environments. It also shows that the *mer* operon is present in many phyla and the identification of homologs in organisms in which Hg detoxification mechanisms were not suspected were found, suggesting that the associated genes (*merA*, *merB*) have been conserved (Christakis et al., 2021).

Finally, microbial communities can also resist Hg-induced stress by interacting within the rhizosphere; for example, a study showed that the coinoculation of fungal species increased bioaccumulation (Pietro-Souza et al., 2020). As mentioned above, phages may also have an important role because in addition to being potential vectors of Hg resistance genes, they coevolve with microbial populations and influence their dynamics in soil matrices (Chevallereau et al., 2021; Gómez and Buckling, 2011). The impact of the presence of Hg in soil on phage communities would therefore be interesting to study.

It is not yet clear why Hg enters cells since, as mentioned above, Hg has no biological role. There is indeed passive diffusion across the membrane, and some have speculated that there is accidental transport of Hg via transporters for essential metals. However, active transport has also been identified, requiring energy input, which raises questions about the biological value of importing Hg into cells (Kis et al., 2017; Schaefer et al., 2011): are these transport mechanisms included in the mechanisms for coping with Hg toxicity? Or are they incidental and only strains with other resistance pathways survive? These questions deserve further investigation since it is by understanding all the ins and outs of each of these mechanisms that they can then be used as tools for numerous applications such as biomonitoring or bioremediation.

4. Hg biomonitoring in soils

Due to the high toxicity of Hg, its detection has become a major challenge for preserving public health as well as ecosystems; therefore, there is a need for constant monitoring in polluted sites.

4.1. Conventional methods for measuring Hg bioavailability and their limits

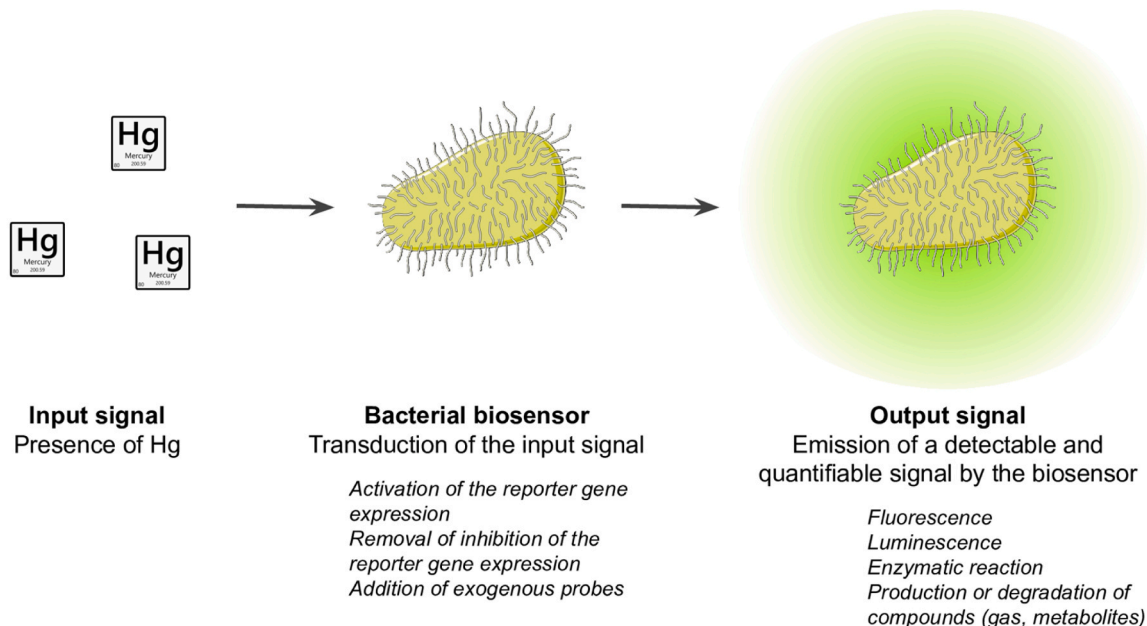
The first step is the collection and isolation of Hg from complex matrices: the methods currently used can be divided into two categories: physical and chemical. Physical methods consist of collecting the soil pore water in which the labile part of Hg can be solubilized. As this type of method is nondestructive, ecosystems can be monitored over the long term (Hojdová et al., 2007; Huang et al., 2020). However, these methods do not allow the quantification of the part of Hg fixed in the solid phase,

which can still be released when environmental parameter vary. Chemical methods are based on the use of chemical reagents to extract labile Hg, with processes such as ion exchange, desorption or proton dissolution (Fernández-Martínez and Rucandio, 2013). However, unlike that with physical methods, the extraction of Hg from soil samples with chemical methods cannot be performed *in situ* and may result in changes in soil parameters due to the use of chemical agents (Huang et al., 2020). After Hg extraction from soils, the concentration can be measured with classical analytical devices, including inductively coupled plasma (ICP) (Favilli et al., 2022), cold vapor and flame atomic absorption spectrometry (AAS) (Kozaki et al., 2021) and flame AAS (Kumar and Gupta, 2012) whose lower limits of Hg detection of the aforementioned

methods are 0.6 µg/L, 0.05 µg/L, and 5 µg/L, respectively (WHO, 2017). Knowing that the guideline for inorganic Hg is 0.6 µg/L in drinking water, the sensitivity of these detection devices should be improved or new detection methods should be developed to ensure the safety of the population (WHO, 2017).

Monitoring toxic compound concentrations in at-risk areas is not an easy task for several reasons. First, technologies for the detection and remediation of heavy metal contamination are not accessible throughout the world, particularly in less-developed countries: on the one hand, there is a lack of technical and financial means, and on the other hand, there is a lack of regulation and legislation frameworks. Moreover, even in countries where the detection methods mentioned

A



B

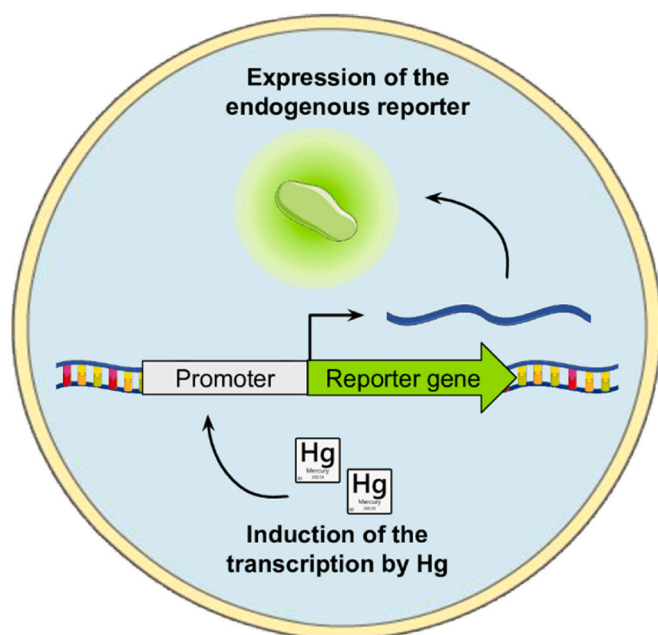


Fig. 3. Bacterial biosensors for Hg monitoring. (A) Operating principle of biosensors. (B) Induction of the endogenous reporter system in bacterial biosensors. The reporter system can be transformed with a plasmid or integrated into the bacterial genome. An example of a fluorescent biosensor is illustrated on (A) and (B).

above are routinely implemented to monitor areas most likely to be contaminated, there are some limits to these analytical technologies: they are expensive, time-consuming and cannot be used for *in situ* analysis, as the apparatus cannot be moved, requiring the samples to be transported to the laboratory (Ekrami et al., 2021; Mahbub et al., 2017b). These issues highlight the need to develop new devices for detecting and monitoring heavy metal concentrations and their bioavailability in contaminated areas.

4.2. Biosensors as a new tool for Hg detection

Recently, the development of biosensors has exploded, particularly to meet this need: by reacting to an input signal (here, the presence of Hg), biosensors are able to transduce this signal by producing an output signal that is ideally proportional to the Hg concentration (Bhalla et al., 2016). These devices have taken several forms in the past, including either exogenous probes that are chemically designed or involved in endogenous molecular mechanisms, such as enzymes, antibodies, and living cells, and among these examples, whole-cell microbial biosensors are thriving in current research (Fig. 3A) (Ma et al., 2022; Ziegler and Göpel, 1998). Indeed, microorganisms have evolved to survive their environment and its variations, and this evolutionary pressure has led to the appearance of regulatory arrays and signaling cascades in response to environmental inputs. Synthetic biology has been able to take advantage of these mechanisms from nature to recreate new genetic circuits by assembling different elements, naturally present in microorganisms or not. By introducing these synthetic constructs into chassis microorganisms, under a plasmidic form or directly into the genome (Fig. 3B), whole-cell microbial biosensors are obtained, and the latter constitute a very interesting alternative to more classical detection methods, hence the increased interest in these analytical devices (Bereza-Malcolm et al., 2014). Indeed, bacterial biosensors are quick and inexpensive to produce, requiring only routine laboratory

techniques and therefore not necessitating high-tech equipment operated by trained laboratory personnel. In addition, whole-cell biosensors can be reused and, more importantly, transported outside the laboratory, thus allowing direct analysis in the field (Ekrami et al., 2021). Finally, what makes them particularly relevant for Hg biomonitoring is the fact that bacterial biosensors are able to detect only the bioavailable forms of Hg in the total amount of Hg in samples.

Many biosensors have been developed in the past to detect heavy metals and in particular Hg; those to be described below are summarised in Table 1. In 1993, Selifonova et al. published their work on one of the first Hg whole-cell biosensors by fusing the *mer* operon from *Serratia marcescens* to the promoterless *luxCDABE* from *Vibrio fischeri*, with *Escherichia coli* as the chassis organism. This bioluminescent biosensor was then tested with water samples enriched in Hg and for bioavailability measurements (Barkay et al., 1997; Rasmussen et al., 2000), demonstrating semiquantitative detection in the nanomolar to micromolar concentration range. Other bioluminescent biosensors have since been developed: in the work of Ivask et al. (2001), the luciferase reporter gene was placed under the dependence of the Hg-inducible regulatory part of the broad-spectrum *mer* operon. These microbial biosensors also coexpressed the gene coding for an organomercurial lyase responsible for the cleavage of Hg-carbon bonds. In this way, both organic and inorganic forms of Hg were detected and therefore induced a bioluminescence signal. The *mer* operon from *S. marcescens* has been reused in other studies, for instance, to regulate the expression of the *gfp* reporter gene: this construct was integrated into the chromosome of *E. coli* with the *attP/attB* recombination system of the λ phage. Genome integration of the biosensor cassette increased its stability, and a linear response from 100 to 1700 nM Hg was obtained, with better cell viability even at high Hg levels (Priyadarshi et al., 2012). Mahbub et al. (2017b) also designed a genome-integrated biosensor using *Sphingobium* SA2 as a chassis organism. This strain was isolated from a Hg-contaminated environment, and the *gfp* gene was inserted directly into its genome

Table 1
Overview of the reporter strategies for biosensors design.

Type of probe	Chassis organism	Design of the reporter system	Assay to test the biosensors	Detection range	Ref.
Endogenous (plasmidic construct)	<i>E. coli</i> HMS174	Promotorless operon <i>luxCDABE</i> from <i>Vibrio fischeri</i> under the control of the <i>mer</i> operon from <i>S. marcescens</i> .	Incubation in natural waters supplemented with various concentrations of HgCl ₂ ; luminescence measurements.	Linear detection range from 0.5 to 50 nM Hg ²⁺ .	Selifonova et al. (1993)
Endogenous (plasmidic construct)	<i>E. coli</i> MC1061	Firefly luciferase gene <i>lucFF</i> and <i>merB</i> under the control of <i>merR/O/P</i> of the <i>mer</i> operon from <i>S. marcescens</i> .	Incubation with organomercurials and HgCl ₂ solutions (made in DMSO); luminescence measurements.	Lower limits of detection around 10 nM for HgCl ₂ , 0.2 nM for MeHgCl and 1 nM for PhHgOA respectively; upper limit of detection around 0.5–1 μ M of Hg.	Ivask et al. (2001)
Endogenous (genome-integrated)	<i>E. coli</i> JM109	Integration with <i>attP/attB</i> from λ phage of the <i>gfp</i> gene under the control of <i>merR/O/P</i> from <i>S. marcescens</i> .	Incubation with HgCl ₂ stock solutions (made in distilled water); fluorescence measurements.	Detection range of Hg ²⁺ from 100 to 1700 nM.	Priyadarshi et al. (2012)
Endogenous (genome-integrated)	<i>Sphingobium</i> SA2	<i>gfp</i> gene fused to the <i>merA</i> gene in the genome of <i>Sphingobium</i> SA2.	Incubation with HgCl ₂ stock solutions (made in distilled water); fluorescence measurements.	Linear detection range of Hg ²⁺ up to 40 nM; saturation of the signal above 40 nM.	Mahbub et al. (2017b)
Exogenous	<i>Photobacterium phosphoreum</i>	Disruption of natural fluorescence by Hg and activation of photoluminescence by hybridisation of bioaccumulated Hg with AIE probes.	Incubation with AIE probes and Hg ²⁺ ; bioluminescence and photoluminescence measurements.	Detection range of Hg ²⁺ from 2 to 8 μ M.	Huang et al. (2019)
Endogenous (plasmidic construct)	<i>E. coli</i> DH5 α	<i>ege</i> gene from <i>Pseudomonas syringae</i> pv. <i>phaseolicola</i> PK2 under the control of <i>merR/O/P</i> .	Incubation with Hg solutions and soil samples; ethylene detection.	Detection range of Hg ²⁺ from 5 to 500 μ M.	Liu et al. (2020)
Exogenous	HeLa cells	Activation of rhodamine-based probes fluorescence by hybridisation with Hg ²⁺ .	Incubation with HgCl ₂ solutions; fluorescence measurements, <i>in vivo</i> imaging and mapping of Hg ²⁺ distribution.	Linear detection range of Hg ²⁺ from 30 nM to 20 μ M.	Yang et al. (2013)
Exogenous	HeLa cells and <i>E. coli</i>	Activation of reaction-based ratiometric fluorescent probes by hybridisation with Hg ²⁺ .	Incubation with natural waters spiked with Hg ²⁺ ; fluorescence measurements and imaging via fluorescence confocal microscopy	Detection of Hg ²⁺ and MeHg with lower limits of detection of 27 nM and 5.8 nM, respectively.	Pan et al. (2018)
Exogenous	n/a	Inactivation of the quenching of a cleavable phosphorothioate RNA fluorescent probe by Hg ²⁺ .	Incubation with PBS solutions and natural waters spiked with Hg ²⁺ ; fluorescence measurements.	Linear detection range of Hg ²⁺ from 1 to 100 nM, with lower limit of detection of 0.118 nM.	Mei et al. (2022)

after the *merA* locus via homologous recombination. The engineered bacterial isolate exhibited a dose-dependent response in a range of Hg concentrations from 0 to 40 nM, which is encouraging for the potential use of biosensors as detection tools for Hg pollution.

Some biosensors have also been designed to detect the bioaccumulation of Hg^{2+} in cells. The mechanism relies on a dual detection of disruption of the quorum sensing system in *Photobacterium phosphoreum* and activation of fluorescence probes. Indeed, the aggregate formation of Hg^{2+} quenches the natural bioluminescence induced by quorum sensing in this strain, and then fluorescence is turned on by the binding of Hg ions to the aggregation-induced emission (AIE) probe. Through these synergistic mechanisms, the accumulation of Hg^{2+} can be detected with high sensitivity and selectivity (Huang et al., 2019).

In the studies mentioned above, reporter genes generate visual output signals, and most of the time, they are widely used because of their ease and speed of measurement. However, soils are complex matrices, and although encouraging results have been obtained for fluorescent and luminescent biosensors with aqueous Hg solutions, other avenues for reporter systems should be investigated. Liu et al. (2020) proposed a system with ethylene production in the presence of Hg that could be more suitable for tests performed with opaque media such as soils. Indeed, the *efe* gene that is involved in the synthesis of ethylene was coupled to the MerR-binding promoter, which was activated proportionally to Hg levels. Gas-reporting microbial biosensors have a detection range from 1 μM to 500 μM and do not react to the presence of other heavy metals. The system is therefore selective towards Hg, which is necessary for the contamination of multiple heavy metals. However, ethylene remains produced in low concentrations, so there is a need for gas chromatography and mass spectrometry analysis, which limits the rapid utilization of biosensors *in situ*.

Other biosensor-based detection methods are being developed, but not necessarily with genetic engineering and the construction of reporter gene circuits. Indeed, exogenous probes are also a promising avenue for Hg monitoring that would preclude the problems associated with the use of genetically modified organisms and their potential spread in contaminated soil. Yang et al. (2013) developed a rhodamine-based probe whose fluorescent signal allowed the mapping of Hg^{2+} sorption on bacterial cell surfaces, highlighting the colocation of Hg with EPS. Other reaction-based fluorescent probes were used as sensors for Hg^{2+} and MeHg and offered a suitable approach for imaging living cells. Their fluorescent signal was improved as the corresponding chemical reaction was irreversible, and ratios of intensities at 2 different emission wavelengths were considered (Pan et al., 2018). Another fluorescent probe has been designed with RNA harboring a cleavable phosphorothioate that was quenched by a metal-organic framework. In the presence of Hg^{2+} , the Hg ions reacted and cleaved the bound probe with the quencher because of its thiophilicity at the cleavage point: the fluorescent signal was therefore turned on in a dose-dependent manner (Mei et al., 2022).

For now, only a few studies have focused on the development of biosensors specific to a given form of Hg. Indeed, the speciation of Hg is difficult to investigate with biosensors-based methods: one study has succeeded in developing a biomonitoring system to detect Hg methylation in several species of the *Desulfobulbaceae* genus (Colin et al., 2018). However, this remains challenging since the vast majority of the developed reporter systems are dependent on the *mer* operon, which is regulated by several Hg species in a non-specific manner.

Some technical difficulties for biosensor-based detection methods can occur, for instance, due to particle-bound pollutants and the interferences and quenching phenomena that result from it: indeed, several particles present in soil matrices are autofluorescent, which induces significant background noise (Tuovinen et al., 2004). The robustness of some of the exogenous biosensors described above has been evaluated for real samples of tap and lake waters spiked with Hg^{2+} , demonstrating a great accuracy (Mei et al., 2022). In addition, some matrices may be contaminated by multiple pollutants, such as other

heavy metals, which can introduce another source of interferences. Therefore, this is necessary to ensure that the biosensors react specifically and proportionally to the presence of Hg alone in order to validate these tools for biomonitoring. Some studies have shown encouraging results, with high specificity of biosensors for Hg compared with other heavy metals, and an absence of interference (Huang et al., 2019; Mei et al., 2022; Priyadarshi et al., 2012). Moreover, the great heterogeneity of soil matrices regarding the spatial distribution of particles and organisms is another problem to overcome. However, the studies presented above show that whole-cell bacterial biosensors could be a reliable method for detecting Hg in polluted matrices. In the future, field kits could be developed (Chouichit et al., 2020; Ma et al., 2020), for instance by immobilizing these biosensors, which would make them a routine analytical device capable of seriously competing with conventional methods of Hg biomonitoring.

5. Bioremediation mediated by Hg-resistant microorganisms

Beyond monitoring applications, the resistance and tolerance mechanisms that microorganisms have acquired in Hg-contaminated environments could be used for bioremediation purposes. Indeed, organisms with these tolerance mechanisms have the capacity to accumulate Hg or to convert it into less toxic forms, which constitutes an interesting prospect for reducing Hg levels in contaminated soils or at least stabilizing them.

5.1. Bioremediation as an alternative to conventional soil rehabilitation technologies

As stated before, bioremediation refers to the use of living organisms to eliminate or neutralize contaminants in polluted areas, with a focus here on Hg detoxification in soil matrices by microbes. Unlike methods commonly used to clean up metal-polluted sites, such as stabilization, soil washing or thermal treatments, bioremediation is neither expensive nor disruptive to ecosystems, as most of the species used are naturally present (Abou-Shanab, 2011; Xu et al., 2015). Physical and chemical removal methods for heavy metals, including Hg, could therefore be replaced by bioremediation techniques. Indeed, microbial-based bioremediation presents many advantages, as mentioned above, and seems more favorable for the development of *in situ* approaches since microorganisms are able to grow in various environments. The pros and cons of each Hg remediation techniques are summarised in Table 2.

5.2. Bioremediation strategies based on the resistance mechanisms of microorganisms

Microorganisms harbor genes that can improve their tolerance to pollutants: in the case of Hg, the bacterial *mer* operon is often associated with other advantageous physiological traits, such as biofilm formation, motility or antibiotic resistance, and bacteria also express metallothionein and polyphosphates, which are metal-scavenging agents involved in Hg resistance and accumulation (Priyadarshane et al., 2022). For instance, the formation of biofilms with Hg-resistant bacteria on a porous carrier material allowed the trapping of Hg^0 produced by microbial reduction reactions, and the aim was to remove Hg from wastewater by implementing this biofilm system at a larger scale in a bioreactor (Wagner-Döbler, 2003). Indeed, biofilm formation could be stimulated by Hg stress and includes the secretion of EPS. These compounds have heavy-metal-chelating potential, which makes them a protective barrier against Hg stress, delaying their propagation into biofilms (de Araújo et al., 2019). Other bioremediation studies focus on the stabilization of Hg by microorganisms using EPS: François et al. (2012) selected Hg-tolerant bacteria from a contaminated site, with a mucoid phenotype indicative of the production of EPS. The particularity of the selected strains was the absence of the Hg volatilization process, which allowed the study of biosorption mechanisms as an alternative

Table 2

Advantages and limitations of the different methods for Hg remediation.

	Soil washing	Stabilisation	Thermal treatment	Bioremediation
Advantages	Full scale and commonly used; well-established technology. Short process and low costs. Easily modular systems for <i>in situ</i> applications. Up to 99% efficiency.	Full scale and commonly used. Compatible with a wide range of soil types. No further treatment required. 20–90% of Hg sequestered.	Full scale and commonly used. Great efficiency. Potential for Hg recovery.	Reduced human exposure to contaminants. No expensive equipment and large-scale transport required. Ecosystem friendly.
Limitations	Unfeasible depending on soil properties. Use of hazardous compounds and production of contaminated waste. Disruptive for ecosystems.	Need to assess long-term performance and need for a long-term monitoring. Interferences with organic matter. Complex to apply on a large scale because of the volumes of soils to be treated.	Production of contaminated waste. Expensive in terms of energy and money. Disruptive for ecosystems (working temperatures from 320° to 700°C).	Pilot scale and ongoing development. Efficiency difficult to evaluate. Need for long-term maintenance. Contaminated organisms to be managed.

resistance pathway. Biosorption can be defined as a metabolically mediated passive process or physicochemical pathways for metal sequestration from the contaminated environment with the help of active biomass (Mukkata et al., 2019). They also compared the sequestration capacity of the dead vs. living biomass after incubation with HgCl_2 , and it appeared that dead biomass sequestered Hg in higher proportions, probably because of the absence of toxicity issues for dead bacteria at high Hg concentrations. Dead bacteria could therefore be fixed on an inert support, with no need for growth conditions (Mukkata et al., 2019). As previously mentioned, similar results have been obtained for fungal necromass, which presented a high biosorption capacity as well, notably due to the presence of lipidic compounds (Maillard et al., 2023). Thus, dead microorganisms appear to have a role to play in Hg fluxes and especially in its extraction from soils. Extracellular sequestration can also be obtained by genetic engineering: in *Saccharomyces cerevisiae*, MerR proteins encoded by the *mer* operon have been displayed at the cell surface to enhance Hg biosorption, as MerR has a high affinity for Hg^{2+} . In addition, the genetically engineered strain presented an increased tolerance to Hg and to pH variations (Wei et al., 2017).

Bioaccumulation can also be considered active biosorption, with great potential for bioremediation, although the relevant pathways need to be further investigated. Some bacteria have been isolated from gold mining sites, and two strains, *Fictibacillus nanhaiensis* and *Bacillus toyonensis*, were distinguished by their high Hg accumulation rates of 82.25% and 81.21%, respectively. In this study, the bioaccumulation capacity was defined by the ratio of the total Hg amount in the bacterial isolate to the total Hg amount in the growth medium (Nurfitriani et al., 2020). It must also be noted that the two isolated bacteria were spore-forming bacteria: only a few studies have investigated the impact of Hg contamination on sporulation, but it seems that Hg has an inhibitory effect on spore formation and germination (Bhajibhuje, 2013; Liu et al., 2018). The presence of spores below a certain threshold in the soil could therefore be an indicator of Hg contamination. Many other studies were based on the isolation of strains from Hg-contaminated environments since microorganisms that have resisted Hg-induced stress are potential candidates for remediation applications. Some Hg-tolerant bacteria from Iranian coastal sediments have shown a 90% removal of Hg in growth medium and a tolerance of up to 45 mg/mL. Modeling with response surface methodology allowed the optimization of parameters (temperature, pH) for bacterial growth to achieve efficient remediation even at high Hg concentrations (Jafari et al., 2014).

Fungal species can also be potential candidates for bioremediation: in Slovakia, autochthonous filamentous fungi from the *Aspergillus*, *Cladosporium*, *Trichoderma* and *Alternaria* genera have been isolated and presented a great capacity for Hg volatilization (Urík et al., 2014). Indeed, this process is the major detoxification mechanism of fungi, and this is supported by the fact that Hg accumulation in fungal biomass did not correspond to Hg uptake from culture media, meaning that there exists another detoxification pathway than biosorption, which is biovolatilization. Enhancing fungal growth in Hg-contaminated areas could

therefore result in natural remediation (Urík et al., 2014). In addition, the potential of fungi as relevant candidates for bioremediation also depends upon their ability to accumulate Hg, even at low concentrations, in soil (Falandysz and Borovička 2012). Already used as bio-indicators for the monitoring of Hg pollution (Wondratschek and Röder, 1993), Hg-accumulating fungi were found to limit the spread of Hg, both in the soil and in the atmosphere, which is increasingly favored in current bioremediation projects (Durand et al., 2020; Maillard et al., 2023). Fungi can also have an increased tolerance due to interactions with plants: for example, 30 endophytic fungi were tested, and growth-promoting traits were identified in some of them, such as indole-3-acetic acid (IAA) and siderophore production and phosphate solubilization (Pietro-Souza et al., 2020). Some candidates for bioremediation were selected because they had great potential to enhance the Hg tolerance and growth of host organisms. In addition, the coinoculation of two or more endophytic fungi was found to increase bioaccumulation in plants while decreasing the Hg^{2+} concentration in soils, which seems to be a promising strategy for reducing Hg levels in soils, and which, unlike volatilization, limits the flow of Hg from one compartment of the biosphere to another, by promoting its stabilisation (Pietro-Souza et al., 2020).

5.3. Contribution of microbial consortia to bioremediation

Bioremediation approaches could be based on microbial consortia instead of isolated species since interactions between different species of soil microorganisms can drive bioremediation processes and increase the resistance of microbial communities to Hg. A comparison of fungal communities between Hg-contaminated and noncontaminated areas showed that Hg stress increases the richness, diversity and number of root endophytic isolates (Pietro-Souza et al., 2017). Indeed, the proportion of fungal strains with antibiosis mechanisms that are able to produce siderophores, amylases, proteases, ligninases, and cellulases was found to be higher in polluted environments, which led to the hypothesis that there are adaptive mechanisms triggered by Hg stress (Pietro-Souza et al., 2017). This confirms that the use of environmental isolates from contaminated sites is an interesting perspective in bioremediation. Similarly, bacteria from a Brazilian wetland presented a greater richness, diversity and abundance if they were isolated in an environment enriched in Hg. Fifty percent of the isolated strains harbored plasmids and the *merA* gene, and generally, they presented higher minimum inhibitory values and multiresistance to metals and antibiotics. Some strains improved corn plant growth in Hg-contaminated soil and helped to reduce up to 87% of the Hg level in the soil while increasing Hg bioaccumulation by up to 94% in corn plants (Mariano et al., 2020).

Other approaches, such as metagenomics, can provide relevant insight into microbial communities and their potential as bioremediators. A metagenomic study of a mining site in Almadén, Spain, contaminated with Hg showed that the most abundant taxa in bulk soil were *Actinobacteria* and *Alphaproteobacteria*, whereas *Proteobacteria*

were the majority in rhizospheric samples (González et al., 2022). This result suggested that roots have a selective effect on the soil microbiome. Functional potential analysis revealed that the cluster related to stress response was among the 10 most abundant, which implied that there was environmental selection pressure because of Hg contamination. Plant growth-promoting traits and nitrogen fixation activities have been detected in metagenome sequences, especially from rhizospheric soil, which could therefore constitute criteria for the selection of candidate strains for bioremediation and genes for engineering with biotechnological purposes (González et al., 2022). Regarding the case of fungi, an environmental barcoding study on fungal communities from a Hg phytomanagement site gave interesting findings on the soil communities that were negatively affected by Hg. In addition, a pattern of mutual exclusion between soil operational taxonomic units has been highlighted (Durand et al., 2017).

Other researchers aimed to find a global way to assess the potential as a bioremediation tool of a given strain among a soil microbial consortium. They therefore developed the Bio-Mercury Remediation Suitability Index (BMRSI), which considers IAA production, 1-AminoCyclopropane-1- Carboxylic acid deaminase (ACCd) activity, phosphate solubilization and siderophore production (that constitute plant growth promoting traits) as well as the maximum bactericidal concentration of Hg. The BMRSI is computed according to the Eq. (1).

$$\text{BMRSI} = [\text{IAA} + \text{ACCd} + \text{SID} + \text{PO}_4^3] + [\text{MBC Hg}] \quad (1)$$

With IAA (mg/mL) corresponding to the IAA concentration in the supernatant of the culture medium, ACCd corresponding to the ACC degradation capacity (1 = presence or 0 = absence), SID (cm) corresponding to the size of the halo formed around a colony after siderophore production, PO_4^3 corresponding to the P solubilisation capacity (1 = presence or 0 = absence) and MBC (mg/mL) corresponding to the maximum bactericidal concentration of Hg.

Each variable is weighted in the equation according to its importance in bioremediation mechanisms. As a result, a numerical value is obtained that reflects the behavior of a considered strain and its potential for bioremediation and biotechnological uses (Robas et al., 2021). The BMRSI was used for the selection of bacterial isolates from a microbial consortium isolated in the Almadén mining site. The study highlighted that Hg contamination in soils negatively affects the effectiveness of plant growth-promoting mechanisms in the following order: siderophore production > phosphate solubilization > ACCd activity > IAA production. Strains with BMRSI values higher than 6.5 were considered relevant candidates for further bioremediation trials with contaminated soils (González et al., 2021). Prior to the development of this index, the bioremediation potential of some strains was already tested with soil samples from Hg-contaminated areas: for example, *Sphingobium* SA2 was selected for its high tolerance to Hg and its biovolatilization capacity. Sixty percent of the total amount of Hg was removed, and microbial bioaugmentation combined with nutrient amendment improved Hg removal and favored revegetation, with an increase in root lengths (Mahbub et al., 2017a).

5.4. Biotechnology-enhanced bioremediation perspectives

The interest in selecting Hg-resistant strains is also to use them as optimized bioremediation tools through biotechnological processes. Genetic engineering has made it possible to develop biosensors, as mentioned above, but there have been other attempts to develop transgenic strains for additional bioremediation purposes. For instance, *Deinococcus radiodurans*, a radiation-resistant strain, was engineered to express the *merA* gene for the remediation of radioactive waste that was cocontaminated with Hg^{2+} (Brim et al., 2000). Other transgenic strains have been developed to improve bioaccumulation: bacteria expressing metallothionein and polyphosphate kinase showed a higher bioaccumulation rate and a greater Hg tolerance (Ruiz et al., 2011), and the

Hg removal efficiency of a recombinant strain of *Rhodopseudomonas palustris* also expressing metallothionein and Hg transport genes was increased by a factor of 3 compared to that of the wild strain (Deng and Jia, 2011). Another transgenic strain was designed from *Bacillus cereus*, selected for its Hg biosorption capacity, and transformed with the *mer* operon. As a result, recombinant bacteria were capable of volatilizing and simultaneously precipitating Hg and removed 100% of Hg from solution (Dash and Das, 2015). In a more recent study, *E. coli* BL21 was transformed with an artificial Hg-resistant operon, and the engineered bacteria exhibited resistance to Hg in several forms (inorganic Hg, MeHg, phenyl Hg), even at high concentrations, and they efficiently removed 43.7% of Hg from polluted wastewater within 24 h (Chang et al., 2018).

There have therefore been many studies that focused on the remediation of Hg-contaminated soils via microbial-based approaches, and a number have been described, although not exhaustively. Fig. 4 gives an overview of the microbial mechanisms on which the bioremediation projects under development are based. This work provides a global perspective of the different techniques and possible uses of microorganisms and their genetic material for remediating complex soil matrices.

6. Perspective of biomonitoring and bioremediation of Hg by microorganisms

An overview of the Hg biogeochemical cycle has highlighted the need for action to limit the impact of Hg emissions, particularly from anthropogenic sources. Indeed, to preserve ecosystems, humans and animals, it is necessary to succeed in reducing Hg levels, particularly in certain areas affected by pollution where health is at risk. The key role of microorganisms in the Hg cycle makes them ideal candidates for the implementation of bioremediation techniques since they possess unique physiological properties that allow them to resist Hg contamination, even at high concentrations. This ability to overcome this stress is due to a set of mechanisms that make microbes capable of Hg uptake reduction in cells, extracellular sequestration, and the conversion of toxic mercurial compounds (MeHg, Hg^{2+}) into Hg^0 , a volatile and less toxic form: all these processes endow microorganisms with great potential for the detoxification and rehabilitation of polluted sites. In addition, they can constitute a suitable tool for monitoring Hg concentration in complex matrices through the development of genetically engineered strains.

However, it is necessary to recall all the issues linked to the use and manipulation of living organisms and that have to be considered in biomonitoring in bioremediation strategies. First of all, the introduction of microbial species that are not endemic to the ecosystem of interest must be carefully considered. These non-native species could potentially compete with the indigenous species and alter the structure of the microbial communities. We therefore recommend using microbial strains from contaminated areas that have been isolated from *in situ* samples. With this in mind, for bioremediation purposes, the BMRSI has been proposed to assess the bioremediation potential of isolated strains (Robas et al., 2021), and it may be beneficial to develop optimised indexes that incorporate for instance quantitative values for ACC degradation capacity and P solubilisation instead of solely evaluating the presence or absence of these traits in strains. Other variables need to be considered, such as the presence of antibiotic resistance genes: in the case of Hg, it has been shown that there is co-selection of Hg and antibiotic resistance genes (Priyadarshane et al., 2022), and care must therefore be taken not to select these multi-antibiotics resistant strains since they present a significant health risk. Moreover, the use of genetically-engineered microorganisms can also have an impact on ecosystems and their integrity. Currently, we have a limited knowledge about the potential effects of introducing genetically modified organisms on untargeted species, and it is challenging to assess the persistence of recombinant DNA in the environment, especially in microorganisms where horizontal transfers and conjugation are widespread (Azad et al.,

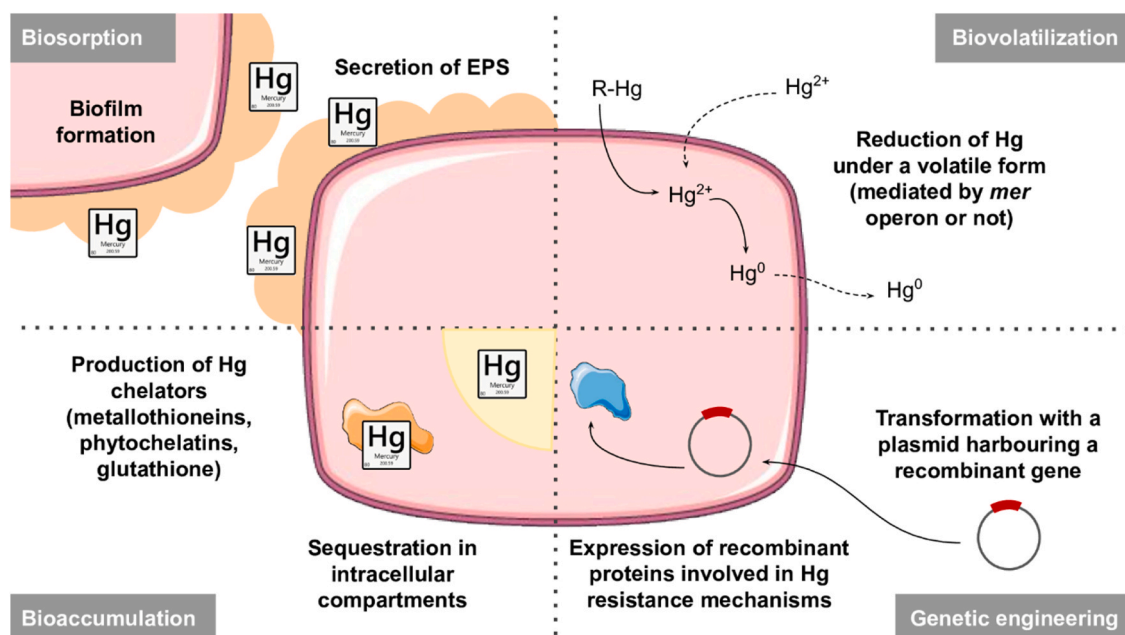


Fig. 4. Overview of the mechanisms described in microorganisms on which current bioremediation technologies are based. EPS: extracellular polymeric substances.

2014; Wu et al., 2021). Implementing suicides mechanisms can be considered to prevent the release of genetically-engineered strains in nature, for instance by introducing lethal genes expressed only in the absence of the pollutant targeted for bioremediation (Azad et al., 2014). Legislation varies from country to country, but there is no doubt that a strict regulatory framework must be put in place. Although this process is time consuming and incurs costs, the evolution of genetically engineered microorganisms in ecosystems must be monitored in order to assess the balance between the environmental damage caused by pollutants and the potential ecological damage caused by these strains. Regarding bioremediation strategies, in our view, biosorption and bioaccumulation should be the preferred mechanisms in candidate microorganisms, because considering the persistence of Hg once in the atmosphere, biovolatilization processes contribute to its propagation and thus to the contamination of new ecosystems that have been preserved until now. It is therefore preferable to stabilize contamination by sequestering Hg externally or within the microorganisms. This also prevents biotic processes such as methylation from occurring, which could increase the toxicity of Hg.

In any case, microbial-based techniques for bioremediation and biomonitoring are more advantageous than conventionally implemented methods, especially in terms of cost, implementation and environmental friendliness, and are therefore a promising avenue for solving Hg pollution issues and are part of the One Health approaches for addressing heavy metal contamination that simultaneously threatens humans, animals and ecosystems.

Ethical approval

The authors confirm that the manuscript has not been simultaneously submitted to any other journal for consideration and has not been previously published.

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CRediT authorship contribution statement

LM performed the literature search and data analysis and drafted the work. SG, NC and MC critically revised the work. All authors reviewed and accepted the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest to disclose regarding the publication of this paper.

Data availability

No data was used for the research described in the article.

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