



Microalgal Metallothioneins and Phytochelatins and Their Potential Use in Bioremediation

Sergio Balzano^{1,2*}, Angela Sardo¹, Martina Blasio¹, Tamara Bou Chahine¹, Filippo Dell'Anno¹, Clementina Sansone¹ and Christophe Brunet¹

¹ Stazione Zoologica Anton Dohrn Napoli (SZN), Naples, Italy, ² NIOZ Royal Netherlands Institute for Sea Research, Den Burg, Netherlands

OPEN ACCESS

Edited by:

Kian Mau Goh,
University of Technology, Malaysia,
Malaysia

Reviewed by:

Alejandra Moenne,
Universidad de Santiago de Chile,
Chile

Ana L. Gonçalves,
University of Porto, Portugal
Veronica Malavasi,
University of Cagliari, Italy

*Correspondence:

Sergio Balzano
sergio.balzano@szn.it

Specialty section:

This article was submitted to
Microbiotechnology,
a section of the journal
Frontiers in Microbiology

Received: 16 January 2020

Accepted: 10 March 2020

Published: 28 April 2020

Citation:

Balzano S, Sardo A, Blasio M, Chahine TB, Dell'Anno F, Sansone C and Brunet C (2020) Microalgal Metallothioneins and Phytochelatins and Their Potential Use in Bioremediation. *Front. Microbiol.* 11:517. doi: 10.3389/fmicb.2020.00517

The persistence of heavy metals (HMs) in the environment causes adverse effects to all living organisms; HMs accumulate along the food chain affecting different levels of biological organizations, from cells to tissues. HMs enter cells through transporter proteins and can bind to enzymes and nucleic acids interfering with their functioning. Strategies used by microalgae to minimize HM toxicity include the biosynthesis of metal-binding peptides that chelate metal cations inhibiting their activity. Metal-binding peptides include genetically encoded metallothioneins (MTs) and enzymatically produced phytochelatins (PCs). A number of techniques, including genetic engineering, focus on increasing the biosynthesis of MTs and PCs in microalgae. The present review reports the current knowledge on microalgal MTs and PCs and describes the state of art of their use for HM bioremediation and other putative biotechnological applications, also emphasizing on techniques aimed at increasing the cellular concentrations of MTs and PCs. In spite of the broad metabolic and chemical diversity of microalgae that are currently receiving increasing attention by biotechnological research, knowledge on MTs and PCs from these organisms is still limited to date.

Keywords: heavy metals, metallothioneins, phytochelatins, microalgal biotechnologies, phycoremediation, cysteine, glutathione, metal-binding proteins

INTRODUCTION

The ability of some microbes to thrive in heavy metal-polluted environments is attracting the interest of the biotechnological industry. Eukaryotic microalgae appear to be particularly suitable to use in bioremediation since they require only sunlight and inorganic nutrients for their growth, can achieve fast growth rates, and are able to compartmentalize heavy metals (HMs) within specific organelles. Biotechnological strategies to improve the removal of HMs from polluted waters made significant progress, although large-scale applications of microalgae-based HM-remediation are still not feasible (Kumar et al., 2015). Two main naturally occurring metal-removal processes are currently under investigation in biotechnological research: passive sorption onto algal biomass (de-Bashan and Bashan, 2010) and active sequestration and transport by specific ligands (Monteiro et al., 2011).

Eukaryotic microalgae are present in six different supergroups (Archaeplastida, Hacrobia, Rhizaria, Excavata, Alveolata, and Heterokontophyta), resulting far more genetically diverse than either terrestrial plants (Archaeplastida), or fungi and animals (Opisthokonta). This leads to a greater diversity in primary and secondary metabolites including organic ligands and metal-binding proteins for microalgae. Since most studies focus on plants,

animals, yeasts, and bacteria, a significant proportion of microalgal metabolites are unknown or uncharacterized to date. Many organic ligands and metal-binding proteins from microalgae are thus likely to be unknown, and some of them might reveal useful for phytoremediation or other biotechnological applications. The far greater genetic, enzymatic, and chemical diversity found in microalgae, compared to terrestrial plants, animals, or fungi (Keeling, 2013), coupled with the ability of microalgae to grow with sunlight and inorganic nutrients, makes photosynthetic microorganisms the best candidates for biotechnological applications and HM remediation. Furthermore, the presence of organelles in which HMs can be enclosed, limiting their detrimental effects to cellular metabolism, makes microbial eukaryotes more suitable for HM remediation compared to photosynthetic bacteria. Massive sequencing of microalgal genomes and transcriptomes (Keeling et al., 2014; Blaby-Haas and Merchant, 2019), carried out in the last decade, provided scientists with a plethora of information available on potential proteins and biosynthetic pathways involved in HM detoxification. This includes both polypeptides directly chelating HMs, such as metallothioneins (MTs) and phytochelatins (PCs), as well as compounds biosynthesized to decrease the oxidative stress induced by HMs (Cobbett and Goldsbrough, 2002).

The structure and function of both MTs and PCs in terrestrial plants, along with mechanisms for HM sequestration and compartmentalization, have been described in detail by Cobbett and Goldsbrough (2002). MTs are currently classified in 15 evolutionarily unrelated families (Capdevila and Atrian, 2011), and most information on phylogeny and structure of metal-binding domains has been elucidated in deep detail for plant MTs (Leszczyszyn et al., 2013). Structure, biosynthetic pathways, and activity regulation of PCs and their precursors, which can also be involved in HM sequestration, have been extensively reviewed (Hirata et al., 2005; Mendoza-Cozatl et al., 2005; Pal and Rai, 2010; Noctor et al., 2012; Filiz et al., 2019). The production of PCs by the different marine microalgae (Kawakami et al., 2006) as well as the role of microalgal PCs in HM detoxification mechanisms have also been elucidated (Perales-Vela et al., 2006), whereas fewer studies focused instead on microbial eukaryotes. Ziller and Fraissinet-Tachet (2018) highlighted the distribution and diversity of MTs across the three domains of life. To date, MTs have been identified in a small fraction of microbial eukaryotes, and detailed studies are available for ciliates only (Gutiérrez et al., 2009, 2015). Here, current knowledge on microalgal metal-binding proteins is described, and the state of art of the application of metal-binding proteins in biotechnological applications, with special attention devoted to phycoremediation, is discussed.

INTERACTIONS BETWEEN MICROALGAE AND HEAVY METALS

Heavy metals are defined as those elements with an atomic number greater than 20 and a density greater than 5 g cm^{-3} . They comprise 67 out of 118 chemical elements (Ali and

Khan, 2018). Iron, zinc, manganese, nickel, copper, cobalt, and molybdenum are essential for cellular metabolism and are part of the so-called metalloproteins, playing an active role in several cellular functions including electron transport and cellular protection against reactive oxygen species (Chadd et al., 1996; Twining and Baines, 2013). In addition, cadmium can also be considered as an essential metal for some microalgae, since it can replace zinc as a catalyst of carbon anhydrase, as found in *Thalassiosira weissflogii* (Lane and Morel, 2000; Xu et al., 2008).

Uptake of metals into living cells typically occurs in two steps: metal adsorption and transport across the cell membrane, with the latter step usually considered to be rate limiting (Levy et al., 2008). During the first stage, which is metabolism independent, the metal ions are adsorbed onto the cell wall through interaction with functional groups, such as polysaccharides and proteins (Das et al., 2008). After adsorption onto the cell wall, metals can penetrate the cell membrane binding to either ion carriers or low molecular weight thiols, such as cysteine, through an active transport process (Narula et al., 2015). The intracellular concentration of HMs ranges from nanomolar to femtomolar (Bruland and Lohan, 2003), and interspecies differences in metal stoichiometry can occur (Loscher, 1999; Twining et al., 2012).

High HM concentrations can inhibit the activity of biologically important molecules, such as enzymes and transport systems, inactivating their functional groups. In addition, HMs can replace essential metal ions in biomolecules and can trigger the over-production of radical oxygen species (ROS) that can, in turn, damage essential biomolecules (Jalmi et al., 2018). Indeed, all HMs are toxic at high concentrations, and their toxicity can vary even within the same taxonomic group. For example, *Phaeodactylum tricorutum* stopped growing at copper(II) concentrations $>1.5 \mu\text{M}$ (Levy et al., 2008), while another diatom, *Thalassiosira pseudonana* is able to grow at copper and cadmium concentrations as high as 3 and $10 \mu\text{M}$, respectively (Biswas and Bandyopadhyay, 2017). Some Chlorophyta appear to tolerate even higher copper concentrations, since *Tetraselmis* sp. and *Dunaliella tertiolecta* can grow with $15 \mu\text{M}$ of dissolved copper (Levy et al., 2008).

Microalgal cells need to avoid both micronutrient deficiency and micronutrient toxicity and thus need to incorporate metals from their surrounding environment at optimal concentrations. A range of mechanisms is applied to maintain optimal concentrations of HMs, such mechanisms include organic ligand formation and exudation, changes in membrane permeability, induction of stress proteins or antioxidants, release of metals into solution, or binding of metals at non-metabolically active sites (Luoma and Rainbow, 2005).

A wide range of organic ligands, also known as transporters, mediates the transport of excess HMs across the cytosol toward chloroplasts, mitochondria, and vacuoles (Cobbett and Goldsbrough, 2002; Mendoza-Cozatl et al., 2002; Aviles et al., 2003; Soldo et al., 2005; Blaby-Haas and Merchant, 2012). Several proteins, known as Group-A transporters, are present across both the plasma and vacuole membranes and allow the transport of metals from the outer environments and the vacuole, respectively, toward the cytoplasm. Group-B transporters are instead present across mitochondrial and vacuolar membranes

and are responsible for decreasing the cytoplasmic concentration of metals (Blaby-Haas and Merchant, 2012). Furthermore, microorganisms can produce other organic ligands, such as porphyrins and siderophores, that are known to chelate iron (Hutchins et al., 1999). Also, the complexation of HMs to organic ligands lowers the toxicity of HMs, and a range of ligands can be released by phytoplankton. Copper(II) supplied to cultures of *D. tertiolecta* was found to be complexed by algal exudates or adsorbed onto cell surfaces (Gonzalez-Davila et al., 1995). It has been suggested that at least 99.9% of copper in seawater is complexed by strong organic ligands and that copper toxicity in the water column would be much higher than that observed without organic complexation (Biswas and Bandyopadhyay, 2017). Organic acids can also chelate metals, as shown in higher plants. For example, Sun et al. (2011) found a positive correlation between both tartaric and malic acid and cadmium concentration within the leaves of *Rorippa globosa*. Ueno et al. (2005) identified a cadmium–malate complex within the leaves of *Thlaspi caerulescens* and suggested that such complexation mostly occur in the vacuoles. Cadmium complexation by citric, malonic, and malic acids was also suggested to occur in the cellular vacuole of *Dittrichia viscosa* (Fernández et al., 2014).

Phytochelatin and MTs are two polypeptides mostly involved in metal binding, and a crucial role is played by the sulfide groups of the cysteine residues present in these polypeptides. Metal cations exhibit indeed a chemical affinity toward the partial negative charge associated with the sulfide groups of cysteine, resulting in the formation of HM–MT and HM–PC complexes. Both MTs and PCs can, thus, scavenge HMs minimizing their detrimental effects within the cell. Peptides or amino acids that do not contain sulfide groups can also play a role in metal binding. For example, *Tetraselmis tetraele* was found to release a cysteine-free peptide (arg–arg–glu) for mercury scavenging (Satoh et al., 1999).

METALLOTHIONEINS IN THE MICROALGAL REALM

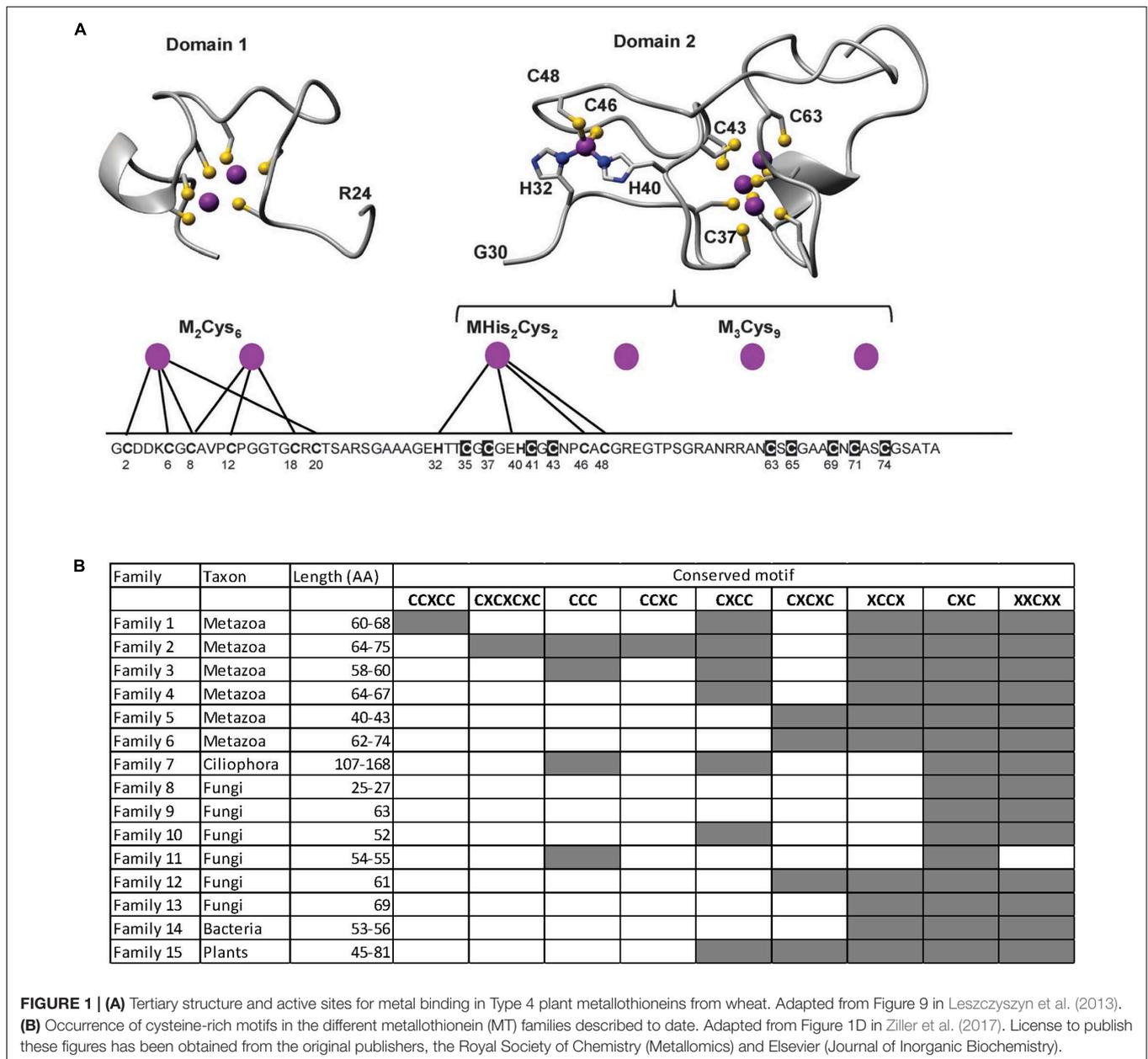
Metallothioneins are ubiquitous in living organisms and play important roles for both the supply of essential metals to the cell and the transport of toxic metals into other organelles (Capdevila and Atrian, 2011). They have also been suggested to act as free-radical scavengers, thus protecting cells from oxidative stress (Thornalley and Vasak, 1985). MTs are located in the cytosol and are typically defined as small (≤ 300 amino acids) proteins containing few ($< 10\%$) aromatic residues and a high proportion (15–35%) of cysteine (Ziller and Fraissinet-Tachet, 2018), which coordinate metal cations. The length and cysteine content of MTs vary among living organisms; for example, MTs from ciliates are longer than those from other microbial eukaryotes (Gutiérrez et al., 2011), and MTs from Metazoa typically exhibit a greater cysteine content (Ziller and Fraissinet-Tachet, 2018). MTs from plants have been characterized in more detail compared to MTs from other taxa. They have been grouped in four types, and both tertiary structure and metal-binding mechanisms have been elucidated for type 4 plant MTs (Leszczyszyn et al., 2013).

Cysteine and, to a lesser extent, histidine residues play a crucial role in metal binding (Leszczyszyn et al., 2010). Each metal cation can be coordinated by several cysteine units (Figure 1A). Type 4 plant MTs consist of a short N-terminal metal-binding domain with a cysteine-rich region and a longer C-terminal domain containing two cysteine-rich regions (Figure 1A). The secondary and tertiary structure of MTs change after HM binding (Leszczyszyn et al., 2013), and HM–MT complexes can vary in shape according to the number of bound cations (Bell and Vallee, 2009). MTs are difficult to classify based on their 3D structure because MT folding varies according to the number and type of coordinated cations.

MTs consist of at least 15 distinct families resulting from convergent evolution as evidenced by the very low sequence similarity occurring among them (Capdevila and Atrian, 2011). Each MT family includes evolutionarily related proteins from organisms belonging to the same taxonomic group. Nine different cysteine-rich conserved motifs have been found across the different MT families (Figure 1B): XXCXX, CXC, XCCX, CXCXC, CXCC, CCXC, CCC, CXCXCXC, and CCXCC. Each MT family contains one or more family-specific motifs (Ziller and Fraissinet-Tachet, 2018). To date, the majority of known eukaryotic MTs have been identified in fungi, Metazoa, plants, and ciliates, whereas very few studies focused on other eukaryotic classes (Ziller and Fraissinet-Tachet, 2018). Based on the arrangements of cysteine in cysteine-rich motifs, Capdevila and Atrian (2011) classified MTs into six families that include one family of proteins from Metazoa, six families from fungi, one from ciliates, one from bacteria, and one from plants (Figure 1B). Since MTs from different families are phylogenetically unrelated, they exhibit a huge variability in length and cysteine-rich motifs (Figure 1B). High variability in the amino acid sequence of MTs can be observed even within the same family of evolutionarily related proteins. For example, Leszczyszyn et al. (2013) reported a broad MT heterogeneity within plants (Family 15), and plant MTs were classified into four types, based on the number and position of the different cysteine-rich motifs within their primary amino acid sequence (Leszczyszyn et al., 2013). Yet, metatranscriptomic analysis of soil microbiome revealed the presence of new cysteine-rich proteins that are involved in cadmium and zinc tolerance (Lehembre et al., 2013), suggesting that the diversity of MTs in nature is much wider than that currently known with more MT families likely to occur.

Information on MTs from microbial eukaryotes different from ciliates is thus either missing or scarce (Ziller and Fraissinet-Tachet, 2018). Indeed, 5,833 eukaryotic proteins from GenBank¹ (November 2019) are annotated as MTs, and only 153 of them are associated with microbial eukaryotes for a total of 27 genera represented (Figure 2A and Table 1). Most known MTs from microbial eukaryotes are associated with ciliates (e.g., *Tetrahymena* and *Paramecium*), Apusozoa (*Thecamonas*), as well as parasitic Apicomplexa (e.g., *Babesia*, *Plasmodium*, *Theileria*) or Amebae (*Entamoeba*), and six microalgal genera (*Aureococcus*, *Chlorella*, *Nannochloropsis*, *Ostreococcus*, *Symbiodinium*, and *Thalassiosira*) include marine

¹www.ncbi.nlm.nih.gov/



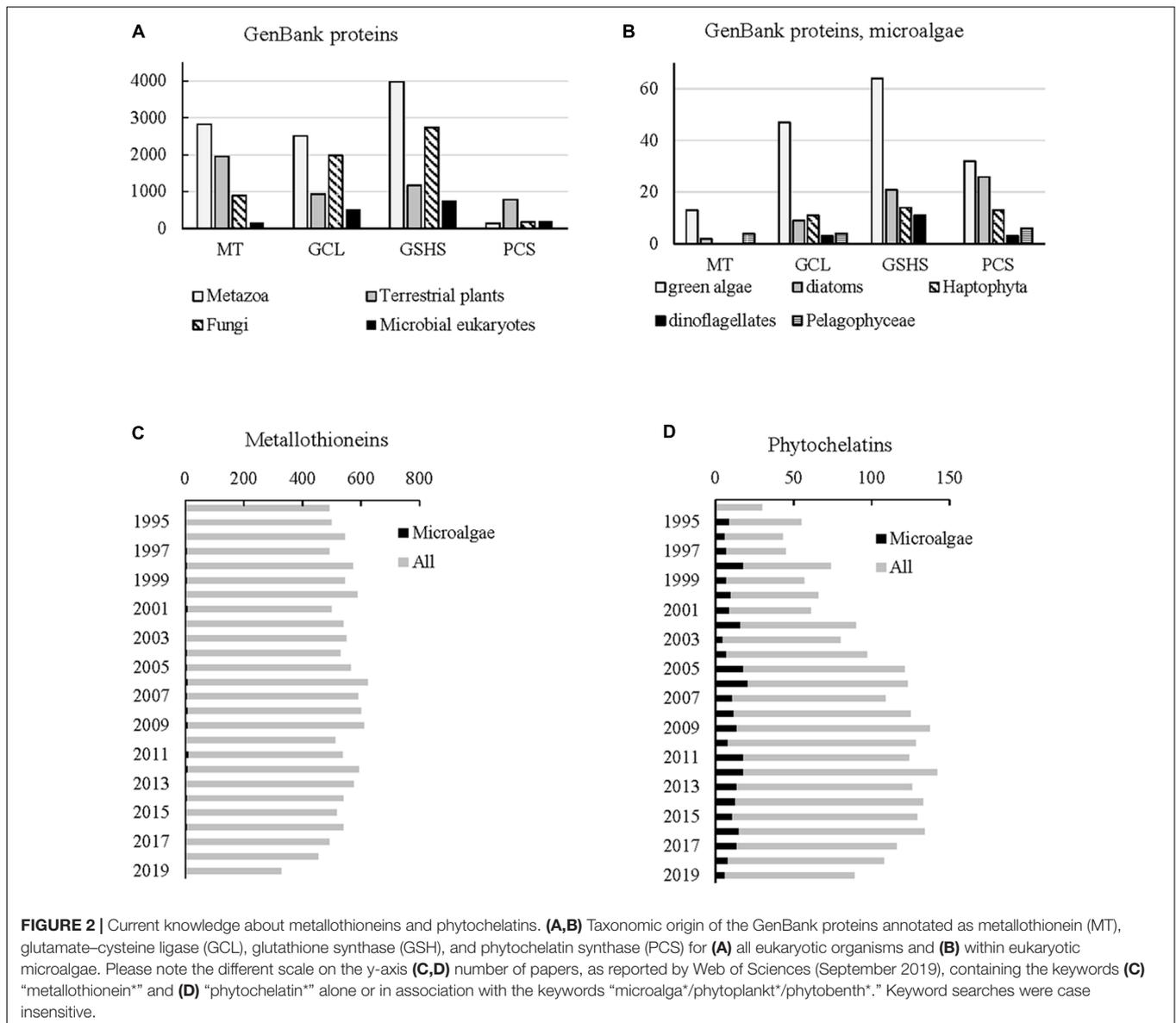
representatives (Table 1). Although diatoms, dinoflagellates, and Haptophyta account for the vast majority of marine primary production, MTs have been predicted only in *Symbiodinium microadriaticum* (dinoflagellates; GenBank accession number OLP85454) and *T. pseudonana* (diatom; XP_002296843). Similarly, 137 peer-reviewed articles on microalgal MTs were published between 1995 and September 2019², accounting for barely 1% of 13,761 MT-related articles (Figure 2C). Because of the high genetic diversity encompassed in eukaryotic microalgae, potentially reflecting a broad diversity of MTs, the scarcity of MT-related publications focusing on microalgae is surprising. Since MTs are highly diverse and some microalgae are known

to survive in HM-contaminated environments, potentially possessing novel MT forms, future research should focus on the discovery of new MTs from microalgae following both *in silico* and experimental approaches.

GLUTATHIONE AND PHYTOCHELATINS IN THE MICROALGAL REALM

Glutathione (GSH) is a tripeptide consisting of a glutamate unit bound through the carboxyl group of its side chain to the amino group of a cysteine molecule (γ -glutamylcysteine), which is, in turn, bound to a glycine unit through a peptide bond (Figure 3A). GSH is the main redox buffer in eukaryotes

²www.webofknowledge.com



and is mostly involved in the defense against oxidative (Meister and Anderson, 1983) and metal (Ahner et al., 2002) stress. *Emiliania huxleyi* cultured with up to 148 nM copper was found to release thiols, as well as GSH and cysteine, in their surrounding environment (Leal et al., 1999), suggesting a role of GSH in microalgae–metal interactions. GSH also acts as a precursor of PCs. PCs were formerly known as class III MTs and were initially identified in higher plants (Grill et al., 1985). PCs are involved in the transport of HMs from the cytosol into the vacuole in higher plants and yeasts (Cobbett and Goldsbrough, 2002). *Arabidopsis thaliana* and *Schizosaccharomyces pombe* showed an increase in sensitivity to cadmium and copper after inhibition of GSH biosynthesis (Clemens et al., 1999).

While GSH is composed by a γ -glutamylcysteine unit bound to a glycine residue, PCs consist of multiple units of

γ -glutamylcysteine bound to a terminal glycine unit (**Figure 3A**). The typical formula of PCs is $(\gamma\text{glu-cys})_n\text{-gly}$ with $2 < n < 10$ (Cobbett and Goldsbrough, 2002; Hirata et al., 2005). Cysteine residues present within different GSH units of a PC can form disulfide bonds and, similarly to MTs, PCs are able to chelate metallic cations through the thiol groups occurring in the side chain of the cysteine residues (**Figure 3B**). HM cations have been suggested to coordinate up to four sulfide groups from one or more PCs, within an HM–PC complex (Hirata et al., 2005). Apart from glycine, terminal amino acid residues, such as glutamine, glutamic acid, serine, alanine, and β -alanine, can also occur in PCs. Overall, nine different PC variants are known to date (Dennis et al., 2019).

While HMs are stably complexed by PCs in the cytosol, the lower pH present within cellular vacuoles promotes the cleavage of HM–PC complexes (Shanab et al., 2012).

TABLE 1 | Genera of microbial eukaryotes in which metallothioneins have been predicted and eventually described¹².

Supergroup	Phylum	Class	Genus	No of proteins
Alveolata	Ciliophora	Oligohymenophorea	<i>Tetrahymena</i>	65
Alveolata	Apicomplexa		<i>Babesia</i>	14
Amoebozoa	Conosa	Archamoebae	<i>Entamoeba</i>	8
Alveolata	Apicomplexa		<i>Plasmodium</i>	7
Alveolata	Apicomplexa		<i>Theileria</i>	7
Alveolata	Ciliophora	Oligohymenophorea	<i>Paramecium</i>	5
Apusozoa	Apusomonadidae	Apusomonadidae Group II	<i>Thecamonas</i>	4
Opisthokonta	Choanoflagellida	Choanoflagellata	<i>Monosiga</i>	4
Stramenopiles	Ochrophyta	Pelagophyceae	<i>Aureococcus</i>	4
Excavata	Metamonada	Parabasalia	<i>Trichomonas</i>	3
Stramenopiles		Oomycota	<i>Phytophthora</i>	3
Alveolata	Ciliophora	Oligohymenophorea	<i>Ichthyophthirius</i>	2
Alveolata	Dinoflagellata	Dinophyceae	<i>Symbiodinium</i>	2
Amoebozoa	Evosea	Eumycetozoa	<i>Cavenderia</i>	2
Excavata	Discoba	Heterolobosea	<i>Naegleria</i>	2
Opisthokonta	Choanoflagellida	Choanoflagellata	<i>Salpingoeca</i>	2
Opisthokonta	Mesomycetozoa	Filasterea	<i>Capsaspora</i>	2
Stramenopiles	Ochrophyta	Bacillariophyta	<i>Thalassiosira</i>	2
Stramenopiles	Oomycetes	Oomycetes	<i>Peronospora</i>	2
Alveolata	Ciliophora	Spirotrichea	<i>Stylonychia</i>	1
Amoebozoa	Conosa	Mycetozoa-Dictyostelea	<i>Dictyostelium</i>	1
Excavata	Discoba	Euglenozoa	<i>Trypanosoma</i>	1
Stramenopiles	incertae sedis	Opalinata	<i>Blastocystis</i>	1
Archaeplastida	Mamiellophyceae	Mamiellophyceae	<i>Ostreococcus</i>	1
Archaeplastida	Chlorophyta	Trebouxiophyceae	<i>Chlorella</i>	6
Archaeplastida	Chlorophyta	Trebouxiophyceae	<i>Micractinium</i>	2
Stramenopiles	Ochrophyta	Eustigmatophyceae	<i>Nannochloropsis</i>	1

¹Data from GenBank (November 2019). ²Phototrophic species are in bold.

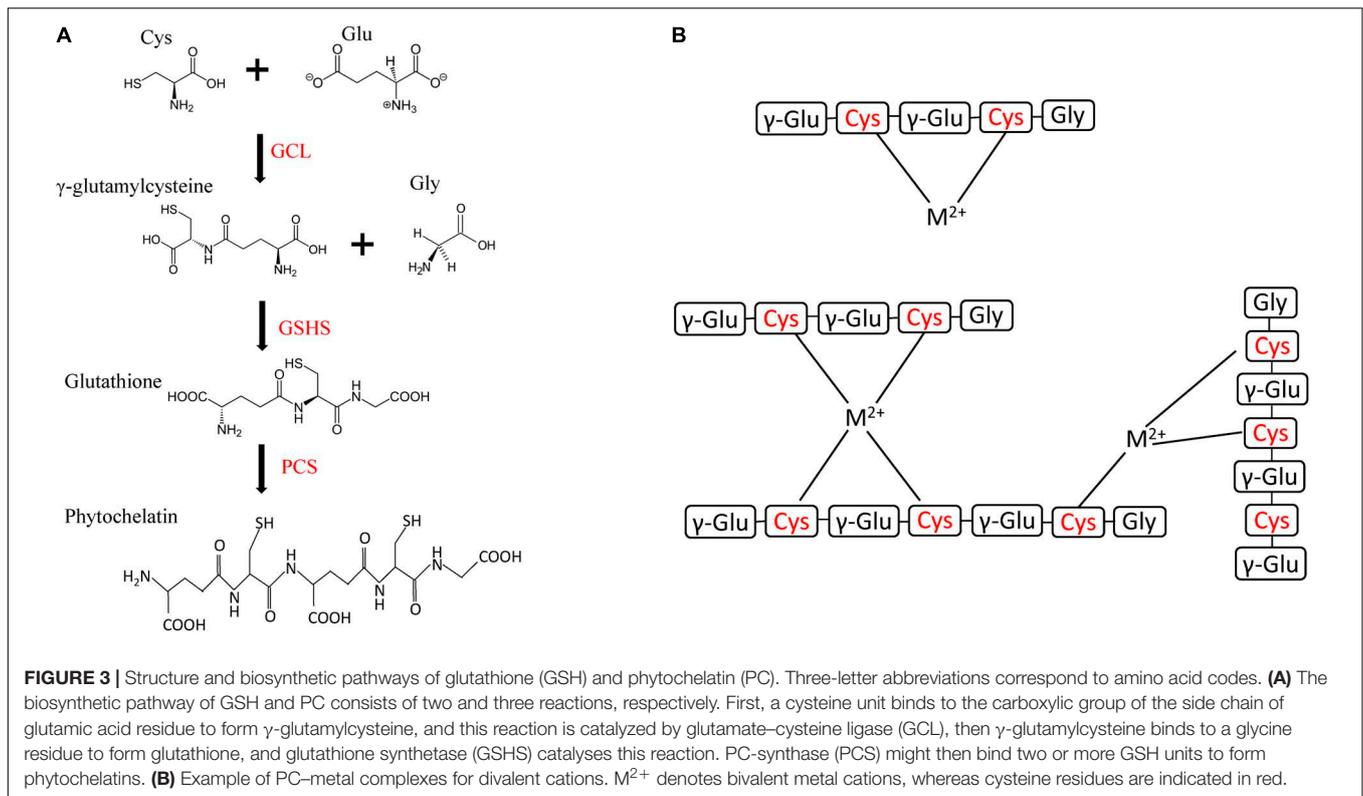
Greater concentrations of HMs in the vacuole, compared to the cytosol have been reported in plants (Sun et al., 2011), and HMs in the vacuoles are likely to be chelated by malic and citric acids (Haydon and Cobbett, 2007). *Chlamydomonas reinhardtii* was found to accumulate silver, mercury, and cadmium in the vacuole (Howe and Merchant, 1992), with cadmium found to be complexed by acetic and malic acids (Penen et al., 2017). High concentrations of HMs lead to an increase in vacuole size and numbers, as reported for Chlorophyta (Levy et al., 2008) and diatoms (Nassiri et al., 1997). In addition, HMs can also accumulate within other cellular organelles; *Euglena gracilis*, a vacuole-free species, typically accumulates HM complexes into chloroplasts (Mendoza-Cozatl et al., 2002) and mitochondria (Aviles et al., 2003).

Both GSH and PCs are enzymatically produced rather than genetically encoded (Figure 3A). GSH is biosynthesized after two subsequent biosynthetic reactions: the formation of γ -glutamylcysteine from cysteine and glutamic acid, catalyzed by glutamate-cysteine ligase (GCL), and the ligation of glutamylcysteine with glycine, catalyzed by the GSH synthetase (GSHS), to form GSH (Musgrave et al., 2013). GSH can eventually be bound to other γ -glutamylcysteine units for the biosynthesis of PCs (Grill et al., 1989;

Cobbett and Goldsbrough, 2002), and such reaction is typically catalyzed by PC synthases (PCs). It has been suggested that the two genes involved in GSH biosynthesis did not evolve together.

The genes coding for GCL likely arose in cyanobacteria and were then transferred to eukaryotes by horizontal gene transfer (Copley and Dhillon, 2002). In contrast to cyanobacterial GCLs, eukaryotic GCLs possess two additional cysteine residues forming a disulfide bridge playing a role against oxidative stress (Musgrave et al., 2013). Overall, GCL genes evolved into three distantly related groups, two of which are present in eukaryotes, Group II and Group III (Copley and Dhillon, 2002). GCL proteins from Group II are mostly present in heterotrophic eukaryotes, whereas GCLs from group III have a highly conserved ALXAXSPFXXGK motif (Musgrave et al., 2013) and are present in plants and microalgae. In contrast, it has been suggested that prokaryotic and eukaryotic GSHS evolved independently because of the high genetic divergence between each other (Musgrave et al., 2013).

Phytochelatin synthases were first identified and described in plants and are highly conserved in their N-terminal domain (Filiz et al., 2019). Genes coding PCs are also present in yeasts (Clemens et al., 1999), Metazoa (Rigouin et al., 2013), and bacteria (Hirata et al., 2005). Most PCs from plants have a plastidial origin,



since they share high similarities with cyanobacterial PCSs (Hirata et al., 2005), while species adapted to low pH environments (i.e., high HM solubility), such as *Chlamydomonas acidophila* and *Dunaliella acidophila*, imported their PCS from bacteria by horizontal gene transfer (Olsson et al., 2017).

Phytochelatin and their biosynthetic enzymes are likely to be present in all microalgae; they have been reported in different species of diatoms, dinoflagellates, Haptophyta, and Chlorophyta (Ahner et al., 1995). The vast majority of genes involved in the biosynthetic pathway of PCs (GCL, GSHT, and PCS) and available on GenBank have been identified or characterized in Metazoa, fungi, and terrestrial plants; similar to MTs, less information on GCLs, GSHTs, and PCSs from microalgae is available (Figure 2A). Known microalgal genes coding GCLs, GSHTs, and PCSs have been mostly sequenced from Chlorophyta (Figure 2B). Published literature on GSH and PCs from microalgae makes also a tiny proportion of the total GSH- and PC-related studies (Figure 2D). Because of the increasing importance of phycoremediation strategies, the development of strains that can tolerate high HM concentrations is of primary importance, and biotechnological research in the coming years is likely to focus on increasing MT and PC content of microalgae. The high chemical diversity typically encompassed in microalgae suggests that some of them might contain PC variants unknown to date. Characterizing PCs and their biosynthetic genes in microalgae might lead to the discovery of new PC variants. Furthermore, knowledge of the transcription factors for these

genes might help genetic engineering in developing new, HM-tolerant, strains.

The cellular levels of PCs increase at increasing HM concentrations, indicating that the biosynthesis of PCs is induced by metals (Grill et al., 1987; Ahner and Morel, 1995). Ahner and Morel (1995) observed that PC biosynthesis is taxa specific and metal specific. Although PC concentrations in *T. weissflogii* and *Tetraselmis maculata* were found to be the highest in the presence of cadmium compared to copper, zinc, and lead, *T. maculata* exhibited much higher levels of PC induction than *T. weissflogii* in cultures supplemented with zinc (Ahner and Morel, 1995). Moreover, PC concentrations in *E. huxleyi* were comparable in the presence of either cadmium or copper (Ahner and Morel, 1995), whereas Hirata et al. (2001) reported a higher PC induction with zinc compared to cadmium in *D. tertiolecta* (Hirata et al., 2001). Since the HM concentration required to induce GCL, GSHT, and PCS varies according to the type of metal, HMs exhibiting relatively low toxicity levels can be added to culture media to enhance the cellular abundance of PCs, thus developing phenotypes more tolerant to HMs. For example, zinc addition to microalgal cultures was proven to increase tolerance toward cadmium, as found for *E. gracilis* (Sanchez-Thomas et al., 2016) and *D. tertiolecta* (Tsuji et al., 2002). The increase in PCs in the presence of HMs can also be an indirect effect. PCs have also been shown to scavenge hydrogen peroxide and superoxide radicals, suggesting to counteract oxidative stress (Tsuji et al., 2002; Hirata et al., 2005); May and Leaver (1993) demonstrated indeed an induction of GSH by hydrogen peroxide in *A. thaliana*. Yet, the biosynthesis

of both GSH and PCs in *D. tertiolecta* might be induced by radical species generated under high zinc concentrations (Tsuji et al., 2002).

METALLOTHIONEINS AND PHYTOCHELATINS IN HEAVY METAL PHYCOREMEDIATION

Because of their high genetic, functional, and chemical diversity, as well as their ability to grow and build biomass at faster rates than terrestrial plants, microalgae are receiving increasing attention for several biotechnological applications including the production of metal-binding molecules for phycoremediation purposes.

Phycoremediation consists of the removal of HMs from contaminated waters and sediments using the ability of microalgae to incorporate metal cations from their surrounding environment. In addition to their fast growth rates and their high chemical diversity, microalgae exhibit an additional advantage because of their high surface-to-volume ratio that allows HM uptake at faster rates than larger organisms. Chlorophyta, from the genera *Chlamydomonas*, *Chlorella*, and *Scenedesmus*, are mostly used for HM remediation purposes (Table 2). Since HM uptake starts with metal adsorption onto the cell wall, and such uptake is driven by the electrochemical affinity occurring between metal cations and polar groups of cell wall polymers (Kumar et al., 2015), the use of dead microalgal biomass for HM phycoremediation is also under investigation (Kumar et al., 2015). The use of dead biomass prevents the risk of ecosystem contamination if allochthonous species are used and is particularly suitable in those highly contaminated environments in which microalgal growth is inhibited (Arica et al., 2005). However, HM uptake can be faster if living microalgae are used; in this case, passive adsorption is coupled with an active transport of HMs within the cytosol, ultimately leading to HM compartmentalization into vacuoles, chloroplasts, or mitochondria (Mendoza-Cozatl and Moreno-Sanchez, 2005; Soldo et al., 2005; Penen et al., 2017). Among the main biochemical features involved in HM uptake, the primary active transporters, metal-binding proteins, as well as enzymatically produced metal-binding peptides should be of primary interest.

The intracellular transport of HMs and their accumulation within specific organelles is thus controlled by MTs, PCs, as well as other HM-binding molecules, such as polyphosphate bodies. While HM biosorption rates can be improved by manipulating the physico-chemical conditions (temperature, pH) to which both HMs and microalgal substrates are exposed, in order to enhance HM bioaccumulation, researchers have recombinantly expressed import-storage systems, which include channels, secondary carriers, as well as primary active transporters (Diep et al., 2018). Although most of the genetic manipulations aimed to improve metal uptake have been carried out on bacteria (Deng and Jia, 2011), yeasts (Shen et al., 2012), or higher plants (Ueno et al., 2011), a few studies focused on microalgae (Cai et al., 1999; Ibuot et al.,

2017). HM-phycoremediation can be improved, in the next decade, by searching new metal-binding peptides naturally occurring in microalgae, as well as by applying genetic engineering techniques aimed at developing mutants able to tolerate high HM concentrations and to rapidly uptake and immobilize such metals.

METALLOTHIONEINS AND PHYTOCHELATINS: OTHER BIOTECHNOLOGICAL APPLICATIONS

Since MTs increase at increasing HM concentrations, biotechnological research for biomonitoring applications also focuses on MTs for developing “biosensors” to detect HM pollution. Most of these applications are based on the immobilization of MTs onto metal electrodes. HM-MT interactions are then recorded based on changes in capacitance (Corbisier et al., 1999) or potential (Adam et al., 2005). Alternatively, whole-cell based biosensors have been also developed. MTs from the ciliate *Tetrahymena thermophila* were fused with luciferase gene to develop *T. thermophila* mutants, and luciferase activity was measured in the presence of cadmium, copper, zinc, lead, arsenic, and mercury exhibiting correlation to HM concentration (Amaro et al., 2011). Overall eukaryote-based biosensors for HM detection have been developed for yeasts, microalgae, and ciliates, with the latter preferred because of the lack of cell wall in the vegetative phase of ciliates (Gutiérrez et al., 2015). HMs can indeed rapidly penetrate the cytosol of cell wall-free species, subsequently interacting with modified MTs. Cell wall-free stages are thus more suitable for the development of biosensors, including MT-based biosensors. Cell wall-free mutants of *C. reinhardtii* possessing MTs fused with yellow fluorescent proteins, have been used to monitor the intracellular accumulation of mercury, cadmium, lead, zinc, and copper (Rajamani et al., 2014).

In addition to biosensors and bioremediation, the use of MTs and PCs for metal transport can also have some applications in Biomedical Sciences. In general, MTs or PCs might be used either to supply essential metals to humans or animals, or to remove toxic HMs from contaminated tissues of living organisms. The strong affinity of MTs and PCs toward HMs, including essential metals, such as copper and zinc, suggests indeed their potential use as drug transporters in patients affected by anemia or other blood diseases, in order to increase absorption of essential metals introduced by the diet.

In the human body, various isoforms of MTs are expressed in a tissue-specific pattern and may play distinct roles in the different cell types (Wunderlich et al., 2010; Summermatter et al., 2017). The distribution of the different MT isoforms in tissues might be linked to a disease. For example, an increase in gene expression of certain MT isoforms has been reported for breast, renal, bladder, prostate, and thyroid cancer (Chung et al., 2008; Thirumoorthy et al., 2011; Gumulec et al., 2014), whereas MTs were found to be downregulated in patients affected by liver cancer (Thirumoorthy et al., 2011), indicating that the

TABLE 2 | Microalgal species mostly used in heavy metal phycoremediation.

Metal	Species	Taxon	Type of biomass	Maximum removal yield (mg g ⁻¹)	References
Arsenic	<i>Chlorella coloniales</i>	Chlorophyta	Live	8.97	Jaafari and Yaghmaeian, 2019
	<i>Scenedesmus quadricauda</i>	Chlorophyta	Live	89	Zhang et al., 2013
Cadmium	<i>Chaetoceros calcitrans</i>	Centric diatom	Live	1060	Sjarul and Arifin, 2012
	<i>Chlamydomonas reinhardtii</i>	Chlorophyta	Immobilized	79.7	Bayramoğlu et al., 2006
	<i>Chlorella sorokiniana</i>	Chlorophyta	Live	43	Yoshida et al., 2006
	<i>Chlorella sorokiniana</i>	Chlorophyta	Immobilized	192	Akhtar et al., 2003
	<i>Chlorella vulgaris</i>	Chlorophyta	Live	58.4	Aksu and Dönmez, 2006
	<i>Cladophora fracta</i>	Chlorophyta	Live	4.08	Lamaia et al., 2005
	<i>Desmodesmus pleiomorphus</i>	Chlorophyta	Live	85.3	Monteiro et al., 2010
	<i>Nannochloropsis oculata</i>	Eustigmatophyceae	Live	100.4	Zhou et al., 1998
	<i>Planothidium lanceolatum</i>	Pennate diatom	Live	275.51	Sbihi et al., 2014
	<i>Pseudochlorococcum typicum</i>	Chlorophyta	Live	5.48	Shanab et al., 2012
	<i>Scenedesmus abundans</i>	Chlorophyta	Live	574	Terry and Stone, 2002
	<i>Scenedesmus obliquus</i>	Chlorophyta	Live	175.6	Mehta and Gaur, 2005
	<i>Tetraselmis chuii</i>	Chlorophyta	Live	292.6	da Costa and de Franca, 1998
Chromium					
Cr ⁶⁺	<i>Chlamydomonas reinhardtii</i>	Chlorophyta	Live	18.2	Arica et al., 2005
	<i>Chlorella vulgaris</i>	Chlorophyta	Live	33.8	Dönmez et al., 1999; Aksu and Dönmez, 2006
	<i>Dunaliella</i> sp.1	Chlorophyta	Live	58.3	Dönmez and Aksu, 2002
	<i>Dunaliella</i> sp.2	Chlorophyta	Live	45.5	Dönmez and Aksu, 2002
	<i>Scenedesmus incrassatulus</i>	Chlorophyta	Live	4.4	Jácome-Pilco et al., 2009
Cr ³⁺	<i>Chlorella sorokiniana</i>	Chlorophyta	Immobilized	69.26	Akhtar et al., 2008
	<i>Chlorella miniata</i>	Chlorophyta	Live	41.12	Han et al., 2006
	<i>Oedogonium</i> sp.	Chlorophyta	Live	75	Bakatula et al., 2014
Cobalt	<i>Chlorella coloniales</i>	Chlorophyta	Live	9.24	Jaafari and Yaghmaeian, 2019
	<i>Oedogonium</i> sp.	Chlorophyta	Live	70	Bakatula et al., 2014
Copper	<i>Asterionella formosa</i>	Pennate diatom	Live	1.1	Tien et al., 2005
	<i>Aulacoseira varians</i>	Centric diatom	Live	2.29	Tien et al., 2005
	<i>Chlamydomonas reinhardtii</i>	Chlorophyta	Live	6.42	Macfie and Welbourn, 2000
	<i>Chlorella fusca</i>	Chlorophyta	Live	3.2	Dönmez et al., 1999
	<i>Chlorella pyrenoidosa</i>	Chlorophyta		2.4	Yan and Pan, 2002
	<i>Chlorella sorokiniana</i>	Chlorophyta	Live	46.4	Yoshida et al., 2006
	<i>Chlorella</i> spp.	Chlorophyta	Live	220	Doshi et al., 2006
	<i>Chlorella vulgaris</i>	Chlorophyta	Free	76.71	Mehta and Gaur, 2001
	<i>Desmodesmus</i> sp.	Chlorophyta	Live	33.4	Rugnini et al., 2017
	<i>Planothidium lanceolatum</i>	Pennate diatom	Live	134.32	Sbihi et al., 2014
	<i>Scenedesmus obliquus</i>	Chlorophyta	Live	1.8	Yan and Pan, 2002
	<i>Oedogonium</i> sp.	Chlorophyta	Live	75	Bakatula et al., 2014
Lead	<i>Chlamydomonas reinhardtii</i>	Chlorophyta	immobilized	380.7	Bayramoğlu et al., 2006
	<i>Chlorella vulgaris</i>	Chlorophyta	Live	17.2	Sandau et al., 1996
	<i>Oedogonium</i> sp.	Chlorophyta	dried	145	Gupta and Rastogi, 2008
	<i>Pseudochlorococcum typicum</i>	Chlorophyta	Live	4.49	Shanab et al., 2012
Mercury	<i>Chlamydomonas reinhardtii</i>	Chlorophyta	immobilized	106.6	Bayramoğlu et al., 2006
	<i>Oedogonium</i> sp.	Chlorophyta	Live	60	Bakatula et al., 2014
	<i>Pseudochlorococcum typicum</i>	Chlorophyta	Live	15.13	Shanab et al., 2012
Nickel	<i>Chlorella miniata</i>	Chlorophyta	Live	1.37	Wong et al., 2000
	<i>Chlorella</i> spp.	Chlorophyta	Live	122	Doshi et al., 2006
	<i>Chlorella</i> spp.	Chlorophyta	Immobilized	28.5	Maznah et al., 2012
	<i>Chlorella vulgaris</i>	Chlorophyta	Live	15.4	Al-Rub et al., 2004
	<i>Chlorella miniata</i>	Chlorophyta	Live	0.6	Wong et al., 2000
<i>Oedogonium</i> sp.	Chlorophyta	Live	65	Bakatula et al., 2014	
Zinc					
	<i>Chlorella vulgaris</i>	Chlorophyta	Live	9.3	Rugnini et al., 2017
	<i>Chlorella sorokiniana</i>	Chlorophyta	Live	42	Yoshida et al., 2006
	<i>Planothidium lanceolatum</i>	Pennate diatom	Live	118.66	Sbihi et al., 2014
	<i>Scenedesmus obliquus</i>	Chlorophyta	Live	836.5	Monteiro et al., 2011
	<i>Scenedesmus subspicatus</i>	Chlorophyta	Live	72.06	Schmitt et al., 2001
	<i>Oedogonium</i> sp.	Chlorophyta	Live	70	Bakatula et al., 2014

expression of MTs is cancer specific rather than universal to all human tumors. This suggests that tumor prognosis might be either directly or indirectly linked with both gene expression and isoform type of MTs. Because of this relationship between tumors and MT expression, biomedical research might focus on developing MT-based biomarkers and diagnostic tools for cancer prognosis.

Furthermore, since MTs can scavenge radical species exhibiting some antioxidant properties, cosmeceutical and nutraceutical application of microalgae enriched in MTs are also under investigation. Antioxidant properties of MTs are due to their high cysteine content that induces the formation of metal–thiolate clusters (Carpenè et al., 2007). Zhang et al. (2006) evaluated the impact of UV-B on *C. reinhardtii* and found an increased survival rate for mutants engineered with the insertion of a human MT gene compared to the wild type. MTs have been suggested to regulate cell growth and protecting the body against oxidative stress (Kumari et al., 1998) and to mediate zinc homeostasis (Krezel and Maret, 2017), being directly involved in immune system functionality.

CURRENT ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF MTs AND PCs

Despite the attention toward MTs for biotechnological purposes, the chemical and molecular determination of MTs is yet to be fully revised. The main chemical methods applied to date are mostly based on spectrophotometry and chromatography. A simple technique for MT determination is based on the quantification of thiol-rich compounds, as described by Ellman (1959). This method has been successfully applied to investigate cadmium tolerance in *P. tricornutum* (Torres et al., 1997) and *Dunaliella salina* (Folgar et al., 2009). This technique is thus based on the quantification of sulfide groups, and GSH is usually used for calibration purposes.

Metallothionein structure can be determined using ^{113}Cd - and ^1H - nuclear magnetic resonance (Duncan et al., 2008), electron paramagnetic resonance (Krepkiy et al., 2003), Raman spectroscopy (Giner et al., 2018), circular dichroism (Yang et al., 2019), Mossbauer spectroscopy (Afonso et al., 2004), and X-ray absorption near-edge structure spectroscopy (Manceau et al., 2019). Mass spectrometry (MS) can be coupled with electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) (Ryvolova et al., 2011). MS-ESI has the advantage to preserve the integrity of the molecular ions and is very effective for a precise determination of the molecular weight of the peptides, whereas MS-MALDI is typically coupled with time-of-flight instruments for unambiguous identification of the primary structure of proteins (Liu and Schey, 2005).

Inductively coupled plasma-mass spectrometry (ICP-MS) allows measuring the metal content of proteins even at very low concentrations (Ryvolova et al., 2011; Hauser-Davis et al., 2012). Chromatographic methods for MT

determination include size exclusion chromatography (Wolf et al., 2009), ion exchange (Infante et al., 2006), and two-dimensional high-performance liquid chromatography (HPLC) coupled with ICP-MS (Miyayama et al., 2007). Resonance light scattering (RLS) was applied using Eosin Y as a dye (Xiao et al., 2015) or phenantroline-Cu chelate as a probe (Xiao et al., 2014) and revealed a cost effective and rapid technique for the determination of MTs at nanomolar levels.

Metallothioneins can also be determined using immunoassays, Western blot, and mRNA sequencing. The enzyme-linked immunosorbent assays (ELISA) is the most common immunochemical method to detect MTs, and is used in both humans and experimental animals (Saito et al., 2013; Nakazato et al., 2014). Sequencing of mRNA has been used to determine the basal expression (Kim et al., 2002) of MTs or variations in their expression levels after exposure to metals (Wu et al., 2008) or for medical purposes (Nagamine et al., 2005). Western blot and mRNA sequencing were also applied for pharmacological (Grzegorzolka et al., 2019) or cytotoxic assays (Li et al., 2019).

Similarly to MTs, PC concentration in cells can also be measured using HPLC-based techniques. The molecular mass of PCs can be predicted from the number of GSH monomers, as well as the presence and the nature of bound metals. A database that includes the molecular masses of all detected and predicted PCs in plants has been recently published (Dennis et al., 2019). Since both MTs and PCs contain cysteine residues, many analytical techniques used for the determination of MTs can also be applied to detect and quantify PCs within biomass. GSH and PCs are typically quantified using HPLC based on postcolumn reaction with Ellman's reagent (Tukendorf and Rauser, 1990). This technique was applied to quantify the PC content in *Chlorella vulgaris* (Bajguz, 2011). The concentrations of cysteine, GSH, and PCs can also be determined simultaneously using HPLC with electrochemical detection (Potesil et al., 2005) or gradient elution (Wu et al., 2016).

CHALLENGES FOR GENETIC ENGINEERING

Genetic engineering techniques aimed at increasing the cellular concentrations of metal-binding proteins have been mostly applied to bacteria, yeasts, and terrestrial plants. Cellular uptake of HMs from the surrounding environment can be improved by increasing the cellular concentrations of primary active transporters. Modified bacteria (*Lactobacillus plantarum*) and plants (*T. caerulea*) exhibiting increased cellular proportions of primary active transporters were indeed found to uptake larger amounts of cadmium compared to the wild types (Deng et al., 2007; Ueno et al., 2011). Similarly, increased copper uptake rates were found in engineered *Enterobacter hirae* (Zagorski and Wilson, 2004). Since polyphosphate bodies are also known to bind metals

(Wang and Dei, 2006), overexpression of polyphosphate kinases has been applied to improve mercury uptake (Kiyono et al., 2003).

The microalgal species most widely used for genetic engineering is the model Chlorophyta *C. reinhardtii* (Leon-Banares et al., 2004). The first application of genetic engineering aimed at improving HM uptake dates back to 20 years and focused on the expression of exogenous MTs in *C. reinhardtii* (Cai et al., 1999); the resulting transgenic strain exhibited a higher growth rate in the presence of 40 μM cadmium, compared to the wild type. MT genes from *Festuca rubra* (higher plant) were cloned into the plastidial genome of *C. reinhardtii* resulting in a mutant capable of growing in the presence of cadmium concentrations as high as 80 μM (Han et al., 2008).

However, it has been shown that overexpression of MTs may not be sufficient to improve the metal uptake, since high abundances of these proteins may trigger the aggregation of misfolded forms, leading to the formation of inefficient or unstable proteins and resulting in lower metal uptake rates (Diep et al., 2018). In contrast, the overexpression of MT fusion proteins, resulting from the combination of MTs and soluble proteins, can lead to increased metal uptake rates. Chicken MTs were fused with yellow fluorescent proteins and expressed in *C. reinhardtii* to monitor the intracellular accumulation of different metals (Rajamani et al., 2014); the engineered *C. reinhardtii* revealed highly efficient as a biosensor for mercury, cadmium, lead, and, to a lesser extent, zinc and copper.

Other applications of MT fusion proteins were carried out using bacteria, yeasts, or plants as expression hosts. For example, the fusion of MT genes from *Corynebacterium glutamicum* with histidine-tagged proteins, and the cloning of the resulting MT fusion protein into *Escherichia coli*, led to increased accumulation of lead, zinc, and cadmium compared to the wild type (Jafarian and Ghaffari, 2017). Two common MT-based fusion proteins, used in genetic engineering to improve metal bioaccumulation, result from the fusion of MTs with either green fluorescence proteins or glutathione-S-transferase (GST) (Diep et al., 2018). Ruta et al. (2017) fused 11 plant MTs with myristoylated green fluorescent proteins in order to target the fusion product toward the inner face of the cell membrane in *Saccharomyces cerevisiae*. Recombinant yeasts were then found to accumulate greater amounts of cobalt, copper, zinc, and cadmium compared to the wild type (Ruta et al., 2017). Green fluorescent proteins have also been fused with human MTs, and the resulting fusion gene cloned in *S. cerevisiae* led to an increase in copper accumulation (Geva et al., 2016). MTs from *Oryza sativa* were fused with GST, and they were expressed in *E. coli*; the recombinant bacteria were found to accumulate greater amounts of mercury (Shahpiri and Mohammadzadeh, 2018). GST-MTs developed from *Pisum sativum* were cloned into the photosynthetic bacterium *Rhodospseudomonas palustris*, and the recombinant *R. palustris* was found to be more tolerant to mercury (Deng and Jia, 2011). Kim et al. (2005) cloned GST-MT into *E. coli* and showed increased uptake of cadmium from the recombinant bacterium compared to

the wild type. *E. coli* mutants containing human MT also exhibited higher cadmium and arsenic uptakes compared to the wild type (Jahnke et al., 2011). Human MTs have also been cloned into the genome of *Mesorhizobium huakuii*, a bacterial symbiont of leguminous roots, to improve cadmium uptake (Sriprang et al., 2002). Ruiz et al. (2011) cloned MTs from a mouse into *E. coli* for mercury remediation purposes. The HM uptake rates of engineered strains might also vary according to the intracellular localization of MTs. For example, engineered *E. coli* with MTs expressed in the periplasmic compartment exhibited a higher cadmium biosorption efficiency compared to the recombinant strains, in which MTs had been targeted toward the cytosol (Kao et al., 2006). In contrast, Sauge-Merle et al. (2012) found greater bioaccumulation rates in *E. coli* overexpressing MT fusion proteins in the periplasm only for copper, whereas cadmium, arsenic, mercury, and zinc were better adsorbed by recombinant *E. coli* with MT fusion proteins targeted toward the cytosol. A recent study expressed MT fusion proteins in *E. coli* and extracted the resulting proteins to make a biosorbent used for the removal of lead and zinc (Mwandira et al., 2020).

Genetic engineering also focused on enzymatically produced peptides, such as PCs. Overexpression of GCL, GSHS, and PCS enzymes has also been applied to increase the cellular concentration of PCs (Diep et al., 2018). For example, Olsson et al. (2017) increased cadmium tolerance in *E. coli* by cloning and expressing a PCS from *C. acidophila*. However, the intracellular environment is typically more reduced than the outer environment in both prokaryotic (Bessette et al., 1999) and eukaryotic (Go and Jones, 2008) cells, thus affecting the formation of disulfide bridges within MTs. Also, the overexpression of both MTs and PCs in cells increases the demand for cysteine biosynthesis negatively impacting the growth.

Genetic engineering techniques thus contributed in developing microbial mutants capable of greater HM uptake from the environment, especially through the enhanced expression of MTs. However, despite the great potential of eukaryotic microalgae for HM phytoremediation, the number of microalgal mutant developed for this purpose is still limited. This is likely due to the difficulties in genetically modifying eukaryotic cells compared to prokaryotes, and to the little information available on MTs from microbial eukaryotes. Future progresses in genetic engineering should facilitate the development of eukaryotic mutants. Sequencing new genomes and metagenomes from polluted environments and bioinformatic mining of pre-existing databases might help in finding novel microalgal MTs.

AUTHOR CONTRIBUTIONS

AS gave her contribution on analytical techniques for the determination of metallothioneins and phytochelatins. AS and TC investigated current strategies for heavy metal

phycoremediation. MB reviewed genetic engineering techniques to improve heavy metal uptake from microalgae. FD and CS highlighted potential applications of metallothioneins in pharmaceuticals and nutraceuticals as well as in biomedical sciences. SB and CB conceived the manuscript, which was drafted by SB and critically reviewed and approved by all the co-authors.

REFERENCES

- Adam, V., Petrlova, J., Potesil, D., Zehnalek, J., Sures, B., Trnkova, L., et al. (2005). Study of metallothionein modified electrode surface behavior in the presence of heavy metal ions-biosensor. *Electroanalysis* 17, 1649–1657. doi: 10.1002/elan.200403264
- Afonso, C., Hathout, Y., and Fenselau, C. (2004). Evidence for zinc ion sharing in metallothionein dimers provided by collision-induced dissociation. *Int. J. Mass Spectrom.* 231, 207–211. doi: 10.1016/j.ijms.2003.10.007
- Ahluwalia, S. S., and Goyal, D. (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresour. Technol.* 98, 2243–2257. doi: 10.1016/j.biortech.2005.12.006
- Ahner, B. A., Kong, S., and Morel, F. M. M. (1995). Phytochelatin production in marine algae. 1. An interspecific comparison. *Limnol. Oceanogr.* 40, 649–657. doi: 10.4319/lo.1995.40.4.0649
- Ahner, B. A., and Morel, F. M. M. (1995). Phytochelatin production in marine algae. 2. Induction by various metals. *Limnol. Oceanogr.* 40, 658–665. doi: 10.4319/lo.1995.40.4.0658
- Ahner, B. A., Wei, L. P., Oleson, J. R., and Ogura, N. (2002). Glutathione and other low molecular weight thiols in marine phytoplankton under metal stress. *Mar. Ecol. Progr. Ser.* 232, 93–103. doi: 10.3354/meps232093
- Akhtar, N., Iqbal, J., and Iqbal, M. (2003). Microalgal-luffa sponge immobilized disc: a new efficient biosorbent for the removal of Ni(II) from aqueous solution. *Lett. Appl. Microbiol.* 37, 149–153. doi: 10.1046/j.1472-765x.2003.01366.x
- Akhtar, N., Iqbal, M., Zafar, S. I., and Iqbal, J. (2008). Biosorption characteristics of unicellular green alga *Chlorella sorokiniana* immobilized in loofa sponge for removal of Cr(III). *J. Environ. Sci.* 20, 231–239. doi: 10.1016/s1001-0742(08)60036-4
- Aksu, Z., and Dönmez, G. (2006). Binary biosorption of cadmium(II) and nickel(II) onto dried *Chlorella vulgaris*: Co-ion effect on mono-component isotherm parameters. *Process Biochem.* 41, 860–868. doi: 10.1016/j.procbio.2005.10.025
- Ali, H., and Khan, E. (2018). What are heavy metals? Long-standing controversy over the scientific use of the term 'heavy metals' – proposal of a comprehensive definition. *Toxicol. Environ. Chem.* 100, 6–19. doi: 10.1080/02772248.2017.1413652
- Al-Rub, F. A. A., El-Naas, M. H., Benyahia, F., and Ashour, I. (2004). Biosorption of nickel on blank alginate beads, free and immobilized algal cells. *Process Biochem.* 39, 1767–1773. doi: 10.1016/j.procbio.2003.08.002
- Amaro, F., Turkewitz, A. P., Martin-Gonzalez, A., and Gutierrez, J. C. (2011). Whole-cell biosensors for detection of heavy metal ions in environmental samples based on metallothionein promoters from *Tetrahymena thermophila*. *Microb. Biotechnol.* 4, 513–522. doi: 10.1111/j.1751-7915.2011.00252.x
- Arica, M. Y., Tüzün, I., Yalçın, E., Ince, Ö., and Bayramoğlu, G. (2005). Utilisation of native, heat and acid-treated microalgae *Chlamydomonas reinhardtii* preparations for biosorption of Cr(VI) ions. *Process Biochem.* 40, 2351–2358. doi: 10.1016/j.procbio.2004.09.008
- Aviles, C., Loza-Tavera, H., Terry, N., and Moreno-Sanchez, R. (2003). Mercury pretreatment selects an enhanced cadmium-accumulating phenotype in *Euglena gracilis*. *Arch. Microbiol.* 180, 1–10. doi: 10.1007/s00203-003-0547-2
- Bajuz, A. (2011). Suppression of *Chlorella vulgaris* growth by cadmium, lead, and copper stress and its restoration by Endogenous Brassinolide. *Arch. Environ. Contam. Toxicol.* 60, 406–416. doi: 10.1007/s00244-010-9551-0
- Bakatula, E. N., Cukrowska, E. M. I., Weiersbye, I. M., Mihaly-Cozmuta, L., Peter, A., and Tutu, H. (2014). Biosorption of trace elements from aqueous systems in gold mining sites by the filamentous green algae (*Oedogonium* sp.). *J. Geochem. Explor.* 144, 492–503. doi: 10.1016/j.gexplo.2014.02.017
- Bayramoğlu, G., Tuzun, I., Celik, G., Yilmaz, M., and Arica, M. Y. (2006). Biosorption of mercury(II), cadmium(II) and lead(II) ions from aqueous system by microalgae *Chlamydomonas reinhardtii* immobilized in alginate beads. *Int. J. Miner. Process.* 81, 35–43. doi: 10.1016/j.minpro.2006.06.002
- Bell, S. G., and Vallee, B. L. (2009). The metallothionein/thionein system: an oxidoreductive metabolic zinc link. *ChemBiochem* 10, 55–62. doi: 10.1002/cbic.200800511
- Besette, P. H., Åslund, F., Beckwith, J., and Georgiou, G. (1999). Efficient folding of proteins with multiple disulfide bonds in the *Escherichia coli* cytoplasm. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13703–13708. doi: 10.1073/pnas.96.24.13703
- Biswas, H., and Bandyopadhyay, D. (2017). Physiological responses of coastal phytoplankton (Visakhapatnam, SW Bay of Bengal, India) to experimental copper addition. *Mar. Environ. Res.* 131, 19–31. doi: 10.1016/j.marenvres.2017.09.008
- Blaby-Haas, C. E., and Merchant, S. S. (2012). The ins and outs of algal metal transport. *Biochim. Biophys. Acta Mol. Cell Res.* 1823, 1531–1552. doi: 10.1016/j.bbamcr.2012.04.010
- Blaby-Haas, C. E., and Merchant, S. S. (2019). Comparative and functional Algal Genomics. *Annu. Rev. Plant Biol.* 70, 605–638. doi: 10.1146/annurev-arplant-050718-095841
- Bruland, K., and Lohan, C. M. (2003). “Controls of trace metals in seawater,” in *Treatise Geochemistry*, eds H. D. Holland and K. K. Turekian (Amsterdam: Elsevier), 23–47. doi: 10.1016/b0-08-043751-6/06105-3
- Cai, X., Brown, H. C., Adhiya, J., Traina, S. J., and Sayre, R. T. (1999). Growth and heavy metal binding properties of transgenic chlamydomonas expressing a foreign Metallothionein gene. *Int. J. Phytoremediation* 1, 53–65. doi: 10.1007/s11427-008-0136-3
- Capdevila, M., and Atrian, S. (2011). Metallothionein protein evolution: a miniassay. *J. Biol. Inorg. Chem.* 16, 977–989. doi: 10.1007/s00775-011-0798-3
- Carpenè, E., Andreani, G., and Isam, G. (2007). Metallothionein functions and structural characteristics. *J. Trace Elem. Med. Biol.* 21, 35–39. doi: 10.1016/j.jtemb.2007.09.011
- Chadd, H., Newman, E. J., Mann, N. H., and Carr, N. G. (1996). Identification of iron superoxide dismutase and a copper/zinc superoxide dismutase enzyme activity within the marine cyanobacterium *Synechococcus* sp WH 7803. *FEMS Microbiol. Lett.* 138, 161–165. doi: 10.1111/j.1574-6968.1996.tb08150.x
- Chung, R., Penkowa, S. M., Dittmann, J., King, C. E., Bartlett, C., and Asmussen, J. W. (2008). Redefining the role of metallothionein within the injured brain – extracellular metallothioneins play an important role in the astrocyte-neuron response to injury. *J. Biol. Chem.* 283, 15349–15358. doi: 10.1074/jbc.M708446200
- Clemens, S., Kim, E. J., Neumann, D., and Schroeder, J. I. (1999). Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. *EMBO J.* 18, 3325–3333. doi: 10.1093/emboj/18.12.3325
- Cobbett, C., and Goldsbrough, P. (2002). Phytochelatin and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.* 53, 159–182. doi: 10.1146/annurev.arplant.53.100301.135154
- Copley, S. D., and Dhillon, J. K. (2002). Lateral gene transfer and parallel evolution in the history of glutathione biosynthesis genes. *Genome Biol.* 3:research0025.1–research0025.16.
- Corbisier, P., van der Lelie, D., Borremans, B., Provoost, A., de Lorenzo, V., and Brown, N. L. (1999). Whole cell- and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples. *Anal. Chim. Acta* 387, 235–244. doi: 10.1016/s0003-2670(98)00725-9
- da Costa, A. C. A., and de Franca, F. P. (1998). The behaviour of the microalgae *Tetraselmis chuii* in cadmium-contaminated solutions. *Aquacult. Int.* 6, 57–66.
- Das, N., Vimala, R., and Karthika, P. (2008). Biosorption of heavy metals – an overview. *Indian J. Biotechnol.* 7, 159–169.
- de-Bashan, L. E., and Bashan, Y. (2010). Immobilized microalgae for removing pollutants: review of practical aspects. *Bioresour. Technol.* 101, 1611–1627. doi: 10.1016/j.biortech.2009.09.043

FUNDING

This work was carried out by temporary and permanent staff of the SZN and publication fees have been paid using SZN research fundings from SB and AS. MB is a Ph.D. student with a doctoral fellowship funded by SZN.

- Deng, X., and Jia, P. (2011). Construction and characterization of a photosynthetic bacterium genetically engineered for Hg²⁺ uptake. *Bioresour. Technol.* 102, 3083–3088. doi: 10.1016/j.biortech.2010.10.051
- Deng, X., Yi, X. E., and Liu, G. (2007). Cadmium removal from aqueous solution by gene-modified *Escherichia coli* JM109. *J. Hazard. Mater.* 139, 340–344. doi: 10.1016/j.jhazmat.2006.06.043
- Dennis, K., Uppal, K. K., Liu, K. H., Ma, C. Y., Liang, B., Go, Y. M., et al. (2019). Phytochelatin database: a resource for phytochelatin complexes of nutritional and environmental metals. *Database J. Biol. Databases Curation* 2019:baz083. doi: 10.1093/database/baz083
- Diep, P., Mahadevan, R., and Yakunin, A. F. (2018). Heavy metal removal by bioaccumulation using genetically engineered microorganisms. *Front. Bioeng. Biotechnol.* 6:157. doi: 10.3389/fbioe.2018.00157
- Dönmez, G., and Aksu, Z. (2002). Removal of chromium(VI) from saline wastewaters by *Dunaliella* species. *Process Biochem.* 38, 751–762. doi: 10.1016/s0032-9592(02)00204-2
- Dönmez, G. C., Aksu, Z., Öztürk, A., and Kutsal, T. (1999). A comparative study on heavy metal biosorption characteristics of some algae. *Process Biochem.* 34, 885–892. doi: 10.1016/s0032-9592(99)00005-9
- Doshi, H., Ray, A., Kothari, L. I., and Gami, B. (2006). Spectroscopic and scanning electron microscopy studies of bioaccumulation of pollutants by algae. *Curr. Microbiol.* 53, 148–157. doi: 10.1007/s00284-005-0401-7
- Duncan, K. E. R., Kirby, C. W., and Stillman, M. J. (2008). Metal exchange in metallothioneins - a novel structurally significant Cd-5 species in the alpha domain of human metallothionein 1a. *FEBS J.* 275, 2227–2239. doi: 10.1111/j.1742-4658.2008.06375.x
- Ellman, G. L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82, 70–77. doi: 10.1016/0003-9861(59)90090-6
- Fernández, R., Fernandez-Fuego, D., Bertrand, A., and Gonzalez, A. (2014). Strategies for Cd accumulation in *Dittrichia viscosa* (L.) Greuter: role of the cell wall, non-protein thiols and organic acids. *Plant Physiol. Biochem.* 78, 63–70. doi: 10.1016/j.plaphy.2014.02.021
- Filiz, E., Saracoglu, I. A., Ozyigit, I. I. II, and Yalcin, B. (2019). Comparative analyses of phytochelatin synthase (PCS) genes in higher plants. *Biotechnol. Biotechnol. Equip.* 33, 178–194. doi: 10.1080/13102818.2018.1559096
- Folgar, S., Torres, E., Perez-Rama, M., Cid, A., Herrero, C., and Abalde, J. (2009). *Dunaliella salina* as marine microalga highly tolerant to but a poor remover of cadmium. *J. Hazard. Mater.* 165, 486–493. doi: 10.1016/j.jhazmat.2008.10.010
- Geva, P., Kahta, R., Nakonechny, F., Aronov, S., and Nisnevitch, M. (2016). Increased copper bioremediation ability of new transgenic and adapted *Saccharomyces cerevisiae* strains. *Environ. Sci. Pollut. Res.* 23, 19613–19625. doi: 10.1007/s11356-016-7157-4
- Giner, M. T., Jimenez-Marti, E., Arasa, R. B., Tinti, A., Di Foggia, M., Chatgililoglu, C., et al. (2018). Analysis of the soybean metallothionein system under free radical stress: protein modification connected to lipid membrane damage. *Metallomics* 10, 1792–1804. doi: 10.1039/c8mt00164b
- Go, Y.-M., and Jones, D. P. (2008). Redox compartmentalization in eukaryotic cells. *Biochim. Biophys. Acta* 1780, 1273–1290. doi: 10.1016/j.bbagen.2008.01.011
- Gonzalez-Davila, M., Santanacasio, J. M., Perezpena, J., and Millero, F. J. (1995). Binding of Cu(II) to the surface and exudates of the alga *Dunaliella tertiolecta* in seawater. *Environ. Sci. Technol.* 29, 289–301. doi: 10.1021/es00002a004
- Grill, E., Löffler, S., Winnacker, E. L., and Zenk, M. H. (1989). Phytochelatin, the heavy metal binding peptides of plants, are synthesized from glutathione by a specific gamma-glutamylcysteine depeptidyl transpeptidase (Phytochelatin synthase). *Proc. Natl. Acad. Sci. U.S.A.* 86, 6838–6842. doi: 10.1073/pnas.86.18.6838
- Grill, E., Winnacker, E. L., and Zenk, M. H. (1985). Phytochelatin. The principal heavy metal complexing peptides of higher plants. *Science* 230, 674–676. doi: 10.1126/science.230.4726.674
- Grill, E., Winnacker, E. L., and Zenk, M. H. (1987). Phytochelatin, a class of heavy metal binding peptides from plants, are functionally analogous to metallothioneins. *Proc. Natl. Acad. Sci. U.S.A.* 84, 439–443. doi: 10.1073/pnas.84.2.439
- Grzegorzolka, J., Gomulkiewicz, A., Olbromski, M., Glatzel-Plucinska, N., and Piotrowska, A. (2019). Expression of tesmin (MTL5) in non-small cell lung cancer: a preliminary study. *Oncol. Rep.* 42, 253–262. doi: 10.3892/or.2019.7145
- Gumulec, M., Raudenska, J., Adam, V., Kizek, R., and Masarik, M. (2014). Metallothionein - Immunohistochemical cancer biomarker: a meta-analysis. *PLoS One* 9:e85346. doi: 10.1371/journal.pone.0085346
- Gupta, V. K., and Rastogi, A. (2008). Biosorption of lead(II) from aqueous solutions by non-living algal biomass *Oedogonium* sp and *Nostoc* sp - a comparative study. *Colloids Surf. B Biointerfaces* 64, 170–178. doi: 10.1016/j.colsurfb.2008.01.019
- Gutiérrez, J. C., Amaro, F., Diaz, S., de Francisco, P., Cubas, L. L., and Martin-Gonzalez, A. (2011). Ciliate metallothioneins: unique microbial eukaryotic heavy-metal-binder molecules. *J. Biol. Inorg. Chem.* 16, 1025–1034. doi: 10.1007/s00775-011-0820-9
- Gutiérrez, J. C., Amaro, F., and Martin-Gonzalez, A. (2009). From heavy metal-binders to biosensors: ciliate metallothioneins discussed. *Bioessays* 31, 805–816. doi: 10.1002/bies.200900011
- Gutiérrez, J. C., Amaro, F., and Martin-Gonzalez, A. (2015). Heavy metal whole-cell biosensors using eukaryotic microorganisms: an updated critical review. *Front. Microbiol.* 6:8. doi: 10.3389/fmicb.2015.00048
- Han, S., Hu, Z., and Lei, A. (2008). Expression and function analysis of the metallothionein-like (MT-like) gene from *Festuca rubra* in *Chlamydomonas reinhardtii* chloroplast. *Sci. China C Life Sci.* 51, 1076–1081. doi: 10.1007/s11427-008-0136-3
- Han, X., Wong, Y. S., and Tam, N. F. Y. (2006). Surface complexation mechanism and modeling in Cr(III) biosorption by a microalgal isolate, *Chlorella miniata*. *J. Colloid Interface Sci.* 303, 365–371. doi: 10.1016/j.jcis.2006.08.028
- Hauser-Davis, R. A., Goncalves, R. A., Zioli, R. L., and de Campos, R. C. (2012). A novel report of metallothioneins in fish bile: SDS-PAGE analysis, spectrophotometry quantification and metal speciation characterization by liquid chromatography coupled to ICP-MS. *Aquat. Toxicol.* 116, 54–60. doi: 10.1016/j.aquatox.2012.03.003
- Haydon, M. J., and Cobbett, C. S. (2007). Transporters of ligands for essential metal ions in plants. *New Phytol.* 174, 499–506. doi: 10.1111/j.1469-8137.2007.02051.x
- Hirata, K., Tsuji, N., and Miyamoto, K. (2005). Biosynthetic regulation of phytochelatin, heavy metal-binding peptides. *J. Biosci. Bioeng.* 100, 593–599. doi: 10.1263/jbb.100.593
- Hirata, K., Tsujimoto, Y., Namba, T., Ohta, T., Hirayanagi, N., Miyasaka, H., et al. (2001). Strong induction of phytochelatin synthesis by zinc in marine green alga, *Dunaliella tertiolecta*. *J. Biosci. Bioeng.* 92, 24–29. doi: 10.1016/s1389-1723(01)80193-6
- Howe, G., and Merchant, S. (1992). Heavy metal-activated synthesis of peptides in *Chlamydomonas reinhardtii*. *Plant Physiol.* 98, 127–136. doi: 10.1104/pp.98.1.127
- Hutchins, D. A., Witter, A. E., Butler, A., and Luther, G. W. (1999). Competition among marine phytoplankton for different chelated iron species. *Nature* 400, 858–861. doi: 10.1038/23680
- Ibuot, A., Dean, A. P., McIntosh, O. A., and Pittman, J. K. (2017). Metal bioremediation by CrMTP4 over-expressing *Chlamydomonas reinhardtii* in comparison to natural wastewater-tolerant microalgae strains. *Algal Res. Biomass Biofuels Bioprod.* 24, 89–96. doi: 10.1016/j.algal.2017.03.002
- Infante, H. G., Van Campenhout, K., Blust, R., and Adams, F. C. (2006). Anion-exchange high performance liquid chromatography hyphenated to inductively coupled plasma-isotope dilution-time-of-flight mass spectrometry for speciation analysis of metal complexes with metallothionein isoforms in gibel carp (*Carassius auratus gibelio*) exposed to environmental metal pollution. *J. Chromatogr. A* 1121, 184–190. doi: 10.1016/j.chroma.2006.04.035
- Jaafari, J., and Yaghmaei, K. (2019). Optimization of heavy metal biosorption onto freshwater algae (*Chlorella coloniales*) using response surface methodology (RSM). *Chemosphere* 217, 447–455. doi: 10.1016/j.chemosphere.2018.10.205
- Jácome-Pilco, C. R., Cristiani-Urbina, E., Flores-Cotera, L. B., Velasco-García, R., Ponce-Noyola, T., and Cañizares-Villanueva, R. O. (2009). Continuous Cr(VI) removal by *Scenedesmus incrasatulus* in an airlift photobioreactor. *Bioresour. Technol.* 100, 2388–2391. doi: 10.1016/j.biortech.2008.10.053
- Jafarian, V., and Ghaffari, F. (2017). A unique metallothionein-engineered in *Escherichia coli* for biosorption of lead, zinc, and cadmium; adsorption or adsorption? *Microbiology* 86, 73–81. doi: 10.1134/s0026261717010064
- Jahnke, J., Mahlmann, D. M., Jacobs, P., and Priefer, U. B. (2011). The influence of growth conditions on the cell dry weight per unit biovolume of *Klebsormidium*

- flaccidum* (Charophyta), a typical ubiquitous soil alga. *J. Appl. Phycol.* 23, 655–664. doi: 10.1007/s10811-010-9557-z
- Jalmi, S. K., Bhagat, P. K., Verma, D., Noryang, S., Tayyeba, S., and Singh, K. (2018). Traversing the links between heavy metal stress and plant signaling. *Front. Plant Sci.* 9:12. doi: 10.3389/fpls.2018.00012
- Kao, W. C., Chiu, Y. P., Chang, C. C., and Chang, J. S. (2006). Localization effect on the metal biosorption capability of recombinant mammalian and fish metallothioneins in *Escherichia coli*. *Biotechnol. Prog.* 22, 1256–1264. doi: 10.1021/bp060067b
- Kawakami, S. K., Gledhill, M., and Achterberg, E. P. (2006). Production of phytochelatins and glutathione by marine phytoplankton in response to metal stress. *J. Phycol.* 42, 975–989. doi: 10.1111/j.1529-8817.2006.00265.x
- Keeling, P. J. (2013). The number, speed, and impact of plastid Endosymbioses in eukaryotic evolution. *Annu. Rev. Plant Biol.* 64, 583–607. doi: 10.1146/annurev-arplant-050312-120144
- Keeling, P. J., Burki, F., Wilcox, H. M., Allam, B., Allen, E. E., and Amaral-Zettler, L. A. (2014). The marine microbial eukaryote transcriptome sequencing project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. *PLoS Biol.* 12:e1001889. doi: 10.1371/journal.pbio.1001889
- Kim, D., Garrett, S. H., Sens, M. A., Somji, S., and Sens, D. A. (2002). Metallothionein isoform 3 and proximal tubule vectorial active transport. *Kidney Int.* 61, 464–472. doi: 10.1046/j.1523-1755.2002.00153.x
- Kim, K. S., Lee, B. S., Wilson, D. B., and Kim, E. K. (2005). Selective cadmium accumulation using recombinant *Escherichia coli*. *J. Biosci. Bioeng.* 99, 109–114. doi: 10.1263/jbb.99.109
- Kiyono, M., Omura, H., Omura, T., Murata, S., and Pan-Hou, H. (2003). Removal of inorganic and organic mercurials by immobilized bacteria having mer-ppk fusion plasmids. *Appl. Microbiol. Biotechnol.* 62, 274–278. doi: 10.1007/s00253-003-1282-y
- Krepkiy, D. W., Antholine, E., and Petering, D. H. (2003). Properties of the reaction of chromate with metallothionein. *Chem. Res. Toxicol.* 16, 750–756. doi: 10.1021/tx020074j
- Krezel, A., and Maret, W. (2017). The functions of metamorphic metallothioneins in zinc and copper metabolism. *Int. J. Mol. Sci.* 18:1237. doi: 10.3390/ijms18061237
- Kumar, K. S., Dahms, H. U., Won, E. J., Lee, J. S., and Shin, K. H. (2015). Microalgae – a promising tool for heavy metal remediation. *Ecotoxicol. Environ. Safety* 113, 329–352. doi: 10.1016/j.ecoenv.2014.12.019
- Kumari, M. V., Hiramatsu, R. M., and Ebadi, M. (1998). Free radical scavenging actions of metallothionein isoforms I and II. *Free Radic. Res.* 29, 93–101. doi: 10.1080/10715769800300111
- Lamaia, C., Kruatrachuea, M., Pokethitiyooka, P., Upathamb, E. S., and Soonthornsarathool, V. (2005). Toxicity and accumulation of lead and cadmium in the filamentous green alga *Cladophora fracta* (O.F. Müller ex Vahl) Kützing: a laboratory study. *Sci. Asia* 31, 121–127.
- Lane, T. W., and Morel, F. M. (2000). A biological function for cadmium in marine diatoms. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4627–4631. doi: 10.1073/pnas.090091397
- Leal, M. F. C., Vasconcelos, M., and van den Berg, C. M. G. (1999). Copper-induced release of complexing ligands similar to thiols by *Emiliania huxleyi* in seawater cultures. *Limnol. Oceanogr.* 44, 1750–1762. doi: 10.4319/lo.1999.44.7.1750
- Lehembre, F., Doillon, D., David, E., Perrotto, S., Baude, J., Foulon, J., et al. (2013). Soil metatranscriptomics for mining eukaryotic heavy metal resistance genes. *Environ. Microbiol.* 15, 2829–2840. doi: 10.1111/1462-2920.12143
- Leon-Banares, R., Gonzalez-Ballester, D., Galvan, A., and Fernandez, E. (2004). Transgenic microalgae as green cell-factories. *Trends Biotechnol.* 22, 45–52. doi: 10.1016/j.tibtech.2003.11.003
- Leszczyszyn, O. I., Imam, H. T., and Blindauer, C. A. (2013). Diversity and distribution of plant metallothioneins: a review of structure, properties and functions. *Metallomics* 5, 1146–1169. doi: 10.1039/c3mt00072a
- Leszczyszyn, O. I., White, C. R. J., and Blindauer, C. A. (2010). The isolated Cys(2)/His(2) site in EC metallothionein mediates metal-specific protein folding. *Mol. Biosyst.* 6, 1592–1603. doi: 10.1039/c002348e
- Levy, J. L., Angel, B. M., Stauber, J. L., Poon, W. L., Simpson, S. L., Cheng, S. H., et al. (2008). Uptake and internalisation of copper by three marine microalgae: comparison of copper-sensitive and copper-tolerant species. *Aquat. Toxicol.* 89, 82–93. doi: 10.1016/j.aquatox.2008.06.003
- Li, H., Malyar, R. M., Zhai, N., Wang, H., Liu, K., and Liu, D. (2019). Zinc supplementation alleviates OTA-induced oxidative stress and apoptosis in MDCK cells by up-regulating metallothioneins. *Life Sci.* 234:116735. doi: 10.1016/j.lfs.2019.116735
- Liu, Z. Y., and Schey, K. L. (2005). Optimization of a MALDI TOF-TOF mass spectrometer for intact protein analysis. *J. Am. Soc. Mass Spectrom.* 16, 482–490. doi: 10.1016/j.jasms.2004.12.018
- Loscher, B. M. (1999). Relationships among Ni, Cu, Zn, and major nutrients in the Southern Ocean. *Mar. Chem.* 67, 67–102. doi: 10.1016/s0304-4203(99)00050-x
- Luoma, S. N., and Rainbow, P. S. (2005). Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environ. Sci. Technol.* 39, 1921–1931. doi: 10.1021/es048947e
- Macfie, S. M., and Welbourn, P. M. (2000). The cell wall as a barrier to uptake of metal ions in the unicellular green alga *Chlamydomonas reinhardtii* (Chlorophyceae). *Arch. Environ. Contam. Toxicol.* 39, 413–419. doi: 10.1007/s002440010122
- Manceau, A., Bustamante, P., Haouz, A., Bourdineaud, J. P., Gonzalez-Rey, M., and Lemouchi, C. (2019). Mercury(II) binding to metallothionein in *Mytilus edulis* revealed by high energy-resolution XANES spectroscopy. *Chem. Eur. J.* 25, 997–1009. doi: 10.1002/chem.201804209
- May, M. J., and Leaver, C. J. (1993). Oxidative stimulation of glutathione synthesis in *Arabidopsis thaliana* suspension cultures. *Plant Physiol.* 103, 621–627. doi: 10.1104/pp.103.2.621
- Maznah, W. O. W., Al-Fawwaz, A. T., and Surif, M. (2012). Biosorption of copper and zinc by immobilised and free algal biomass, and the effects of metal biosorption on the growth and cellular structure of *Chlorella* sp. and *Chlamydomonas* sp. isolated from rivers in Penang, Malaysia. *J. Environ. Sci. China* 24, 1386–1393. doi: 10.1016/s1001-0742(11)60931-5
- Mehta, S. K., and Gaur, J. P. (2001). Characterization and optimization of Ni and Cu sorption from aqueous solution by *Chlorella vulgaris*. *Ecol. Eng.* 18, 1–13. doi: 10.1016/s0925-8574(00)00174-9
- Mehta, S. K., and Gaur, J. P. (2005). Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Crit. Rev. Biotechnol.* 25, 113–152. doi: 10.1080/07388550500248571
- Meister, A., and Anderson, M. E. (1983). Glutathione. *Annu. Rev. Biochem.* 52, 711–760.
- Mendoza-Cozatl, D., Devars, S., Loza-Tavera, H., and Moreno-Sanchez, R. (2002). Cadmium accumulation in the chloroplast of *Euglena gracilis*. *Physiol. Plant.* 115, 276–283. doi: 10.1034/j.1399-3054.2002.1150214.x
- Mendoza-Cozatl, D., Loza-Tavera, H., Hernandez-Navarro, A., and Moreno-Sanchez, R. (2005). Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. *FEMS Microbiol. Rev.* 29, 653–671. doi: 10.1016/j.femsre.2004.09.004
- Mendoza-Cozatl, D. G., and Moreno-Sanchez, R. (2005). Cd²⁺ transport and storage in the chloroplast of *Euglena gracilis*. *Biochim. Biophys. Acta Bioenerg.* 1706, 88–97. doi: 10.1016/j.bbabi.2004.09.010
- Miyayama, T., Ogra, Y., and Suzuki, K. T. (2007). Separation of metallothionein isoforms extracted from isoform-specific knockdown cells on two-dimensional micro high-performance liquid chromatography hyphenated with inductively coupled plasma-mass spectrometry. *J. Anal. Atomic Spectrom.* 22, 179–182. doi: 10.1039/b613662c
- Monteiro, C. M., Castro, P. M. L., and Malcata, F. X. (2010). Cadmium removal by two strains of *Desmodesmus pleiomorphus* Cells. *Water Air Soil Pollut.* 208, 17–27. doi: 10.1007/s11270-009-0146-1
- Monteiro, C. M., Castro, P. M. L., and Malcata, F. X. (2011). “Microalga-mediated bioremediation of heavy metal-contaminated surface waters,” in *Bio-management of Metal-Contaminated Soils*, eds M. S. Khan, A. Zaidi, R. Goel, and J. Musarrat (Dordrecht: Springer), 365–385. doi: 10.1007/978-94-007-1914-9_16
- Musgrave, W., Yi, B. H., Kline, D., Cameron, J. C., Wignes, J., Dey, S., et al. (2013). Probing the origins of glutathione biosynthesis through biochemical analysis of glutamate-cysteine ligase and glutathione synthetase from a model photosynthetic prokaryote. *Biochem. J.* 450, 63–72. doi: 10.1042/BJ20121332
- Mwandira, W., Nakashima, K., Togo, Y., Sato, T., and Kawasaki, S. (2020). Cellulose-metallothionein biosorbent for removal of Pb(II) and Zn(II) from

- polluted water. *Chemosphere* 246:125733. doi: 10.1016/j.chemosphere.2019.125733
- Nagamine, T., Suzuki, K., Kondo, T., Nakazato, K., Kakizaki, S., Takagi, H., et al. (2005). Interferon-alpha-induced changes in metallothionein expression in liver biopsies from patients with chronic hepatitis C. *Can. J. Gastroenterol. Hepatol.* 19, 481–486. doi: 10.1155/2005/262597
- Nakazato, K., Tomioka, S., Nakajima, K., Saito, H., Kato, M., Kodaira, T., et al. (2014). Determination of the serum metallothionein (MT)1/2 concentration in patients with Wilson's disease and Menkes disease. *J. Trace Elem. Med. Biol.* 28, 441–447. doi: 10.1016/j.jtemb.2014.07.013
- Narula, P., Mahajan, A., Gurnani, C., Kumar, C., and Mukhija, S. (2015). Microalgae as an indispensable tool against heavy metals toxicity to plants: a review. *Int. J. Pharm. Sci. Rev. Res.* 31, 86–93.
- Nassiri, Y., Mansori, J. L., Wery, J., Ginsburger-Vogel, T., and Amiand, J. C. (1997). Ultrastructural and electron energy loss spectroscopy studies of sequestration mechanisms of Cd and Cu in the marine diatom *Skeletonema costatum*. *Arch. Environ. Contam. Toxicol.* 33, 147–155. doi: 10.1007/s002449900236
- Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B., et al. (2012). Glutathione in plants: an integrated overview. *Plant Cell Environ.* 35, 454–484. doi: 10.1111/j.1365-3040.2011.02400.x
- Olsson, S., Penacho, V., Puente-Sanchez, F., Diaz, S., Gonzalez-Pastor, J. E., and Aguilera, A. (2017). Horizontal gene transfer of phytochelatin synthases from bacteria to extremophilic Green Algae. *Microb. Ecol.* 73, 50–60. doi: 10.1007/s00248-016-0848-z
- Pal, R., and Rai, J. P. N. (2010). Phytochelatin: peptides involved in heavy metal detoxification. *Appl. Biochem. Biotechnol.* 160, 945–963. doi: 10.1007/s12010-009-8565-4
- Penen, F., Isaure, M. P., Dobritzsch, D., Bertalan, I., Castillo-Michel, H., and Proux, O. (2017). Pools of cadmium in *Chlamydomonas reinhardtii* revealed by chemical imaging and XAS spectroscopy. *Metallomics* 9, 910–923. doi: 10.1039/c7mt00029d
- Perales-Vela, H. V., Pena-Castro, J. M., and Canizares-Villanueva, R. O. (2006). Heavy metal detoxification in eukaryotic microalgae. *Chemosphere* 64, 1–10. doi: 10.1016/j.chemosphere.2005.11.024
- Potesil, D., Petrlova, J., Adam, V., Vacek, J., Klejduš, B., Zehnalek, J., et al. (2005). Simultaneous femtomole determination of cysteine, reduced and oxidized glutathione, and phytochelatin in maize (*Zea mays* L.) kernels using high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. A* 1084, 134–144. doi: 10.1016/j.chroma.2005.06.019
- Rajamani, S., Torres, M., Falcao, V., Gray, J. E., Coury, D. A., Colepicolo, P., et al. (2014). Noninvasive evaluation of heavy metal uptake and storage in microalgae using a fluorescence resonance energy transfer-based heavy metal biosensor. *Plant Physiol.* 164, 1059–1067. doi: 10.1104/pp.113.229765
- Rigouin, C., Vermeire, J. J., Nylin, E., and Williams, D. L. (2013). Characterization of the phytochelatin synthase from the human parasitic nematode *Ancylostoma ceylanicum*. *Mol. Biochem. Parasitol.* 191, 1–6. doi: 10.1016/j.molbiopara.2013.07.003
- Rugini, L., Costa, G., Congestri, R., and Bruno, L. (2017). Testing of two different strains of green microalgae for Cu and Ni removal from aqueous media. *Sci. Total Environ.* 601, 959–967. doi: 10.1016/j.scitotenv.2017.05.222
- Ruiz, O., Alvarez, N. D., Gonzalez-Ruiz, G., and Torres, C. (2011). Characterization of mercury bioremediation by transgenic bacteria expressing metallothionein and polyphosphate kinase. *BMC Biotechnol.* 11:8. doi: 10.1186/1472-6750-11-82
- Ruta, L. L., Lin, Y. F., Kissen, R., Nicolau, I., Neagoe, A. D., and Ghenea, S. (2017). Anchoring plant metallothioneins to the inner face of the plasma membrane of *Saccharomyces cerevisiae* cells leads to heavy metal accumulation. *PLoS One* 12:e0178393. doi: 10.1371/journal.pone.0178393
- Ryvolova, M., Krizkova, S., Adam, V., Beklova, M., Trnkova, L., Hubalek, J., et al. (2011). Analytical methods for metallothionein detection. *Curr. Anal. Chem.* 7, 243–261. doi: 10.2174/15734110110107030243
- Saito, H., Nakazato, K., Kato, M., Kodaira, T., Akutsu, T., Tokita, Y., et al. (2013). Determination of metallothionein-3 by a competitive enzyme-linked immunosorbent assay in experimental animals. *J. Toxicol. Sci.* 38, 83–91. doi: 10.2131/jts.38.83
- Sanchez-Thomas, R., Moreno-Sanchez, R., and Garcia-Garcia, J. D. (2016). Accumulation of zinc protects against cadmium stress in photosynthetic *Euglena gracilis*. *Environ. Exp. Bot.* 131, 19–31. doi: 10.1016/j.envexpbot.2016.06.009
- Sandau, E., Sandau, P., and Pulz, O. (1996). Heavy metal sorption by microalgae. *Acta Biotechnol.* 16, 227–235. doi: 10.1002/abio.370160402
- Satoh, M., Karaki, E., Kakehashi, M., Okazaki, E., Gotoh, T., and Oyama, Y. (1999). Heavy metal-induced changes in non-proteinaceous thiol levels and heavy metals binding peptide in *Tetraselmis tetraathele* (Prasinophyceae). *J. Phycol.* 35, 989–994. doi: 10.1046/j.1529-8817.1999.3550989.x
- Sauge-Merle, S., Lecomte-Pradines, C., Carrier, P., Cuine, S., and DuBow, M. (2012). Heavy metal accumulation by recombinant mammalian metallothionein within *Escherichia coli* protects against elevated metal exposure. *Chemosphere* 88, 918–924. doi: 10.1016/j.chemosphere.2012.04.015
- Sbihi, K., Cherifi, O., Bertrand, M., and El Gharmali, A. (2014). Biosorption of metals (Cd, Cu and Zn) by the freshwater diatom *Planothidium lanceolatum*: a laboratory study. *Diatom Res.* 29, 55–63. doi: 10.1080/0269249x.2013.872193
- Schmitt, D., Müller, A., Csögör, Z., Frimmel, F. H., and Posten, C. (2001). The adsorption kinetics of metal ions onto different microalgae and siliceous earth. *Water Res.* 35, 779–785. doi: 10.1016/s0043-1354(00)00317-1
- Shahpiri, A., and Mohammadzadeh, A. (2018). Mercury removal by engineered *Escherichia coli* cells expressing different rice metallothionein isoforms. *Ann. Microbiol.* 68, 145–152. doi: 10.1007/s13213-018-1326-2
- Shanab, S., Essa, A., and Shalaby, E. (2012). Bioremediation capacity of three heavy metals by some microalgae species (Egyptian Isolates). *Plant Signal. Behav.* 7, 392–399. doi: 10.4161/psb.19173
- Shen, M. W., Shah, Y. D., Chen, W., and Da Silva, N. (2012). Enhanced arsenate uptake in *Saccharomyces cerevisiae* overexpressing the Pho84 phosphate transporter. *Biotechnol. Prog.* 28, 654–661. doi: 10.1002/btpr.1531
- Sjarul, M., and Arifin, D. (2012). Phytoremediation of Cd²⁺ by marine Phytoplankton *Tetraselmis chuii* and *Chaetoceros calcitrans*. *Int. J. Chem.* 4, 69–74.
- Soldo, D., Hari, R., Sigg, L., and Behra, R. (2005). Tolerance of *Oocystis niphrocytioides* to copper: intracellular distribution and extracellular complexation of copper. *Aquat. Toxicol.* 71, 307–317. doi: 10.1016/j.aquatox.2004.11.011
- Sriprang, R., Hayashi, M., Yamashita, M., Ono, H., Saeki, K., and Murooka, Y. (2002). A novel bioremediation system for heavy metals using the symbiosis between leguminous plant and genetically engineered rhizobia. *J. Biotechnol.* 99, 279–293. doi: 10.1016/s0168-1656(02)00219-5
- Summermatter, S., Bouzan, A., Pierrel, E., Melly, S., Stauffer, D., and Gutzwiller, S. (2017). Blockade of metallothioneins 1 and 2 increases skeletal muscle mass and strength. *Mol. Cell. Biol.* 37:e00305-16.
- Sun, R. L., Zhou, Q. X., and Wei, S. H. (2011). Cadmium accumulation in relation to organic acids and nonprotein thiols in leaves of the recently found Cd hyperaccumulator *Rorippa globosa* and the Cd-accumulating plant *Rorippa islandica*. *J. Plant Growth Regul.* 30, 83–91. doi: 10.1007/s00344-010-9176-6
- Terry, P. A., and Stone, W. (2002). Biosorption of cadmium and copper contaminated water by *Scenedesmus abundans*. *Chemosphere* 47, 249–255. doi: 10.1016/s0045-6535(01)00303-4
- Thirumorthy, N., Sunder, A. S., Kumar, K. M., Kumar, M. S., Ganesh, G., and Chatterjee, M. (2011). A review of metallothionein isoforms and their role in pathophysiology. *World J. Surg. Oncol.* 9:54. doi: 10.1186/1477-7819-9-54
- Thornalley, P. J., and Vasak, M. (1985). Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochim. Biophys. Acta* 827, 36–44. doi: 10.1016/0167-4838(85)90098-6
- Tien, C.-J., Sigge, D. C., and White, K. N. (2005). Copper adsorption kinetics of cultured algal cells and freshwater phytoplankton with emphasis on cell surface characteristics. *J. Appl. Phycol.* 17, 379–389. doi: 10.1007/s10811-005-5555-y
- Torres, E., Cid, A., Fidalgo, P., Herrero, C., and Abalde, J. (1997). Long-chain class III metallothioneins as a mechanism of cadmium tolerance in the marine diatom *Phaeodactylum tricornutum* Bohlin. *Aquat. Toxicol.* 39, 231–246. doi: 10.1016/s0166-445x(97)00034-9
- Tsuji, N., Hirayanagi, N., Okada, M., Miyasaka, H., Hirata, K., Zenk, M. H., et al. (2002). Enhancement of tolerance to heavy metals and oxidative stress in *Dunaliella tertiolecta* by Zn-induced phytochelatin synthesis. *Biochem. Biophys. Res. Commun.* 293, 653–659. doi: 10.1016/s0006-291x(02)00265-6

- Tukendorf, A., and Rauser, W. E. (1990). Changes in glutathione and phytochelatins in roots of maize seedlings exposed to cadmium. *Plant Sci.* 70, 155–166. doi: 10.1016/0168-9452(90)90129-c
- Twining, B., Baines, S., Vogt, S. B., and Nelson, D. M. (2012). Role of diatoms in nickel biogeochemistry in the ocean. *Global Biogeochem. Cycles* 26, 1–9.
- Twining, B. S., and Baines, S. B. (2013). “The trace metal composition of marine phytoplankton,” in *Annual Review of Marine Science*, Vol. 5, eds C. A. Carlson and S. J. Giovannoni (Palo Alto, CA: Annual Reviews), 191–215. doi: 10.1146/annurev-marine-121211-172322
- Ueno, D., Ma, J. F., Iwashita, T., Zhao, F. J., and McGrath, S. P. (2005). Identification of the form of Cd in the leaves of a superior Cd-accumulating ecotype of *Thlaspi caerulescens* using Cd-113-NMR. *Planta* 221, 928–936. doi: 10.1007/s00425-005-1491-y
- Ueno, D., Milner, M. J., Yamaji, N., Yokosho, K., Koyama, E., Zambrano, M. C., et al. (2011). Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating ecotype of *Thlaspi caerulescens*. *Plant J.* 66, 852–862. doi: 10.1111/j.1365-313X.2011.04548.x
- Wang, W.-X., and Dei, R. C. H. (2006). Metal stoichiometry in predicting Cd and Cu toxicity to a freshwater green alga *Chlamydomonas reinhardtii*. *Environ. Pollut.* 142, 303–312. doi: 10.1016/j.envpol.2005.10.005
- Wong, J. P. K., Wong, Y. S., and Tam, N. F. Y. (2000). Nickel biosorption by two chlorella species, *C. Vulgaris* (a commercial species) and *C. Miniata* (a local isolate). *Bioresour. Technol.* 73, 133–137. doi: 10.1016/S0960-8524(99)00175-3
- Wolf, C. R., Strenziok, R., and Kyriakopoulos, A. (2009). Elevated metallothionein-bound cadmium concentrations in urine from bladder carcinoma patients, investigated by size exclusion chromatography-inductively coupled plasma mass spectrometry. *Anal. Chim. Acta* 631, 218–222. doi: 10.1016/j.aca.2008.10.035
- Wu, S. M., Zheng, Y. D., and Kuo, C. H. (2008). Expression of mt2 and smt-B upon cadmium exposure and cold shock in zebrafish (*Danio rerio*). *Comp. Biochem. Physiol. C Toxicol. Pharm.* 148, 184–193. doi: 10.1016/j.cbpc.2008.05.007
- Wu, Y., Guo, Z. Q., Zhang, W., Tan, Q. G., Zhang, L., Ge, X. L., et al. (2016). Quantitative relationship between cadmium uptake and the kinetics of phytochelatin induction by cadmium in a marine diatom. *Sci. Rep.* 6:35935. doi: 10.1038/srep35935
- Wunderlich, K., Leveillard, A. T., Penkowa, M., Zrenner, E., and Perez, M. T. (2010). Altered expression of metallothionein-I and -II and their receptor megalin in inherited photoreceptor degeneration. *Invest. Ophthalmol. Vis. Sci.* 51, 4809–4820. doi: 10.1167/iovs.09-5073
- Xiao, X., Xue, J., Ding, D., Liao, L., Meng, X. L., Liu, L. Z., et al. (2015). Determination of trace metallothioneins at nanogram levels with Eosin Y by resonance light scattering method. *Int. J. Environ. Anal. Chem.* 95, 520–530. doi: 10.1080/03067319.2015.1048436
- Xiao, X., Xue, J., Liao, L., Chen, X., Zeng, Y., and Wu, Y. (2014). Determination of trace metallothioneins at nanomolar levels using phenanthroline-copper coordination by fluorescence spectra. *Anal. Sci.* 30, 999–1004. doi: 10.2116/analsci.30.999
- Xu, Y., Feng, L., Jeffrey, P. D., Shi, Y. G., and Morel, F. M. M. (2008). Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature* 452, 56–61. doi: 10.1038/nature06636
- Yan, H., and Pan, G. (2002). Toxicity and bioaccumulation of copper in three green microalgal species. *Chemosphere* 49, 471–476. doi: 10.1016/S0045-6535(02)00285-0
- Yang, H. Z., Gu, W. J., Chen, W., Hwang, J. S., and Wang, L. (2019). Metal binding characterization of heterologously expressed metallothionein of the freshwater crab *Sinopotamon henanense*. *Chemosphere* 235, 926–934. doi: 10.1016/j.chemosphere.2019.06.097
- Yoshida, N., Ikeda, R., and Okuno, T. (2006). Identification and characterization of heavy metal-resistant unicellular alga isolated from soil and its potential for phytoremediation. *Bioresour. Technol.* 97, 1843–1849. doi: 10.1016/j.biortech.2005.08.021
- Zagorski, N., and Wilson, D. B. (2004). Characterization and comparison of metal accumulation in two *Escherichia coli* strains expressing either CopA or MntA, heavy metal-transporting bacterial P-type adenosine triphosphatases. *Appl. Biochem. Biotechnol.* 117, 33–48. doi: 10.1385/abab:117:1:33
- Zhang, J., Ding, T., and Zhang, C. (2013). Biosorption and toxicity responses to arsenite (As[III]) in *Scenedesmus quadricauda*. *Chemosphere* 92, 1077–1084. doi: 10.1016/j.chemosphere.2013.01.002
- Zhang, Y.-K., Shen, G.-F., and Ru, B.-G. (2006). Survival of human metallothionein-2 transplastomic *Chlamydomonas reinhardtii* to ultraviolet B exposure. *Acta Biochim. Biophys. Sin.* 38, 187–193. doi: 10.1111/j.1745-7270.2006.00148.x
- Zhou, J. L., Huang, P. L., and Lin, R. G. (1998). Sorption and desorption of Cu and Cd by macroalgae and microalgae. *Environ. Pollut.* 101, 67–75. doi: 10.1016/S0269-7491(98)00034-7
- Ziller, A., and Fraissinet-Tachet, L. (2018). Metallothionein diversity and distribution in the tree of life: a multifunctional protein. *Metallomics* 10, 1549–1559. doi: 10.1039/c8mt00165k
- Ziller, A., Yadav, R. K., Capdevila, M., Reddy, M. S., Vallon, L., Marmesse, R., et al. (2017). Metagenomics analysis reveals a new metallothionein family: sequence and metal-binding features of new environmental cysteine-rich proteins. *J. Inorgan. Biochem.* 167, 1–11. doi: 10.1016/j.jinorgbio.2016.11.017

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Balzano, Sardo, Blasio, Chahine, Dell'Anno, Sansone and Brunet. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.