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Perspectives on nanomaterial-empowered bioremediation of heavy metals by photosynthetic microorganisms

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ABSTRACT

Environmental remediation of heavy metals (HMs) is a crucial aspect of sustainable development, safeguarding natural resources, biodiversity, and the delicate balance of ecosystems, all of which are critical for sustaining life on our planet. The bioremediation of HMs by unicellular phototrophs harnesses their intrinsic detoxification mechanisms, including biosorption, bioaccumulation, and biotransformation. These processes can be remarkably effective in mitigating HMs, particularly at lower contaminant concentrations, surpassing the efficacy of conventional physicochemical methods and offering greater sustainability and cost-effectiveness. Here, we explore the potential of various engineered nanomaterials to further enhance the capacity and efficiency of HM bioremediation based on photosynthetic microorganisms. The critical assessment of the interactions between nanomaterials and unicellular phototrophs emphasised the ability of tailored nanomaterials to sustain photosynthetic metabolism and the defence system of microorganisms, thereby enhancing their growth, biomass accumulation, and overall bioremediation capacity. Key factors that could shape future research efforts toward sustainable nanobioremediation of HM are discussed, and knowledge gaps in the field have been identified. This study sheds light on the potential of nanobioremediation by unicellular phototrophs as an efficient, scalable, and cost-effective solution for HM removal.

1. Introduction

Heavy metals (HMs) are ubiquitous environmental pollutants that originate from various natural and anthropogenic sources. Human activities such as industrial processes, mining, agriculture, and urbanization are among the main contributors alongside natural occurrences such as rock weathering, forest fires, and volcanic activities (Masindi et al., 2021). The HMs are nonbiodegradable and tend to persist in ecosystems and organisms, accumulating in the food chain and posing serious risks to human health. The adverse effects of HMs typically stem from their capacity to stimulate the generation of reactive oxygen species (ROS) and to impede their neutralization by interacting with thiol groups of key proteins involved in cellular defence mechanisms. These interactions lead to oxidative deterioration of biological macromolecules and disruption of cellular homeostasis (Flora et al., 2008). Additionally, certain HMs have the capacity to displace essential metals from protein binding sites, consequently hampering their physiological functions. They may also engage with DNA and nuclear proteins, causing malfunction and damage to vital tissues and organs (Balali-Mood et al., 2021). Thus, the environmental release of HMs is under constant control and strict regulations by the United Nations Economic Commission for Europe under the Environmental Policy "Protocol on heavy metals" and "Air" (UNECE, 2021a; UNECE, 2021b). The European Environment Agency (EEA) considers HMs a priority environmental threat (EEA, 2023) and indicates prevention as the most effective and cost-efficient approach to ensure long term soil health and clean water and air.

HM contaminated sites are generally categorized in two groups: soil and water (including industrial wastewaters and aquatic ecosystems), each requiring tailored decontamination strategies to mitigate their HM content. Different physicochemical and biological remediation approaches have been devised for both types of sites. In the case of contaminated soils, physicochemical methods include HM immobilisation and soil washing. Immobilisation technology adds organic and inorganic substances such as clay, cement, zeolites, minerals, phosphates, and organic composts to soils. These substances reduce the mobility and toxicity of HMs by transforming them into more stable

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Abbreviations

EPS	extracellular polymeric substances
GSH	glutathione
GO	graphene oxide
HMBE	harmful macroalgal bloom extract
HMs	heavy metals
MTs	metallothioneins
MOFs	metal-organic frameworks
MWCNTs	multi-walled carbon nanotubes
NF	nanofibres
NPs	nanoparticles
PCs	phytochelatins
SWCNTs	single-walled carbon nanotubes

phases through sorption, precipitation, and complexation processes (Derakhshan Nejad et al., 2018). Soil washing, on the other hand, involves physically separating the most contaminated soil particles from the bulk fractions and treating them with aqueous chemicals to extract HMs, which are then recovered on a solid substrate (Feng et al., 2020). Physicochemical methods for removing HMs from wastewater include adsorption onto various porous materials, filtration, osmosis, and electrophoresis, chemical methods, electrochemical reduction/oxidation, and photocatalytic treatments (Qasem et al., 2021). These conventional techniques offer relatively rapid, highly controllable and effective removal of the contaminants, however, they suffer from substantial drawbacks, such as incomplete metal extraction, low efficiency at HM concentrations lower than 100 $mg.L^{-1}$, high costs and the risk of producing hazardous byproducts (Qasem et al., 2021; Feng et al., 2020).

The biological methods for remediations of HMs utilise environmentally friendly and, in most cases, cost-effective approaches, which may be suitable for the reconstitution of both contaminated soils and waters. The basis of this strategy, generally termed as bioremediation, is the use of biological agents, such as microorganisms, plants, or enzymes to remove or neutralize HM contaminants. Photosynthetic microorganisms (microalgae, cyanobacteria, and anoxygenic photosynthetic bacteria) are a heterogeneous group of unicellular microbes that share the common ability to use light as a primary energy source for growth. They are non-pathogenic, and their phototrophic nature enables easy, fast, and relatively inexpensive cultivation, which is increasingly attracting attention for the development of various bioremediation applications (Fernández et al., 2021; Chen et al., 2020a). Unicellular phototrophs are broadly represented in the biosphere, inhabiting a wide variety of natural environments and often thriving in harsh conditions such as high salinity, nutritional stress, extreme temperatures, and HM-contaminated environments (Kaewsuk et al., 2010; Mohseni et al., 2021; Grattieri et al., 2022). Most of these organisms possess extraordinary metabolic flexibility that can be tailored through changes in cultivation conditions to achieve desired outcomes (Proietti Tocca et al., 2024). The presence of sub-lethal concentrations of HMs and the consequent ROS accumulation may trigger the carbon partitioning in green algae *Parachlorella kessleri* R-3, thereby promoting lipid production and accumulation (Song et al., 2023). Anoxygenic photosynthetic bacteria can use different electron donors and/or acceptors in their main metabolic pathways in the presence of different photo-oxygen conditions (Chen et al., 2020a). As a specific example, the light-dependent anoxygenic photosynthesis of some purple sulphur bacteria can use electrons from arsenic-containing compounds to support their growth (Edwardson et al., 2014).

Furthermore, autotrophic microorganisms, mainly microalgae and cyanobacteria, readily form functional consortia among themselves and with heterotrophic bacteria. These consortia often outperform individual species in HM bioremediation efficiency due to improved stability of the involved organisms, enhanced tolerance toward contaminants, and the synergistic involvement of multiple HM removal mechanisms (Yang et al., 2021; Subashchandrabose et al., 2011). Photosynthetic microorganisms are also well-suited for developing bioremediation strategies based on immobilised cells. Cell immobilisation offers several advantages, including enhanced capacity for HM removal, the potential for regeneration and reuse of biosorbents, reduced costs associated with cell harvesting, improved overall cost-effectiveness of the remediation process (Chen et al., 2023).

Different studies proved possible the coupling of bioremediation by unicellular phototrophs to the generation of valuable products such as biomass, biofuels and various high value-added compounds (Song et al., 2023; Razaviarani et al., 2023). The possibility of integrating contaminant removal with bio-production is extremely attractive due to its positive impact on the cost/benefit ratio of the overall process. Although photobioreactor technology is rapidly advancing (Abdur Razzak et al., 2023), the cost of cultivating and harvesting phototrophic microorganisms remains a critical issue (Tanvir et al., 2021). The sustainability of these practices is therefore strictly dependent on maximising benefits.

The removal of HMs by unicellular phototrophs is primarily driven by the processes of biosorption and bioaccumulation. Biosorption is typically a passive mechanism, involving the adsorption of metals onto the surface of dead or living cells, while bioaccumulation is an energy-driven process, involving active HM transport across membranes into live cells (Danouche et al., 2021). An exception to this is HM biosorption triggered by the entrapment of the contaminant within the extracellular polymeric substances (EPS) that are products of the cellular metabolism (Momin et al., 2024). The action mechanisms underlying the bioremediation process can vary depending on the microorganisms, operational conditions, and the nature and properties of the HMs. In most cases, the bioremediation process is limited to the restoration of sites and waste products containing low concentrations of HMs. Other limitations of bioremediation by (living) photosynthetic microorganisms stem from their specific nutritional and environmental requirements, including factors such as temperature, pH, oxygen levels, and light availability (Danouche et al., 2021). An important factor influencing the feasibility of the bioremediation process is the cost associated with biomass harvesting (Ferraro et al., 2018; Lalhmunsiama et al., 2017).

Nanotechnology has been gaining momentum in the environmental remediation of different noxious compounds, including HMs. The scientific literature is abundant with surveys and experimental studies exploring the use of nanomaterials, varying in shape, size, and structural properties, to enhance the physicochemical remediation of contaminated air, soil, water, and industrial waste or byproducts (Mahesh et al., 2022; Ali et al., 2023; Verma et al., 2024). Further, the convergence of nanotechnology and biological agents paves the way for the pioneering approach of nanobioremediation, which uses nanotechnology in combination with biological processes to degrade, transform, or eliminate environmental pollutants. Nanobioremediation has successfully been applied in the decontamination of various pollutants (dyes, pesticides, drugs, HMs, etc.), helping to overcome the low efficiency of traditional bioremediation processes in treating heavily polluted sites and demonstrating potential for large-scale and cost-effective environmental restoration (Chaudhary et al., 2023; Malik et al., 2022; Kumar et al., 2023).

In general terms, nanobioremediation refers to the use of nanomaterials to remove various harmful chemicals through biological agents, including the use of nanoparticles synthesised from biomass or living organisms. The research direction in nanobioremediation, which focuses on the synthesis and utilisation of nanomaterials produced by bacteria, algae, fungi, or plants for the removal of HMs, has already been comprehensively reviewed in recent studies and will not be addressed here. In-depth information on this topic can be found in the latest review articles (Tauseef et al., 2023; Sharma et al., 2023; Verma et al., 2024; Kumar et al., 2023; Hoang et al., 2022).

The present work delves into the underexplored topic of the potential of tailored nanoscale materials to support HM mitigation by unicellular

phototrophs, thereby improving the efficiency of bioremediation processes. The field is still relatively new, and a comprehensive understanding of the effectiveness, potential risks, and long-term implications of using nanomaterials to support or even boost bioremediation based on photosynthetic microorganisms is still lacking. Microorganisms are particularly suited for the development of such nanobioremediation strategies due to their high contact surface area and unicellular nature, which facilitate the interaction with nanomaterials and their cellular internalization. The mechanisms of cellular uptake of the nanoscale materials in walled-type cells have not been extensively elucidated. It has been suggested that the dimensions and surface charge of the nanomaterial (Orlanducci et al., 2020; Lambreva et al., 2015), and more recently, the cell cycle stage (Yan et al., 2021), are among the main determinant factors. The uptake of nanomaterials with different shapes and sizes in unicellular phototrophs (mainly microalgae) has been well-documented (Orlanducci et al., 2020; von Moos et al., 2014), often with a lack of cytotoxic effects. Indeed, hormetic effects on culture growth, photosynthetic performance, and accumulation of pigments, proteins, carbohydrates or biomass have been often obtained in various microorganisms due to the presence of nanomaterials (Antal et al., 2022; Zamani, 2023; Zhang et al., 2018b; Bisht et al., 2023; Petit et al., 2010). An intriguing yet still relatively unexplored possibility lies in coupling the extracellular electron transport capabilities of certain unicellular phototrophs (Lea-Smith et al., 2016), with redox-active nanomaterials. This coupling could potentially enhance the efficiency of these microorganisms for reducing and/or removing HMs.

To fill the knowledge gap in the capacity of engineered nanomaterials to sustain the bioremediation process or enhance the stability of the biological component, we critically analysed literature data demonstrating nanomaterial-mediated alterations in the metabolism of unicellular phototrophs and their tolerance to HMs. We further examined the ability of various nanostructured materials to alter the bioavailability and toxicity of HMs to photosynthetic microorganisms and explored the potential implications of these phenomena in the pollutant removal process. This critical literature survey allowed us to identify the putative pathways of interplay between the main metabolic reactions of unicellular phototrophs and nanostructures, and to outline perspectives for the future use of nanomaterials in the bioremediation process. These findings are likely to be of significant interest to the broader scientific community engaged in developing sustainable applications based on microalgae, cyanobacteria, and anoxygenic photosynthetic bacteria. They offer insights into how nanostructured materials can enhance the efficiency and effectiveness of these organisms in various environmental applications, particularly in the realm of bioremediation and sustainable technology development.

2. Main routes for HM bioremediation by photosynthetic microorganisms

Bioremediation facilitated by unicellular phototrophs exploits the intrinsic detoxification mechanisms of the microorganisms, typically unfolding in two distinct processes. Initially, there is a rapid and reversible sorption of HMs onto the cellular surface. This biosorption can be followed by a slower and irreversible phase characterized by metabolically-driven accumulation, wherein HMs are transported across the cell membrane into the cytoplasm, potentially leading to subsequent biotransformation (Danouche et al., 2021). The biosorption is the predominant mechanism involved in the HM removal in different photosynthetic microorganisms (Jeyakumar et al., 2023). Biotransformation reactions, such as reduction or chelation, can also occur extracellularly, as the initial metal transformation may be a prerequisite for their cellular uptake (Merchant et al., 2020; Urzica et al., 2012) or part of the HM detoxification process (Italiano et al., 2018).

2.1. Biosorption of HMs by photosynthetic microorganisms

The key players in the biosorption process are the various functional groups present on the cell wall surface of microorganisms (including EPS), which participate in various physicochemical interactions with HM ions (Fig. 1). The cell wall of unicellular phototrophs is rich in polysaccharides, proteins, and lipids, which contain numerous negatively charged functional groups, including hydroxyl, carboxyl, sulfhydryl, sulphate, phosphoryl, and amide groups (Silhavy et al., 2010; Spain et al., 2021). Extensive evidence suggests that the ion exchange mechanism is a primary driver of the biosorption process in both microalgae and cyanobacteria (Zeraatkar et al., 2016; Talaiekhozani and Rezania, 2017).

Specifically, carboxyl groups within cell wall polysaccharides are known to predominantly facilitate the sorption of HMs, whereas other groups such as sulfonate and amino play a relatively minor role in this process (Mehta and Gaur, 2005; Momin et al., 2024; Spain et al., 2021). The importance of the carboxyl groups in the reversible binding of Ni^{2+} ions has been demonstrated also in the gram-negative purple non-sulphur bacterium *Rhodobacter sphaeroides* (Giotta et al., 2011; Chirizzi et al., 2022). Precipitation, which can either be dependent (via EPS) or independent on cellular metabolism, is also involved in HM removal by some microalgae and cyanobacteria (Ahalya et al., 2003; Momin et al., 2024). EPS, consisting of complex mixtures of biopolymers secreted by microorganism, exhibit a significant capacity for binding HMs and may serve a key role in both bioremediation and nanobioremediation processes. An important characteristic of EPS is their

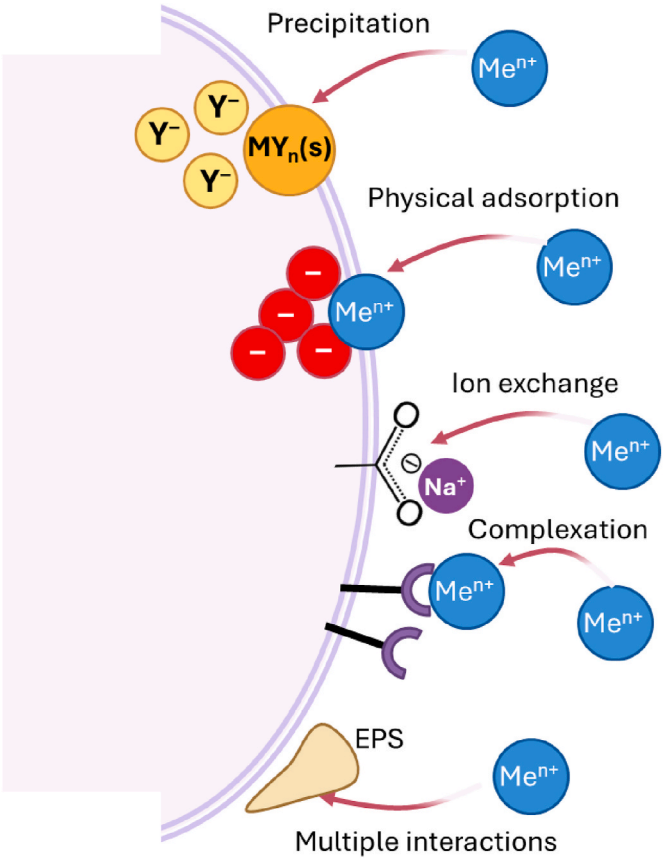

Fig. 1. Biosorption mechanisms of HMs on the surface of photosynthetic microorganisms. Me^{n+} represents a generic HM cation. From top to bottom: (1) precipitation with a precipitating agent Y^-; (2) physical adsorption occurring via Wan der Waals or electrostatic interactions; (3) ion exchange with specific light metal cations (K^+, Na^+, Ca^{2+} and Mg^{2+}); (4) complexation with ligand groups; (5) multiple interactions with the EPS matrix, as outlined in points 1–4.

ability to be induced by external stimuli (Momin et al., 2024), including the presence of nanomaterials (Rhiem et al., 2015).

The efficiency of biosorption process depends on both intrinsic characteristics of the biomass, including among others, culture growth phase and cell concentration, and external factors such as pH, temperature, contact time, co-existence of other ions (Jeyakumar et al., 2023). Indeed, the pursuit of strains with high metal sorption capacity, combined with molecular or nanotechnological strategies to augment the available binding sites on the cellular surface, alongside a comprehensive understanding of sorption mechanisms, are among the main aspects in development of commercially viable applications of this technology (Danouche et al., 2021; Cheng et al., 2019).

Among external factors, the pH of the media is the most important trigger for the sorption and desorption processes of HMs on microorganism surface. It can affect both metal speciation and the availability of cell surface functional groups, as well as the competition among coexisting metal ions (Das and Das, 2013). The biosorption of cationic species increases as pH values rise, as increasing amounts of deprotonated functional groups on the cellular surface become gradually available. Based on their pKa values, different carboxyl groups become active when pH values rise above ~4, phosphate groups get deprotonated at pH above ~7, and hydroxyl or amine groups at pH above ~9 (Chojnacka et al., 2005). Thus, effective biosorption often requires pH conditions that may not fully align with the physiological requirements of unicellular phototrophs. This drawback can be addressed by using dead cells, which allow for the utilisation of a wide range of working pH and temperature, in addition to offering other advantages, such as: i) the ability to store biosorbents for extended periods, ii) absence of HM toxicity effects, iii) no nutrient limitations, and iv) the possibility of chemical regeneration for reuse in adsorption-desorption cycles (Ordóñez et al., 2023). On the other hand, the advantages of biomass of living cells include rapid growth and simultaneous removal of HMs alongside culture proliferation, the ability to remove HMs through a combination of reactions (sorption, accumulatio and/or transformation), and the potential to integrate the bioremediation process with the production of valuable products (Razaviarani et al., 2023).

2.2. Bioaccumulation and biotransformation of HMs by photosynthetic microorganisms

The mechanisms involved in the cellular uptake of HMs can be metal-specific and in some cases also species-specific (Cavet et al., 2003; Sure et al., 2016). In microalgae, anoxygenic photosynthetic bacteria, and cyanobacteria, the active transport of HMs may involve different ion pumps, protein channels, or carrier-mediated transport (Tripathi and Poluri, 2021; Tiwari et al., 2019; Borsetti et al., 2009). Some of the trafficking mechanisms of HMs present in the photosynthetic microorganisms are depicted in Fig. 2. The growing amount of transcriptomic data has revealed tight control over the gene expression levels of families of metal transporter proteins in response to extracellular HMs. These findings shed light also on the intricate mechanisms of metal homeostasis and the role of sulphur metabolism in HM mitigation, particularly in microalgae (Tripathi and Poluri, 2021; Ranjbar and Malcata, 2022).

After entry into the cell, HMs undergo a series of reactions including chelation with thiol-containing compounds to restrict intracellular metal interactions and reduction/oxidation to produce less harmful forms. Chelation is primarily regulated by cysteine-rich biomolecules such as metallothioneins (MTs) and phytochelatins (PCs), which bind metals through numerous sulfhydryl groups (Balzano et al., 2020; Ranjbar and Malcata, 2022). The complexes formed between HMs and chelators are primarily stored in vacuoles or other cellular organelles, such as chloroplasts or mitochondria. Alternatively, HM-chelator complexes may be effused from the cells to preserve the cytoplasm from the presence of free metals (Balzano et al., 2020; Tripathi and Poluri, 2021). In unicellular phototrophs, the pool of cytosolic glutathione (GSH), a tripeptide structurally similar to the PCs, can also participate in HM mitigation, in addition to its role as redox buffer in oxidative stress reactions, in the redox equilibrium of MTs and synthesis of PCs (Ziller and Fraissinet-Tachet, 2018; Balzano et al., 2020). Enzymatic transformation for some of the HMs are also known, such as hexavalent chromium Cr(VI) reduction to Cr(III) by chromate reductase (in *Chlorella vulgaris*) or Hg(II) reduction to Hg(0) by mercuric reductase (in *Selenastrum minutum*, *C. fusca* and *Galdiera sulphuraria*) (Danouche et al., 2021). In the case of *R. sphaeroides*, the Cr(VI) reduction may involve nonspecific soluble reductants, such as GSH, GSH reductase, carbohydrates, cytochromes, NADPH, or ascorbic acid (Italiano et al., 2012), while Au(III) may be transformed to stable, hydrophilic Au(0) nanoparticles through reductions by sugars and capping by proteins and peptides (Italiano et al., 2018). Additional pathways for HM detoxification may involve light-dependent or light-independent reduction, oxidation, methylation, demethylation and others (Liang et al., 2023;

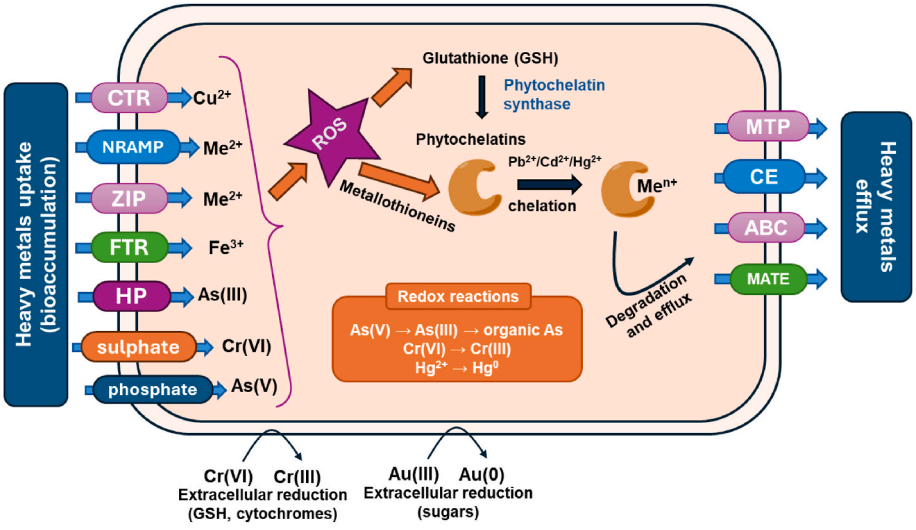

Fig. 2. Scheme of some HM bioaccumulation and biotransformation reactions in photosynthetic microorganisms. Abbreviations for HM uptake channels: Copper Transporter (CTR), Natural Resistance-Associated Macrophage Protein (NRAMP), Zinc transporter proteins and Irt-like Protein (ZIP), Iron Transporter (FTR). Abbreviations for HM efflux channels: Metal Tolerance Proteins (MTP), ATP-binding cassette (ABC), Cation Exchange (CE) and Multidrug And Toxic Compound Extrusion (MATE).

Danouche et al., 2021). For example, bioaccumulation via sunlight-driven bioreduction represents an effective and highly sustainable bioremediation mechanism for the mitigation of high-oxidation-state metal contaminants. The microbial-mediated reduction of Cr(VI) to the less toxic and less soluble Cr(III) is a common bioreduction strategy among various microbial phototrophs, including the purple non-sulphur bacterium *R. sphaeroides* (Rajyalaxmi et al., 2019). Moreover, different purple sulphur bacteria belonging to *Ectothiorhodospiraceae* family may use monothioarsenate as an electron donor to support photoautotrophic (and possibly chemoautotrophic) growth, producing arsenate (Edwardson et al., 2014). The ability of these bacteria to convert thioarsenates through anoxygenic photosynthesis adds a new dimension to the extensively studied arsenic and sulphur geochemistry and could further aid in the development of bioremediation strategies.

For bioremediation purposes, various molecular engineering attempts have been made to increase the overexpression levels of metal transporter proteins and the production of metal-binding peptides, among other strategies, with the goal of enhancing the tolerance to HMs and the bioaccumulation capacity of microorganisms (Ranjbar and Malcata, 2022; Balzano et al., 2020; Tripathi and Poluri, 2021). The cellular ability to conduct metabolism-dependent HM removal is closely linked to the effectiveness of mechanisms aimed at mitigating adverse effects induced by the contaminant. These effects include oxidative stress and consecutive lipid peroxidation, disruptions in ionic transport, enzyme inhibition, and DNA damage (Nowicka, 2022). Maintaining microorganism fitness and antioxidant response is essential for tolerating the stress induced by HMs and potentially increase the organism bioremediation capacity. Various strategies to reduce oxidative stress caused by HMs include well-known antioxidant enzymes, non-enzymatic antioxidants such as proline, ascorbic acid, and GSH, as well as the thioredoxin pool, heat shock proteins, and carotenoids. These components may be directly or indirectly involved in scavenging ROS and maintaining redox equilibrium in the cells (Ranjbar and Malcata, 2022; Nowicka, 2022). In this regard, the potential of nanostructured materials to enhance photosynthetic performance and biomass accumulation, stimulate enzymatic and non-enzymatic pathways of the antioxidant system and HM tolerance in different unicellular phototrophs is worthy to be investigated.

3. Exploring the interface: nanomaterials in HM remediation and their impact on photosynthetic microorganisms

Nanomaterials have emerged as leading-edge tools in mitigating waste products and environmental pollutants, including HMs, standing out with high efficiency and versatility. Despite the impressive variety of nanostructures employed in HM remediation, their efficacy commonly stems from shared features, including their nanoscale size, significant surface area and catalytic activity, abundance of active sites, and easily tuneable surface chemistry. One of the most significant characteristics of nanostructured materials is their substantial surface area relative to their mass. This enhanced surface area allows for a greater portion of the material to come into contact with targeted elements, thereby enhancing its reactivity. Nanoparticles exhibit quantum effects, resulting in relatively lower activation energy for feasible reactions. Thus, nanomaterials possess the ability to penetrate polluted areas inaccessible to larger particles and exhibit higher reactivity toward contaminants susceptible to redox processes. Therefore, in most of the studies dealing with environmental remediation of HMs, nanomaterials have been used as sorbent or photocatalytic agents (O'Carroll et al., 2013; Mahesh et al., 2022; Hoang et al., 2022; Wang et al., 2017; Costa et al., 2019). Among the numerous nanomaterials currently used to mitigate HM pollutants, the following section focuses solely on the most common ones. A specific emphasis was placed on exploring the interactions between various nanomaterials and photosynthetic microorganisms, aiming to assess the feasibility of using these materials to facilitate the bioremediation process.

3.1. Carbon-based nanomaterials

Carbon-based nanomaterials, such as graphene, graphene oxide (GO), fullerenes, multi-walled carbon nanotubes (MWCNTs), and single-walled carbon nanotubes (SWCNTs), are esteemed for their capability in HM removal, owing to their remarkable adsorption capacities and effectiveness in eliminating metal ions (Mahesh et al., 2022). Their distinctive electronic and structural properties, coupled with easily modifiable surface chemistry, further enhance their utility in this regard (Mahesh et al., 2022; Hoang et al., 2022). The main mechanisms that drive the mitigation of HMs by carbon-based nanomaterials (Fig. 3a) include physical adsorption, electrostatic interactions, ion exchange, surface complexation and (co)-precipitation (Duan et al., 2020). The internalization of carbon nanomaterials in microalgae has been demonstrated, however, the mechanisms involved in their cellular uptake are still not fully understood (Orlanducci et al., 2020; Rhiem et al., 2015). The cytotoxicity of carbon nanomaterials in photosynthetic microorganisms has primarily been linked to their ability to induce oxidative stress, promote cell agglomeration, impede nutrient uptake, and release metal catalyst residues (Saxena et al., 2020; Lambreva et al., 2015). This complex, concentration-dependent phenomenon is strongly influenced by factors, such as dimensions, dispersion state, functionalisation, and purity level of the material (Lambreva et al., 2015; Orlanducci et al., 2020). Despite numerous studies demonstrating the acute toxicity of these nanomaterials in various unicellular phototrophs, hormetic effects on Photosystem II (PSII) activity, photosynthetic performance, culture growth, pigment, and biomass accumulation under physiological and stress conditions have been frequently documented for SWCNTs and MWCNTs (Antal et al., 2022; Sun et al., 2020; Zhang et al., 2018a; Zamani, 2023; Liu et al., 2023), hydroxylated nanodiamonds (NDs) (Antal et al., 2023), carbon dots (Zhang et al., 2018b; Xue et al., 2020), etc. For example, intentionally engineered carbon dots were shown to enhance the growth rate and biomass accumulation in *C. vulgaris* cultures (Fig. 4a), accompanied by an increase in Rubisco activity (Zhang et al., 2018b). Carboxylated MWCNTs were able to promote the PSII activity, dry mass and chlorophyll content of microalgal-fungal consortium unlike the pristine and hydroxylated MWCNTs with similar characteristics (Liu et al., 2023). Shortening SWCNTs alleviated the growth inhibition of *Chlamydomonas reinhardtii* induced by the material (Orlanducci et al., 2020), while functionalising MWCNTs with a biocompatible coating such as Gum Arabic reversed the negative effects of nanotubes on the proliferation of *Dunaliella salina* cultures (Zamani, 2023). All these findings strongly support the notion that altering the physicochemical properties of carbon-based nanomaterials can effectively mitigate their adverse effects on the fitness of the photosynthetic microorganisms. This assumption holds considerable implications for the future advancement of nanobiotechnological applications employing carbon nanomaterials. Understanding how targeted modifications to the physicochemical properties of the material can affect its interactions with the microorganisms may enable the development of safer and more efficient nanomaterials for diverse applications, including nanobioremediation.

3.2. Metal and metal oxide nanoparticles

Nanosized metals (Ag, Au, Al, Si) and metal oxides (TiO_2, CuO, ZnO, Fe_3O_4, Fe_2O_3) are also well-suited for HMremoval, offering significant advantages, such as a large surface-to-volume ratio, tuneable size, high reactivity, controllable colloidal stability, and the possibility for easy inclusion in a supporting matrix or functional composites (Ali et al., 2023). A critical issue arises from the strong tendency of NPs to self-aggregate in solution, compromising their effectiveness in removing HMs. The incorporation of metal NPs onto porous supports, including natural materials, synthetic polymeric hosts, and activated carbon, has

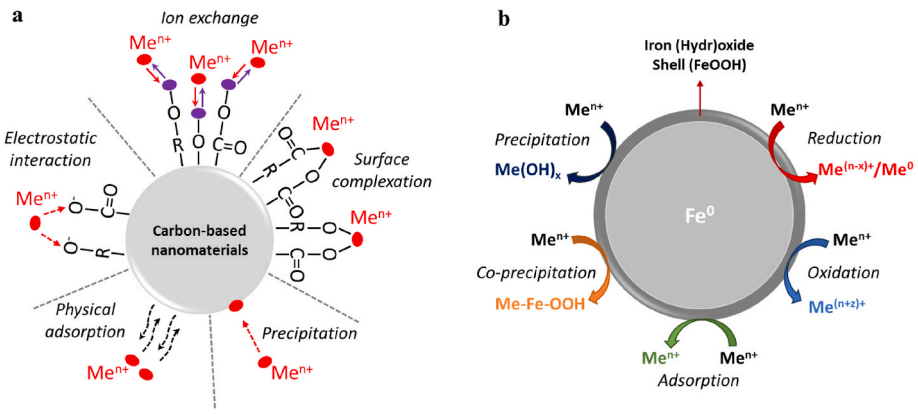

Fig. 3. Interaction mechanisms involved in the remediation of heavy metals by (a) carbon-based nanomaterials (*adapted from (Duan et al., 2020), Copyright (2020) with permission from Elsevier*) and (b) zero-valent iron (Fe^0) (*adapted from (O'Carroll et al., 2013), Copyright (2013) with permission from Elsevier*).

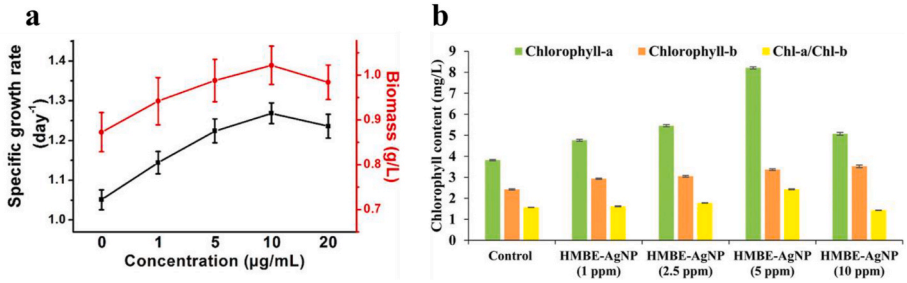

Fig. 4. (a) Enhancement of biomass accumulation and growth in *C. vulgaris* cultures induced by carbon dots (*reprinted with permission from (Zhang et al., 2018b), Copyright (2018) American Chemical Society*); (b) Increase of chlorophyll pigment content in *C. sorokiniana* UUIND6 induced by different concentrations of HMBE-Ag NPs (*from (Bisht et al., 2023) reprinted with permission from The Royal Society of Chemistry*).

been demonstrated to significantly enhance their stability (Kobielska et al., 2018). The interaction with biomass originating from dead or live cells, can also be used to stabilise the NPs in aqueous biohybrid reaction systems (Lalhmunsiama et al., 2017; Blosi et al., 2022). Metal NPs can neutralize HMs through various mechanisms, including adsorption, reduction, oxidation, and (co)-precipitation, thus reducing their toxicity by transforming the pollutants into less toxic and/or less mobile forms (O'Carroll et al., 2013; Li et al., 2024). An example of the different mechanisms involved in the removal of HMs by one of the most studied nanoscale metal particles, zero-valent iron (Fe^0) NPs, is shown in Fig. 3b. The capability of different metal NPs (Ag, Au, TiO2, CuO) to promptly overcome cellular barriers and accumulate inside cells is well-established across various microalgal species (von Moos et al., 2014). The main factors that drive the toxicity of NPs towards unicellular phototrophs include NP size, shape, charge, dispersion state, and coating (Hou et al., 2018). Adverse effects of metal NPs on growth, photosynthesis, and cellular structural integrity are primarily concentration-dependent and have been attributed to the generation of oxidative stress, cell aggregation, light shading, and metal ion release (Liang et al., 2020). Some studies suggest that cyanobacteria, such as the marine dominant *Prochlorococcus*, may be more susceptible to NP-mediated oxidative stress (Ag NPs) compared to eukaryotic autotrophs, likely due to the lack of robust cellular barriers and inadequate antioxidant system (Dedman et al., 2020).

The susceptibility to certain metal NPs can vary among different microalgal species, and some nanoscale metals could exhibit higher toxicity than their corresponding bulk counterparts, as demonstrated by Manzo et al. (2013) for ZnO NPs. However, non-toxic concentrations (here and afterward determined as *functional*) of the nanomaterial have frequently been shown to stimulate photosynthesis, the production of photosynthetic pigments, proteins, and carbohydrates, as well as growth in various unicellular phototrophs (Chen et al., 2020b; Bisht et al., 2023; Vargas-Estrada et al., 2023). This point is discussed in detail in *section 5.1*. Fig. 4b presents the increase in photosynthetic pigments in *C. sorokiniana* cultures enriched with different concentrations of HMBE (harmful macroalgal bloom extract)-Ag NPs (Bisht et al., 2023).

3.3. Dendrimers

Dendrimers are other nanoscale materials gaining strength in the environmental remediation of HMs. They are highly branched and monodispersed macromolecules composed of a central core, interior branch cells (also called generations, Gn, where *n* is the generation number) and terminal branch cells (carrying the functional groups). Their highly controlled composition and architecture provide the benefits of tuneable specificity and reactivity against the targeted contaminant, as well as solubility under specific experimental conditions. Dendrimers are effective sorption agents due to the presence of dense terminal functionality and internal cavities, and have been used in the mitigation of various metal ions (Ni^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}) (Aigbe et al., 2022; Patel et al., 2023; Rincón-Montón et al., 2023). They can be easily conjugated with different NPs to improve specificity or recovery of the material (Iannazzo et al., 2017). Dendrimer molecules can enter the cells of microalgae and cyanobacteria, potentially exhibiting hormetic effects on photosynthetic machinery (Fig. 5a), as well as inducing growth inhibition and oxidative stress at higher concentration (Petit et al., 2010; Tamayo-Belda et al., 2019; Gonzalo et al., 2015). In some studies, a higher level of toxicity has been reported for dendrimers with a higher number of generations (Gonzalo et al., 2015; Petit et al., 2010). The potential of this nanomaterial in bioremediation by photosynthetic organisms remains to be fully explored. To the best of our knowledge, this field has not been extensively studied.

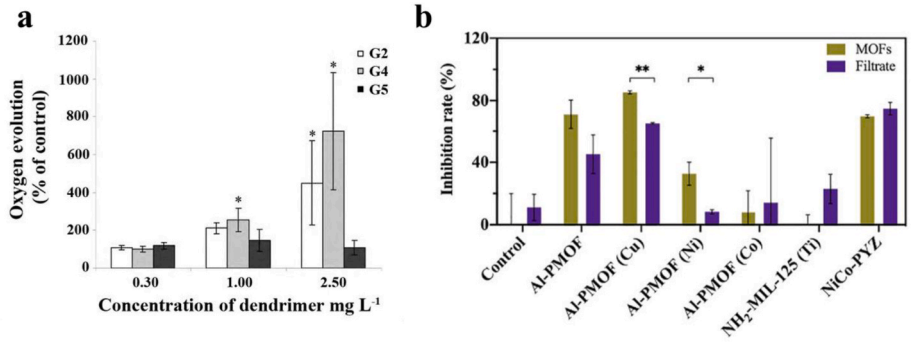

Fig. 5. (a) Hormetic effects of polyamidoamine (PAMAM) dendrimers (G2, G4 and G5) on photosynthetic activity of *Ch. reinhardtii* cultures, as indicated by the rate of O_2 evolution (*reprinted from (Petit* et al., *2010), Copyright (2010) with permission from Elsevier*); (b) Inhibition of *Ch. reinhardtii* growth due to metal ion release induced by MOFs: comparison of the effects of different MOFs (10 $mg.L^{-1}$) and their filtrates, the supernatants of algal culture medium after the removal of MOFs (*reprinted from (Li* et al., *2021), Copyright (2021) with permission from Elsevier*).

3.4. Metal-organic frameworks and nanocomposites

Metal-organic frameworks (MOFs) are emerging group of nanomaterials characterized by varying complexity, assembled by linking different metal ions or metal clusters and organic molecules (linkers) to form three-dimensional matrices. MOFs are highly effective HM adsorbents with improved selectivity due to their vast number of adsorption sites, high specific surface, and adjustable pore sizes (Kobielska et al., 2018). However, different MOFs have been characterized as highly toxic to photosynthetic microorganisms (Li et al., 2021; Johari et al., 2024) mainly due to the release of toxic metal ions from the framework (Fig. 5b). The uncontrolled release could limit the use of MOFs to bioremediation based solely on dead cells, however, this potential application is still awaiting exploration.

Nanocomposites are another class of nanomaterials used to remove HMs, achieved by embedding nanomaterials in a matrix to improve the stability, mechanical strength, and catalytic activity of the material. They usually consist of two or more distinct components with at least one dimension at the nanoscale, and often involve the incorporation of NPs or nanofillers into the matrix (Verma et al., 2024). In general, the resulting composite shows increased capacity and efficiency for HM mitigation compared to the host material, as demonstrated e.g. in studies on chitosan/graphene (Al-Salman et al., 2023) and chitosan/$CaCO_3$ (Hassan et al., 2024) nanocomposites used to neutralize Cd, Cu, Pb, Zn, Cr, or Ni ions. In the case of TiO_2 NPs, the addition of Ag ions and the loading of the NPs into chitosan nanofibres improved the photocatalytic efficiency of TiO_2 for Cr(VI) reduction (Wang et al., 2017). Different nanocomposites may exhibit different levels of toxicity to photosynthetic microorganisms, depending on material structure, composition, surface chemistry, and experimental conditions (material concentration, illumination, pH, etc.). One of the most commonly observed effects is the generation of redox-active species, leading to the induction of oxidative stress, activation of organism antioxidant enzyme system and growth inhibition. Examples include TiO_2/Ag-chitosan nanocomposites deliberately designed to induce biocidal effects in the marine alga *D. salina* (Natarajan et al., 2018), and the use of synergistic photocatalytic reactions to enhance the efficiency of the TiO_2/Ag-chitosan composites in reducing Cr(VI) by loading the nanofibre mats with *C. vulgaris* cells (Wang et al., 2017). Low concentrations of nanocomposites of metal NPs/carbon-based matrices may not inhibit algal growth and may have lower toxicity than the pure metal counterpart. This has been observed for TiO_2/MWCNT-nanofibres in the freshwater alga *Pseudokirchneriella subcapitata* (Malatjie et al., 2022) and Ag-CNT and Pt-CNT composites in *Ch. reinhardtii* cultures (Intrchom et al., 2018). Furthermore, the loading of *R. capsulatus* on Fe_3O_4/biochar nanocomposites, increased the adsorption capability of the bacteria for nutrients, such as NH_4^+ and PO_4^{3-} from wastewater (He et al., 2017b). These findings support the idea of nanocomposite suitability for HM bioremediation by both leaving and dead cells of unicellular phototrophs.

4. Case studies of nanomaterial-mediated bioremediation of HM by photosynthetic microorganisms

There are only a few examples in the literature that illustrate the functional integration of unicellular phototrophs and nanomaterials aimed at enhancing the bioremediation of HMs (Table 1). These experimental approaches demonstrated the beneficial interplay between microorganisms and the high redox activity and surface area of nanomaterial in promoting the efficiency of biotransformation and biosorption processes. The photocatalytic activity of *C. vulgaris* cells and TiO_2/Ag NPs for the removal of Cr(VI) was enhanced when they were incorporated into chitosan nanofibre (NF) mats in a combined nanobiohybrid system (algae-TiO_2/Ag NF) (Wang et al., 2017). The photocatalytic process was induced by strong visible light (>420 nm), capable of both generating electron-hole pairs in the TiO_2/Ag component and inducing oxidative stress within algal cells. The resulting ROS ($^{\bullet}OH$, $^1O_2$ and $O_2^{\bullet-}$), initiated various redox reactions ultimately resulting in the reduction of Cr(VI). Each component of this biohybrid system had a specific role in this complex process (Fig. 6a). The Ag NPs stabilised the charge separation states of the TiO_2/Ag component enhancing its photocatalytic activity against Cr(VI) and promoting the generation of hydroxyl radicals ($^{\bullet}OH$). The latter engage in redox reactions with algal organic matter, consuming both photo-excited holes and $^{\bullet}OH$, thereby stimulating the photocatalytic reduction of Cr(VI) by the NPs. Additionally, NP-mediated ROS and photo-induced oxidative stress within algal cells caused intracellular generation of ROS and organic radicals,

Table 1
Case studies of nanomaterial-enhanced bioremediation of HM by photosynthetic microorganism.

Microorganism	Nanomaterial	Targeted process	Reference
C. vulgaris	TiO_2/Ag NPs incorporated into chitosan nanofibre	Cr(VI) photoreduction	Wang et al. (2017)
C. vulgaris	Al_2O_3 hollow fibre decorated with TiO_2 NPs	Cr(VI) photoreduction	Costa et al. (2019)
C. vulgaris	Fe_3O_4 NPs	Cd^{2+}/Pb^{2+} biosorption	Lalhmunsiama et al. (2017)
Chlorella sp.	Fe_3O_4 NPs, natural magnetic clay functionalized with poly-ethilenimine	Zn^{2+} biosorption	Ferraro et al. (2018)
C. vulgaris	TiO_2 NPs	Cu^{2+} biosorption	Blosi et al. (2022)

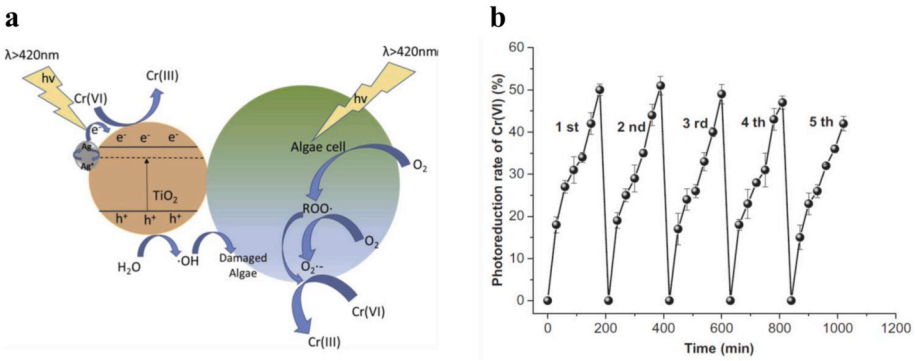

Fig. 6. (a) Proposed mechanism of Cr(VI) photoreduction by algae-TiO_2/Ag hybrid nanofibre mat. (b) algae-TiO_2/Ag NF photoreduction of Cr(VI) repeated test at initial concentration of 10.0 mg.L^{-1} and pH 4.0 (*reprinted from (Wang et al., 2017) Copyright (2017) with permission from Elsevier*).

further contributing to the reduction of Cr(VI). This biohybrid system was able to maintain high photocatalytic activity for 16 h during five consecutive cycles (Fig. 6b). Furthermore, the performance of the algae-TiO_2/Ag NF complex was enhanced by the oxidative damage to the algal cells and their subsequent death, highlighting the potential contribution of inactive biomass to the remediation of Cr(VI).

Similar concepts were exploited in the development of a nanobiohybrid system for Cr(VI) photoreduction, comprising asymmetric alumina (Al_2O_3) hollow fibre impregnated with TiO_2 NPs and externally decorated with *C. vulgaris* cells (Costa et al., 2019). The algae-TiO_2 NF composite outperformed the individual systems in reduction efficiency, and the spatial segregation of the NPs and microalgae reduced the phytotoxicity of the TiO_2, allowing for substantially increase in the operational duration of the system. This hybrid setup maintained over 90% of its efficiency for Cr(VI) reduction after 5 cycles of 12 h each. The authors illustrated the advantages of incorporating nanobiohybrid systems into supporting matrices for enhancing Cr(VI) detoxification efficiency, facilitating nanomaterial recovery, and enabling system reuse.

To facilitate the separation of algal biomass from the system and promote the reusability of the materials, thus improving the bioremediation of HMs in aqueous environments, microalgae were conjugated with magnetic nanoparticles, such as iron oxide NPs. This approach proved to be effective for the biosorption of Cd^{2+} and Pb^{2+} by the dry mass of C. vulgaris (Lalhmunsiama et al., 2017) and for the removal of Zn^{2+} ions by living cells of *Chlorella* sp. (Ferraro et al., 2018). Importantly, coating the cells with the metal NPs did not alter the efficiency of HM biosorption and allowed for high rates of metal removal in several sequential adsorption-desorption cycles (Lalhmunsiama et al., 2017).

Furthermore, it has been demonstrated that the interaction between TiO_2 NPs and *C. vulgaris* cells can enhance the biosorption capacity of algal biomass for Cu ions, both in solution and after spray-freeze drying of the TiO_2-algal nanosols into micrometric granules (Blosi et al., 2022). This experimental approach proved that the counterion interactions between the cells and NPs can increase the contact surface area of the biomass for HM ions, generating highly efficient nanobiohybrid system for Cu^{2+} sorption. It was able to accumulate up to 8650 mg $Cu^{2+}.g^{-1}$ cells after 30 min of incubation, compared to only 103 mg $Cu^{2+}.g^{-1}$ cells in *Chlorella* cells alone. Interestingly, the synergistic effect of the NPs on the microalgal biosorption capacity was observed solely when the NPs were introduced to living cells, highlighting the essential interplay between inorganic and biological components as a prerequisite for the proper functioning of the biohybrid system. Importantly, converting TiO_2-algal nanosols into dried granules could result in notable progress in nanobioremediation utilizing dead biomass, rendering it more manageable and potentially suitable for large scale applications.

5. Perspectives on nanomaterial-mediated bioremediation of HMs by photosynthetic microorganisms

Despite the few experimental approaches implemented to enhance biosorption and photocatalytic activity of photosynthetic microorganisms using nanomaterials, their potential to improve the capacity and efficiency of bioremediation processes remains largely untapped. The broader integration of nanomaterials in bioremediation efforts involving unicellular phototrophs may be driven by two key factors: firstly, the documented beneficial effects of nanomaterials on the growth, HM tolerance and photosynthetic performance of microorganisms, and secondly, the capability of nanomaterials to interact with HMs, thereby modifying their bioavailability and toxicity.

5.1. Hormetic effects of nanomaterials on photosynthetic microorganisms

The positive response to the addition of specific doses of different nanoparticles has been demonstrated for several unicellular phototrophs, as reported in Table 2.

For example, various metal/metal oxide NPs have been shown to beneficially affect the growth and biomass accumulation of photosynthetic bacteria and microalgae, with nanoscale Fe_3O_4 and Fe^0 being among the most commonly reported. In sulphur photosynthetic bacteria, iron is an essential component of the various dehydrogenase enzymes that drive the photosynthetic sulphur oxidation process, contributing to energy generation and ATP synthesis (Wu et al., 2012). Likewise, in microalgae, iron cofactor proteins are the main drivers of the biochemical processes of respiration, photosynthesis and nitrogen fixation (Behnke and LaRoche, 2020). In line with the importance of this element for cellular metabolisms, the addition of Fe^{2+} enhanced nutrient assimilation, stimulated growth, and promoted the synthesis of ATP and photosynthetic pigments in bacterial cells during wastewater treatment (Wu et al., 2012; Sun et al., 2022). It was further demonstrated that Fe^0 NPs (20 mg.L^{-1}) may be even more effective than the same concentration of Fe^{2+} in boosting the biomass production of a consortium of photosynthetic bacteria in artificial wastewater. (Chen et al., 2020b). Similarly, the enrichment of high-rate algal ponds, with carbon-coated Fe^0 NPs resulted in improved biomethane production, mainly due to the enhancement of algal photosynthetic activity and biomass accumulation (Hoyos et al., 2024). An increase in biomass and lipid content was observed also in *Scenedesmus obliquus* and *C. sorokiniana* supplemented with concentrations less than 20 mg.L^{-1} of Fe_3O_4 NPs and Fe^0 NPs, respectively (He et al., 2017a; Vargas-Estrada et al., 2023).

The mechanism by which other metal/metal oxide NPs increase the efficiency of solar-to-chemical production to stimulate the accumulation of photosynthetic pigments, lipids, or biomass in unicellular phototrophs is still not fully understood. The application of Ag NPs in *C. sorokiniana* biohybrid system and TiO_2 NPs to *Coelastrella* sp. M60

Table 2
Examples of hormetic effects induced by various nanomaterials on unicellular phototrophs. The column "targeted parameter" denotes various physiological parameters that have been positively affected at the optimal nanomaterial concentration.

Microorganism	Nanomaterial	Targeted parameter	Reference
Photosynthetic bacteria consortium	Fe^0NPs	biomass production, pigment content, dehydrogenase and succinate dehydrogenase activity	Chen et al. (2020b)
Algal-bacterial consortium	carbon coated Fe^0NPs	photosynthetic activity, bio-mass production, N_2 and P assimilation	Hoyos et al. (2024)
C. sorokiniana	carbon coated Fe^0NPs	photosynthetic activity, bio-mass production, carbohydrate and lipid content	Vargas-Estrada et al. (2023)
S. obliquus	CNTs, nano Fe_2O_3, nano MgO	lipid content	He et al. (2017a)
Rh. faecalis PA2	Fe_3O_4NPs	biomass production, protein and carotenoid content	Chaiyarat and Saejung (2022)
C. sorokiniana	HMBE-AgNPs	growth, photosynthetic pigment content	Bisht et al. (2023)
Coelastrella sp. *M60*	TiO_2NPs	biomass production, astaxan-thin and zeaxanthin content, lipid content	Pushpalatha et al. (2021)
Ch. reinhardtii	PAMAM dendrimer	O_2 evolution, electron transport efficiency, relative amount of active PSII	Petit et al. (2010)
S. obliquus	MWCNTs	growth, biomass production, chlorophyll content, electron transport efficiency	Sun et al. (2020)
C. vulgaris	MWCNTs	dry mass, chlorophyll content, photosynthetic activity	Liu et al. (2023)
Ch. reinhardtii	SWCNTs	photosynthetic activity under photoinhibitory conditions	Antal et al. (2022)
Ch. reinhardtii	hydroxylated NDs	photosynthetic activity under oxidative stress	Antal et al. (2023)
Phaeodactylum tricornutum	CuNPs	algal growth, photosynthetic pigment content	Zhu et al. (2017)
C. vulgaris	Cu/Se nanocar-boxylates	biomass production, photosyn-thetic activity	Mykhaylenko and Zolotareva (2017)
C. regularis (FACHB-729)	carbon dots	growth, content of pigments, carbohydrates, proteins, and lipids	Xue et al. (2020)
C. vulgaris	carbon dots	growth, biomass production, Rubisco activity	Zhang et al. (2018b)

cultures co-exposed to different stress conditions stimulated the algal growth and cellular content of chlorophylls and carotenoids (Bisht et al., 2023; Pushpalatha et al., 2021). These studies raised the hypothesis that functional concentrations of the NPs might trigger the metabolic processes of photosynthetic microorganisms to synthesise compounds capable of counteracting moderate stress conditions, thereby promoting the photosynthesis, antioxidant capacity, and biomass production of the organisms.

Hormetic effects of dendrimers and carbon-based nanomaterials on unicellular phototrophs have also been reported. Low-generation PAMAM dendrimers (G2 and G4) positively affected O_2 production and photosynthetic performance of *Ch. reinhardtii* cultures, most likely by interacting directly with the photosynthetic electron transport chain, increasing the relative amount of active PSII and electron transport efficiency (Petit et al., 2010). At low concentrations ($\leq$5 mg.L^{-1}), stably dispersed MWCNTs, characterized by low levels of catalytic residues, stimulated the cell proliferation and biomass accumulation of *S. obliquus* over an eight-day period. Although the mechanism of this stimulation is not fully uncovered, it has been correlated with an increase in the total chlorophyll content and photosynthetic electron transport efficiency (Sun et al., 2020). A similar increase in dry mass, chlorophylls and photosynthetic activity was observed in microalgal-fungal consortia of *C. vulgaris* and *Ganoderma lucidum* by carboxylated MWCNTs (1.5 mg.L^{-1}), which was beneficial for wastewater remediation (Liu et al., 2023). Furthermore, we have demonstrated that well dispersed SWCNTs with high purity level may protect *Ch. reinhardtii* photosynthesis under photoinhibitory conditions attenuating the loss of photosynthetic activity. The mechanism of this CNT-induced protection could include both the direct attenuation of the damaging reactions and the facilitation of recovery reactions by reducing the excitation pressure in PSII and ROS generation (Antal et al., 2022; Lambreva et al., 2023). Detonation NDs, which are known to be less toxic to microalgae than CNTs, were found to be effective in reducing cellular levels of oxidative stress, thus helping to maintain the photosynthetic activity of *Ch. reinhardtii* cells under conditions associated with oxidative stress (Antal et al., 2023). Thus, the application of functional concentrations of different NPs, dendrimers and carbon-based nanomaterials could benefit the HM bioremediation process by boosting the primary productivity of unicellular phototrophs and/or increasing their capacity to cope with oxidative stress-related conditions. The latter could be particularly relevant for bioremediation processes driven by photosynthetic microorganisms, given the established correlation between HM toxicity and their ability to induce cellular damage via ROS generation.

Another intriguing finding emerged from the interaction between the facultative anaerobic (non-photosynthetic) bacterium *Shewanella xiamenensis* and GO in a study on Cr(VI) bioremediation (Li et al., 2020). This bacterium reduces Cr(VI) through a series of extracellular electron transfer reactions catalysed by c-type cytochromes (c-Cyts). The addition of GO increased the efficiency of the bioreduction process by inducing the overexpression of genes encoding c-Cyts proteins. Interestingly, under these conditions, the c-Cyts proteins targeted the reduction of both Cr(VI) and GO, and the reduced GO synergistically enhanced the removal of Cr(VI) carried out by the bacteria. This interaction resulted in Cr(VI) removal levels of 90–95% within 6 days at an initial Cr(VI) concentration of 60 mg.L^{-1}. No analogous studies in unicellular phototrophs were found. Many photosynthetic microorganisms, being key players in the environmental cycling of HMs, possess a sophisticated machinery of enzymes and transporters for interacting with and transforming metals. For example, the cyanobacterium *Synechocystis* PCC 6803 possesses a complex network of enzymes to cope with the presence of arsenic, that are highly regulated and show differentially expression in response to the different As forms (Sure et al., 2016). Similarly, in most green algae, the plasmalemma membrane hosts specific reductase enzymes for the reduction of trace elements such as iron and copper (Merchant et al., 2020; Urzica et al., 2012). Understanding the interaction between enzymatic pathways involved in the cellular uptake, reduction, and/or extrusion of HM ions and various nanomaterials is crucial to further enhance the biological treatment of these contaminants. Furthermore, there is limited understanding of nanomaterial-induced alterations in the expression of genes associated with both primary and secondary metabolism in photosynthetic microorganisms. In this regard, the observed overexpression of genes involved in amino acid metabolism and photosynthesis in *C. pyrenoidosa* under ND-mediated stress is believed to enhance algal resilience to oxidative

stress (Zhang et al., 2021).

The approach of nanomaterial-mediated overexpression of genes involved in HM detoxification, photosynthesis, and antioxidant enzymes is worth investigating due to its potential to enhance the stability and fitness of microorganism in the bioremediation process. Another promising avenue for HM mitigation that remains largely unexplored is the addition of functional concentrations of nanomaterials to consortia of microorganisms. For example, in a hypothetical consortium involving *S. xiamenensis* and microalgae such as *Chlorella* sp., the incorporation of nanomaterials may enhance bioremediation capacity and efficiency of the consortium by increasing both bacterial bioremediation ability and microalgal photosynthesis and production of organic compounds.

5.2. Antagonistic and synergistic effects of nanomaterials on toxicity and bioavailability of HMs

The application of 100 mg.L^{-1} Fe_3O_4 NPs to cultures of the phototrophic bacterium *Rhodopseudomonas faecalis* PA2 proved to be effective in increasing its tolerance to HMs during the removal of cooking oil co-contaminated with up to 8 mM of Pb, Ni, Co, or Zn ions (Chaiyarat and Saejung, 2022). In this eight-day trail, all tested HMs inhibited the growth and development of *Rh. faecalis* PA2 cells, with Pb and Ni ions being more toxic than the Co and Zn. The presence of NPs reduced the toxicity of all HMs, resulting in an increase in biomass production, protein and carotenoid content of the samples compared to cultures exposed to HM-supplemented cooking oil without NPs. Although the concentration of "free" HM ions in the growth medium was not investigated, the authors speculated that the observed effects were due to the metal-sorbent capacity of the Fe_3O_4 NPs along with their ability to stimulate photosynthetic and metabolic pathways in the cells (Chaiyarat and Saejung, 2022). A similar reduction in HM toxicity, attributed to the presence of metal/metal oxide NPs during co-exposure, has been demonstrated under various experimental conditions across different species. The growth inhibition and oxidative stress in the green alga *S. obliquus*, induced by Cu^{2+} at concentrations up to 0.5 mg.L^{-1}, were decreased by the presence of 0.1 mg.L^{-1} Al_2O_3 NPs (Li et al., 2016). An analogous antagonism between the NPs and HMs on growth, pigment contents and oxidative stress markers has been demonstrated in the freshwater cyanobacterium *Microcystis aeruginosa* (Chen et al., 2015) and the marine planktonic microalga *Chaetoceros muelleri* (Mosleminejad et al., 2024), which were co-exposed to TiO_2 NPs/Cu^{2+} and Ag NPs/Hg^{2+} respectively. Table 3 presents a list of recent studies addressing the influence of nanomaterials on the toxicity of HMs to phototrophic microorganisms. In most cases, the antagonistic effects of non-toxic concentrations of NPs on HM toxicity were attributed to the nanomaterials' capacity to alter the bioavailability of the contaminant. This correlation was further elucidated by Yu et al. (2018), who demonstrated a clear link between the capacity of TiO_2, SiO_2, and Ag NPs to reduce the toxicity of Cd^{2+} and NP ability to "control" the accumulation of the HM ions in *Ch. reinhardtii* cells. A more comprehensive study on the combined toxicity of TiO_2 NPs and Cd^{2+} on the growth of *S. obliquus* quantified both the HM ions adsorbed on NPs and those accumulated in algal cells at different concentration ratios of these two components (Wang et al., 2021). At low TiO_2:Cd^{2+} concentration ratios, the HM-induced inhibition of algal growth was alleviated by the presence of NPs due to the decrease of free Cd^{2+} in the medium (*antagonistic combined toxicity*, Fig. 7, mode 1). By increasing the amount of TiO_2 NPs in the system, the mode of combined toxicity changed to a *partially additive/synergistic* one (Fig. 7 mode 2 and 3), resulting in greater growth inhibition accompanied by structural and oxidative damage to the algal cells.

Table 3
Nanomaterial-modulated attenuation of HM toxicity in photosynthetic microorganisms. The column "targeted HM" denotes the HMs for which attenuation of toxicity was observed.

Microorganism	Nanomaterial	Targeted HM	Reference
Rh. faecalis PA2	Fe_3O_4NPs	Pb, Ni, Co, Zn	Chaiyarat and Saejung (2022)
S. obliquus	Al_2O_3NPs	Cu^{2+}	Li et al. (2016)
M. aeruginosa	TiO_2NPs	Cu^{2+}	Chen et al. (2015)
Chaet. muelleri	AgNPs	Hg^{2+}	Mosleminejad et al. (2024)
Ch. reinhardtii	TiO_2-, SiO_2- and AgNPs	Cd^{2+}	Yu et al. (2018)
S. obliquus	TiO_2NPs	Cd^{2+}	Wang et al. (2021)
Sk. costatum	CNTs	nano-Cu	Zhang et al. (2018a)
S. obliquus	MWCNTs	Cu^{2+}, Cd^{2+}, Zn^{2+}	Sun et al. (2020)
D. salina	MWCNTs	Cd^{2+}	Zamani (2023)
Ch. reinhardtii	MWCNTs	Ag, Pt	Intrchom et al. (2018)

An important point to emphasise is that at high concentrations, the NPs could act as effective carriers of HM ions, facilitating their entry and accumulation into cells. This strong synergism was observed in *S. obliquus* at higher TiO_2 NP:Cd^{2+} ratio (114.8 mg.L^{-1} TiO_2 and 0.011 mg.L^{-1} Cd^{2+}) (Wang et al., 2021), and in the unicellular marine alga *Isochrysis galbana*, where 100 mg.L^{-1} Al_2O_3 NPs enhanced the cellular uptake of Pb ions across a wide concentration range (from 0.25 to 2.0 mg.L^{-1}) (Hu et al., 2018). Although such synergy will undoubtedly result in cellular damage, stimulating the bioaccumulation of HMs could be beneficial for the endpoint in biomass-based remediation.

Evidence of antagonistic combined toxicity between HMs and carbon-based nanomaterials has also been reported in the literature. Mainly due to their prompt interaction with HMs, the CNTs were able to mitigate the adverse effects of nano-Cu as well as Cu, Cd and Zn ions on photosynthetic activity and cell density in the marine diatom *Skeletonema costatum* (Zhang et al., 2018a) and *S. obliquus* (Sun et al., 2020), respectively. Likewise, the presence of CNTs reduced the growth inhibition and membrane damage induced by Cd in *D. salina* (Zamani, 2023) and attenuated the toxicity of Ag and Pt in *Ch. reinhardtii* cultures. In most of these studies, the nanomaterial effects were correlated with alteration of cell capacity to accumulate the HM ions, thus in the context of pollutant bioremediation this factor should be taken into careful consideration.

6. Conclusions

The rapid advancements in nanotechnology, coupled with an increasing understanding of the interactions among nanomaterials, HMs, and unicellular phototrophs, hold significant potential to further promote the development of sustainable solutions for HM mitigation. This progress can lead to cost-effective production of safer and more efficient nanomaterials suitable for nanobioremediation applications, harnessing the metabolic diversity of photosynthetic organisms. The progress in nanomaterial-mediated HM removal by photosynthetic microorganisms may further advance ongoing efforts to enhance the bioremediation process by generating and selecting strains with improved sorption, accumulation, and transformation capabilities. While certain nanoparticles have shown effectiveness in enhancing the biosorption capacity and photoreduction efficiency in HM mitigation processes, further investigation in this field remains largely unexplored. Based on the critical analysis of the interactions between nanomaterials and unicellular phototrophs, we revealed the potential of tailored nanomaterials to sustain photosynthetic metabolism, promote growth, and enhance biomass accumulation, thereby improving bioremediation capacity of these microorganisms. Furthermore, functional concentrations of nanomaterials can also stimulate the antioxidant defence system of the microorganisms, enhancing their ability to cope with oxidative stress conditions and increasing tolerance to HMs.

In this scenario, addressing the knowledge gap regarding the molecular mechanisms underlying cellular responses to nanomaterials is also important. This deeper understanding can lead the optimisation of nanomaterials to enhance microorganism resilience in HM-

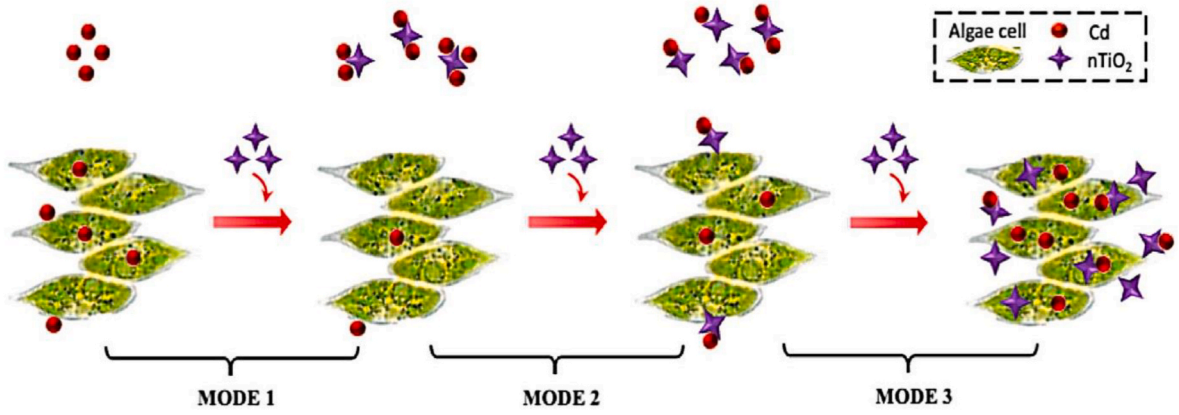

Fig. 7. The different combined toxicity modes of TiO_2 NPs and Cd^{2+} in *S. obliquus*. **Mode 1 - antagonistic effect:** at relatively low concentration ratio of TiO_2 NPs to Cd^{2+}, TiO_2 NPs compete with algae for Cd^{2+} binding, reducing Cd^{2+} entry into the cells and mitigating toxicity. **Mode 2 - partially additive effect:** at moderate concentration ratio of TiO_2 NPs to Cd^{2+}, TiO_2 NPs start inducing mechanical and/or oxidative damage of cell wall/membrane while still compete with algae for Cd^{2+} binding. **Mode 3 - synergistic effect:** at relatively high concentration ratio of TiO_2 NPs to Cd^{2+}, TiO_2 NPs not only damage cell wall/membrane structure, but also promote the entry of Cd^{2+} into the cells, enhancing toxicity *(reprinted from. (Wang et al., 2021), Copyright (2021) with permission from Elsevier).*

contaminated environments. Elucidating these mechanisms will facilitate the development of effective nanomaterial-based strategies for efficient bioremediation and environmental restoration efforts. The present study prospected key factors that could shape future research efforts toward sustainable nanobioremediation of HM, highlighting the importance of interdisciplinary approaches for the development of the next generation of bioremediation solutions using unicellular phototrophs.

Author contribution

Francesco Milano: Writing – original draft, Writing – review & editing, Visualisation; Livia Giotta: Writing – review & editing; Maya D. Lambreva: Conceptualisation, Writing – original draft, Writing – review & editing, Visualisation, Validation.

Declaration of competing interest

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

Data availability

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

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