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# The roles of phytohormones in metal stress regulation in microalgae

Hai Ngoc Nguyen[1,2] · Anna B. Kisiala[2] · R. J. Neil Emery[2]

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**Abstract**

The constant spread of heavy metal contamination creates an increasing global environmental issue that results in considerable deterioration of land and water ecosystems leading to a decline in the health of plants, animals and humans. Novel, algal-based filtration technologies have been gaining a great deal of attention given their eco-friendly, effective and easy to implement processes. This review focuses on the potential roles that phytohormones can play in heavy metal stress response in microalgae. It emphasizes phytohormone efficiency and proposes the use of these signaling molecules for enhanced metal stress alleviation in microalgae. Furthermore, future implications for algal-based filtration technologies involving modifications of phytohormone metabolism towards improved heavy metal biodegradation rates are presented.

**Keywords** Algae-based bioremediation · Heavy metal stress · Microalgae · Phytohormone

## Introduction

### Heavy metals: pollution, toxicity, and bio-removal

Heavy metal (HM) pollution is a worldwide environmental concern. Various industrial processes and the use of chemicals in many areas deliberately or accidentally release toxic HMs into the environment. Effluent wastes are released to rivers and wetlands causing water pollution, seriously damaging ecosystems and threatening human health (Afonne and Ifediba 2020). Among many HMs that originate mainly from agro-industrial wastewaters, nickel (Ni), copper (Cu), cadmium (Cd), arsenic (As), lead (Pb), and chromium (Cr) are considered as priority pollutants, owing to their high toxicity and non-biochemically degradable properties (Zeraatkar et al. 2016; Azimi et al. 2017). Exposure to HM contaminants leads to cytotoxicity, carcinogenicity, teratogenicity, and mutagenicity, thus increasing the risk of cancer and cancer-related diseases (Zeraatkar et al. 2016; Afonne and Ifediba 2020). Several physical and chemical methods can be used for HM removal from the environment, including replacement or washing of soil, metal precipitation, oxidation, ion exchange, or adsorption in water. However, these methods are costly and time- and labor-consuming and, in natural ecosystems, their success is often limited due to the large areas of contaminated land or water. These methods can also create a form of secondary pollution (Khalid et al. 2017).

Bio-removal of HMs has therefore been gaining a great deal of attention given its eco-friendly, effective, and easy to operate processes. Algal cells present diverse adaptive responses and physiochemical mechanisms to clean up metal contamination from water, which make them interesting material to explore for their bioremediation potential (Zeraatkar et al. 2016; Qin et al. 2020).

### Microalgae: powerful organisms for bioremediation

The use of microalgae as bioremediation agents has become a promising solution for cleaning up HMs from the environment (Qin et al. 2020). Algae acclimate to, accumulate, and remove HMs from the polluted habitats using diverse defense systems, including tolerance against HM toxicity through ROS detoxification (Moenne et al. 2016) and bio-removal of HMs via bioadsorption, bioaccumulation, and chelation (Urrutia et al. 2019; Khatiwada et al. 2020). Algae-based bioremediation has been a focus of attention owing to the associated removal capacity, low cost, time and energy saving, and ease of handling operations (Urrutia et al. 2019). As a downstream bioremediation process, the remaining algal biomass could be disposed of with a much lower environmental footprint or used in industrial processing to produce biofuel or algal-

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✉ Hai Ngoc Nguyen
hainguyen@trentu.ca

[1] Trent University, Environmental and Life Science Program, Peterborough, Canada

[2] Department of Biology, Trent University, Peterborough, Canada

derived, beneficial compounds. These advantages could be intensified through the application of phytohormones (Piotrowska-Niczyporuk et al. 2012; Sytar et al. 2019; Zhao et al. 2019a).

## Phytohormones: elegant and effective signal molecules to enhance bioremediation

Phytohormones are the signaling molecules that are responsible for many important plant physiological activities, and they also play a pivotal role in HM stress tolerance in higher plants (Bücker-Neto et al. 2017). In microalgae, phytohormones help to increase cell growth, protein, and metabolite accumulation and they can improve abiotic stress tolerance (Zhao et al. 2019a). Enhanced algal biomass production can be achieved through the direct supply of phytohormones, such as the exogenous treatment of CKs (induction of growth rate, pigment and monosaccharides content) (Piotrowska and Czerpak 2009; de Jesus Raposo and de Morais 2013), auxin (boosted biomass, lipid and fatty acid (FA) accumulation) (Liu et al. 2017b; Salama et al. 2017), abscisic acid (ABA) (enhanced cell concentration, lipid and triacylglycerol content) (Contreras-Pool et al. 2016), gibberellins (GAs) (high value polyunsaturated FA production) (Udayan et al. 2018), or ethylene (ET) (saturated FA accumulation) (Kim et al. 2016).

Many findings linking phytohormones with various abiotic stress responses in algae have been reported in examples such as ABA (improved drought stress tolerance) (Kobayashi et al. 1997), nitrogen depletion (Sulochana and Arumugam 2016), brassinosteroids (BRs) (involved in short-term heat stress response) (Bajguz 2009) and auxin (improved salt stress tolerance) (El Arroussi et al. 2015). Phytohormones such as GAs, auxin, and CKs enhance HM stress tolerance and yield of *Chlorella vulgaris* (Falkowska et al. 2011; Piotrowska-Niczyporuk et al. 2012). Phytohormones are also helpful in reducing HM toxicity by increasing antioxidant enzyme activities (Piotrowska-Niczyporuk et al. 2012). Additionally, phytohormones are possibly beneficial in alleviating the negative effects of HMs via the inducement of non-enzymatic antioxidant components, such as proline and astaxanthin (Gao et al. 2012a, b; Lee et al. 2016). Algal cells with optimized phytohormone profiles would present improved growth and stress tolerance and open new windows for future water waste treatment practices (Fig. 1).

The above evidence shows that phytohormones can provide a new approach for developing effective and environmentally friendly bioremediation methods and resilient algal bio-filtration formulations. Thus, this review focuses on the current knowledge of the functional aspects and proposes phytohormone signaling pathways involved in algal HM stress response. Furthermore, novel concepts are emphasized using modern integrated -omic techniques to realize algal-based bioremediation technologies.

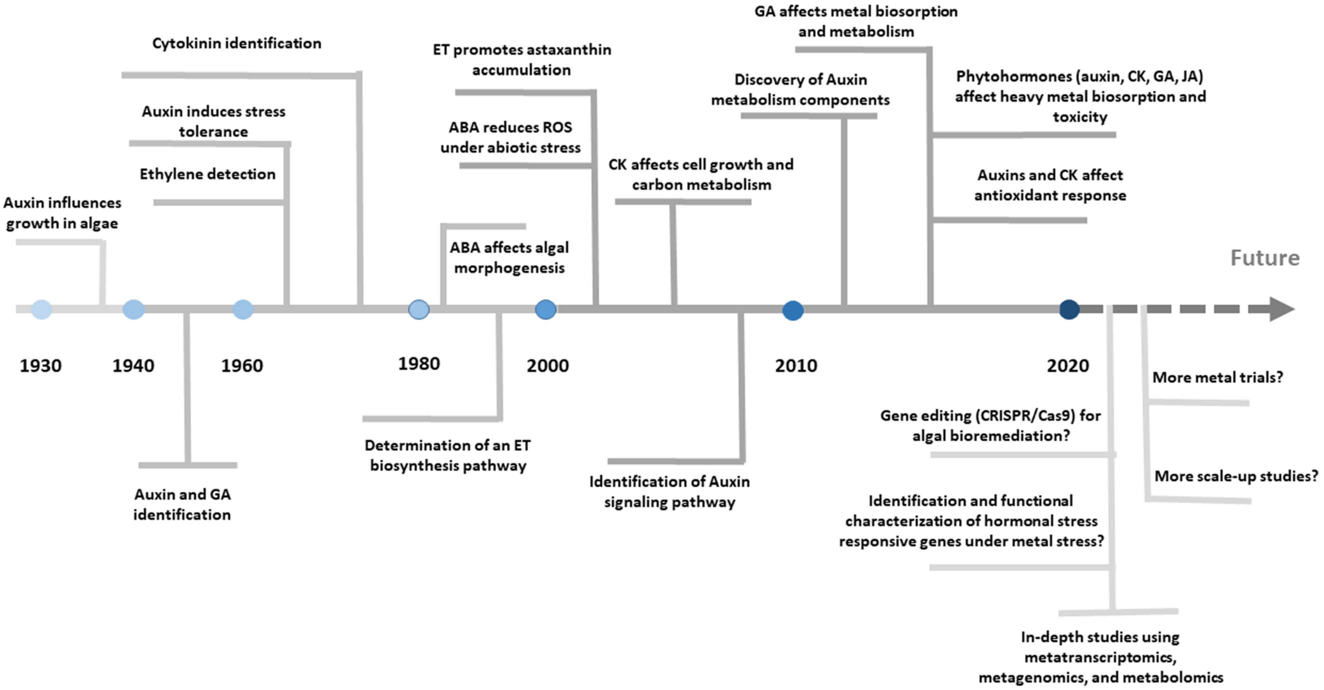

**Fig. 1** Timeline identifying important scientific milestones in phytohormone research in algae. CK cytokinin, ET ethylene, GA gibberellin, JA jasmonic acid, ROS reactive oxygen species, SA salicylic acid. Based on Pratt (1938); Mowat (1965); Stewart et al. (1968); Jacobs et al. (1985); Zhang et al. (1989); Maillard et al. (1993); Kobayashi et al. (1997); Yoshida et al. (2004); Lau et al. (2009); Piotrowska and Czerpak (2009); Le Bail et al. (2010); Falkowska et al. (2011); Piotrowska-Niczyporuk et al. (2012); Piotrowska-Niczyporuk and Bajguz (2014); Lu and Xu (2015); Lee et al. (2016); Liu et al. (2017a); Tiwari et al. (2018)

## Exogenous phytohormone application can advance algae-based bioremediation

Phytohormones are critical for a wide range of HM stress tolerance mechanisms in higher plants (Shukla et al. 2017; Table 1). Exogenous treatment with phytohormones have been utilized to impact a broad spectrum of physiological and biochemical processes in algae, including abiotic stress tolerance, biomass enhancement, and accumulation of FAs, oil, and other valuable metabolites. However, regulatory functions of phytohormones in HM stress alleviation in algae remain relatively unexplored as compared with higher plants. To date, the model green alga *C. vulgaris* is the most successful algal system with significant influences of phytohormones for HM stress tolerance. Among the HMs, cadmium (Cd), lead (Pb), and copper (Cu) are highly phytotoxic metals that negatively impact general cellular metabolism and disturb ROS balance. Metal bio-adsorption and reduction of oxidative-induced damage under HM exposure can be directly controlled by CKs, GAs and auxin (Table 1).

## CKs, GAs, and auxin regulate Cu, Pb, and Cd tolerance through the ROS detoxification

### Multiple roles of CKs in HM stress tolerance

CKs are responsible for many important physiological activities, and they also play a pivotal role in HM stress tolerance (Mohan et al. 2016; Jalmi et al. 2018). The manipulations of CKs during HM stress have targeted either CK biosynthesis/degradation or enhanced levels via exogenous CK treatment, and these have revealed promising outcomes in higher plants (Table 1). For example, exogenous CK treatment mitigated Cd-induced damage in pea seedlings (Al-Hakimi 2007). Increased endogenous CK levels in tobacco resulting from overexpression of a CK biosynthetic gene (IPT) enhanced plant tolerance to Cu stress, and this was explained by an increased transcriptional pattern of a metallothionein-like gene (Thomas et al. 2005). In *Arabidopsis* (*A. thaliana*), CK depletion caused by mutations in the *ipt1*, *ipt3*, *ipt5*, and *ipt7* genes activated selenium (Se) tolerance; induced activities of catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX); and increased glutathione (GSH) content (Jiang et al. 2019). CK-deficient *Arabidopsis* and tobacco plants overexpressing a CK degradation enzyme (CKX1) presented higher accumulations of thiol compounds like phytochelatins (PCs), leading to improved As tolerance (Mohan et al. 2016).

CKs regulate a wide range of important cellular processes in both micro- and macroalgal species including photorespiration in *Chlamydomonas reinhardtii* (Tian et al. 2006), carbon metabolism in *C. vulgaris* (Piotrowska and Czerpak 2009), or photosynthesis in *Gracilaria caudata* (Souza and Yokoya 2016). Exogenous application of CKs and its combined treatment with ABA induced 1.4-fold increase in cell yield in *Euglena gracilis* after 144 h of cultivation (Noble et al. 2014). CK supply improved salt stress tolerance in *Haematococcus pluvialis* and *Dunaliella salina* grown under 10% salinity (3.2-fold and 4.1-fold increase in cell number, respectively) (de Jesus Raposo and de Morais 2013). When exposed to nitrogen depletion, a time-series transcriptional analysis showed an activation of the ABA biosynthetic pathway and antagonistic transcription of CK biosynthesis genes, indicating there are antagonistic roles between CKs and ABA (Lu et al. 2014). In the context of hormonal cross-talk, CKs (10 nM tZ) interacted synergistically with BR (10 nM brassinolide (BL)) in *C. vulgaris*, leading to improved cell growth (almost 4 time increase in cell density) and accumulation of proteins (2.5-fold increase), chlorophyll (at least 3-fold increase), and monosaccharides (3-fold increase) (Bajguz and Piotrowska-Niczyporuk 2014). Regarding HM stress tolerance, CKs alleviated toxic effects of Cd, Pb, and Cu on growth of *C. vulgaris* (order of CKs stimulating properties: DPU (N,N′-diphenylurea) > CPPU (forchlorfenuron) > TDZ (thidiazuron) > tZ (trans-zeatin) > BA ($N^6$-benzyladenine) > Kin (kinetin) > 2iP (2-isopentenyladenine)) (Piotrowska-Niczyporuk et al. 2012).

Apart from regulatory functions in multiple physiochemical processes, CKs have a capacity to control scavenger ROS and protect cellular proteins, pigments, and sugars. This makes CKs outstanding molecules for algal HM tolerance. Exogenous application of CKs stimulated antioxidant enzymes (superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT)) and caused ascorbate and GSH accumulation (over 2-fold higher with various HM treatment), reducing oxidative stress expressed by lipid peroxidation and hydrogen peroxide level in *C. vulgaris*. With respect to metabolite levels under metal exposure (Cd/Cu/Pb), CKs improved protein content (11–85% recovery of protein), carotenoids (60–71%), and monosaccharide accumulation (order of stimulating properties: DPU > CPPU > TDZ > Z > BA > Kin > 2iP) (Piotrowska-Niczyporuk et al. 2012). Exogenous CK (tZ) application reduced Pb toxicity through the manipulation of the endogenous phytohormones (auxins and CKs) and phytochelatin (PC) precursor levels in the green algae, *Acutodesmus obliquus* (Piotrowska-Niczyporuk et al. 2020). The above studies show that CKs help control metal stress tolerance in algae through the modification of the ROS detoxification system and regulation of main cellular pathways.

### GAs associate with ROS systems during HM stress

In higher plants, exogenous GA treatment increased antioxidant potential and reduced oxidative damage under Cr, Cd, and Ca stresses (Meng et al. 2009; Gangwar et al. 2011; Siddiqui et al. 2011; Zhu et al. 2012). At a molecular level,

**Table 1** Role of phytohormones in plant stress response and their potential for HM bioremediation in microalgae. ABA abscisic acid, As arsenic, *aux1-7* and *pin2-1* auxin polar transport mutants, BR brassinosteroid, Ca calcium, CAT catalase, Cd cadmium, CK cytokinin, CKX cytokinin oxidase/dehydrogenase, Cr chromium, Cu copper, ET ethylene, *Etr1-1* and *etr1-3 mutant* ethylene receptor mutants, FA fatty acid, GA gibberellin, GSH glutathione, HM heavy metal, IPT isopentenyl transferase, JA jasmonic acid, Ni nickel, Pb lead, PC phytochelatin, ROS reactive oxygen species, SA salicylic acid, Se selenium, SOD superoxide dismutase, Zn zinc. *Exogenous treatment studies, (↓) decrease, (↑) increase

| Phytohormone | Function in HM tolerance in higher plants | Phytohormone application mode in higher plants | Physiological impact in HM stress response in higher plants | Relevant finding in microalga* | Potential implication for HM bioremediation using microalgae |
|---|---|---|---|---|---|
| **CK** | Key role in HM stress tolerance | Exogenous treatment | ↓ Cd damage (Al-Hakimi 2007) | ↑ cell number and metabolites (*Chlorophyceae*), ↑ pigment content and protein (*Gracilariales, Rhodophyta*) (Piotrowska and Czerpak 2009; Souza and Yokoya 2016)<br>↓ metal absorption, ↑ Cd, Pb, and Cu tolerance (*Chlorella vulgaris*) (Piotrowska-Niczyporuk et al. 2012)<br>↑ Pb stress tolerance in *Acutodesmus obliquus* (Piotrowska-Niczyporuk et al.2020) | Improvement of HM stress tolerance |
| | | Overexpression of *CKX1* gene | ↑ As tolerance, ↑ PC compounds (Mohan et al. 2016) | | |
| | | Overexpression of IPT gene | ↑ Cu stress tolerance, ↑ expression of metallothionein gene (Thomas et al. 2005) | | |
| | | Mutants of *ipt1 3 5 7* genes | ↓ Se uptake, ↑ antioxidant levels (Jiang et al. 2019) | | |
| **ABA** | Response to Zn, Pb, and As stress (Atici et al. 2005; Souza and Yokoya 2016) | Exogenous treatment | ↑ ROS enzyme activities under Pb exposure (Wang et al. 2013)<br>↑ Zn detoxification (Shi et al. 2015) | ↑ growth, biomass, and lipid yield under nitrogen starved conditions (*Scenedesmus quadricauda*) (Sulochana and Arumugam 2016)<br>Associated with CK, auxin, and Br in ↑ Pb stress tolerance in *Acutodesmus obliquus* (Talarek-Karwel et al. 2020) | Enhancement of microalga growth rate, cell viability, lipid content, and stress tolerance |
| **Auxin** | Response to Cd, Zn, Cu, and Pb (Luo et al. 2016) | *aux1-7* and*pin2-1* mutants | No effect on lateral root formation under Cd stress (Hu et al. 2013) | ↑ enzymatic and non-enzymatic antioxidant systems, ↓ ROS accumulation (*Chlorella vulgaris*) (Piotrowska-Niczyporuk and Bajguz 2014)<br>↓ stress symptoms, ↓ HM biosorption, restoring algal growth and primary metabolite level (*Chlorella vulgaris*) (Piotrowska-Niczyporuk et al. 2012)<br>↑ phytochelatins, ↑ Pb stress tolerance in *Acutodesmus obliquus* (Piotrowska-Niczyporuk et al.2020) | Improvement of HM stress tolerance |
| | A crucial role in HM stress tolerance | Exogenous treatment | Interaction with miRNA, ↑ As stress tolerance (Srivastava et al. 2013)<br>↑ toxicity amelioration (Pandey and Gupta 2015)<br>↑metal phytoextraction for Pb (Hadi et al. 2010) | | |
| **GA** | Regulate HM stress through ROS system | Exogenous treatment | ↓ Cd toxicity, ↓ Cd uptake transporter gene expression (Zhu et al. 2012)<br>↑Ni stress (Siddiqui et al. 2011)<br>↓ROS damages under Cr and Cd stress (Meng et al. 2009; Gangwar et al. 2011) | ↑ Pb and Cd stress tolerance (*Chlorella vulgaris*) (Falkowska et al. 2011)<br>↓HM biosorption, ↑ Cd, Cu, and Pb stress tolerance (*Chlorella vulgaris*) (Piotrowska-Niczyporuk et al. 2012) | Improvement of HM stress tolerance |

**Table 1** (continued)

| Phytohormone | Function in HM tolerance in higher plants | Phytohormone application mode in higher plants | Physiological impact in HM stress response in higher plants | Relevant finding in microalga* | Potential implication for HM bioremediation using microalgae |
|---|---|---|---|---|---|
| **ET** | Transcriptional response to Cr, Cd, Pb (Cao et al. 2009; Schellingen et al. 2014; Trinh et al. 2014) | *ein2-1* mutant | ↑Pb resistance through GSH-dependent mechanism and a GSH-independent AtPDR12-mediated mechanism (Cao et al. 2009) | ↑ growth and astaxanthin (antioxidant) accumulation (*Haematococcus pluvialis*) (Lee et al. 2016)<br>↑ transcriptional level of SOD and CAT genes (*Haematococcus pluvialis*) (Lee et al. 2016) | Enhancement of HM stress tolerance via ROS detoxification |
| | | *etr1-1* and *etr1-3* mutant | ↑ Li tolerance (Bueso et al. 2007) | | |
| **SA** | Provide HM stress resistance | Exogenous treatment | ↑ Cd stress tolerance (Belkadhi et al. 2016)<br>↓ ROS, ↑ Cd stress tolerance (Zhang et al. 2011)<br>↑ Cd toxicity, regulate Cd uptake, photosynthesis and antioxidant enzymes, (Shi et al. 2009) | ↑ astaxanthin content, (Raman and Ravi 2011)<br>↑ transcriptional level of carotenoid genes (*Haematococcus pluvialis*) (Gao et al. 2012b) | Enhancement of HM stress tolerance via antioxidant system |
| **JA** | Association with ROS and regulation of toxic action of HMs | Exogenous treatment | ↑ Cu and Cd tolerance, ↑ GSH and PC accumulation (Maksymiec et al. 2007)<br>↑ Ni tolerance (Sirhindi et al. 2015; Hanaka et al. 2016)<br>↑ As stress tolerance via interplay with miRNA (Srivastava et al. 2013) | ↑ astaxanthin accumulation and carotenoid gene expression (*Haematococcus pluvialis*) (Lu et al. 2010; Gao et al. 2012a)<br>↑ cell density, total oil content, alter FA composition and FA biosynthetic gene expression (*Chlorella vulgaris*) (Jusoh et al. 2015) | Elevation of oil content and HM stress tolerance |
| **BR** | Regulate antioxidant, PC level, improve HM stress tolerance | Exogenous treatment | ↓ Cr accumulation, ↑ROS detoxification (Sharma et al. 2016)<br>↓ Zn toxicity (Li et al. 2016)<br>↓ Cd toxicity (Sharma and Bhardwaj 2007)<br>↑ Zn tolerance through glutathione-ascorbate dependent detoxification pathway (Ramakrishna and Rao 2015; Wu et al. 2016) | ↑ABA during heat stress (*Chlorella vulgaris*) (Bajguz 2009)<br>Synergistic action with auxin on the growth, and antioxidant response (*Chlorella vulgaris*) (Bajguz and Piotrowska-Niczyporuk 2013)<br>interact synergistically with CKs, ↑cell number, protein, chlorophyll and monosaccharides (*Chlorella vulgaris*) (Bajguz and Piotrowska-Niczyporuk 2014)<br>↑ Pb stress tolerance and modified hormone homeostasis (Talarek-Karwel et al. 2020) | Improvement of HM stress tolerance |

GAs reduce Cd toxicity via suppression of expression of a Cd uptake transporter gene. The functions of GAs in providing protection to HM stress through ROS systems are presented in Table 1. Many deleterious effects of HM toxicities (Cu, Cd, and Pb) on the growth of *C. vulgaris* were reduced by supplementing cultures with gibberellin ($GA_3$) (Piotrowska-Niczyporuk et al. 2012). Under the concentration of 100 µM of Cu, Cd, and Pb, $GA_3$ had a positive effect on protein (6–14% increase), chlorophyll *a* (20–29% increase), chlorophyll *b* (28–52% increase), monosaccharides (∼30% increase), ascorbate (52–76% increase), and GSH (58–79% increase) content (Piotrowska-Niczyporuk et al. 2012). $GA_3$ significantly improved carotenoid content, and SOD and CAT activities under Cu exposure, surpassing its effect when exposure involved similar concentrations of Cd and Pb (Piotrowska-Niczyporuk et al. 2012). Hence, GAs appear to be involved in HM stress tolerance in algae and to be associated with photosynthesis pathways and ROS networks (Fig. 2). Moreover, $GA_3$ ($10^{-5}$ M) induced the biosorption capacity of *C. vulgaris*, thus enhancing cell capacity to bioaccumulate

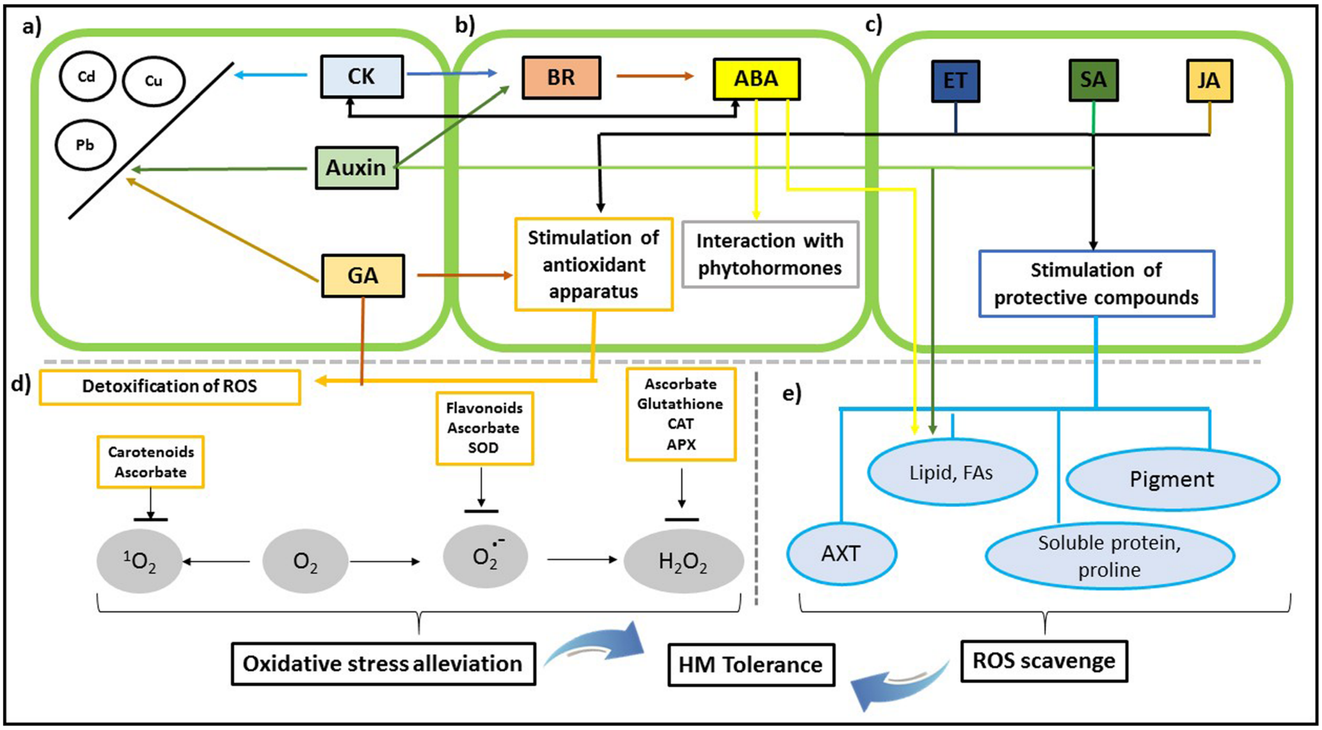

**Fig. 2** The proposed model of action of phytohormones in HM stress tolerance in algae. **a** ABA, CKs, and GAs' function as regulators of Cu, Pb, and Cd biosorption and toxicity. **b** Proposed synergistic functions of BRs with other hormones. **c** Regulatory action of ABA, ET, JA, and SA under HM stress through antioxidant systems. **d** Key ROS processing pathways under regulation of phytohormones during HM stress in algae (adapted from Mhamdi and Van Breusegem (2018). **e** Non-enzymatic oxidant system associated with phytohormones under HM treatment. ABA abscisic acid, APX ascorbate peroxidase, BRs brassinosteroids, CAT catalase, Cd cadmium, CK cytokinin, Cu copper, ET ethylene, FA fatty acid, GA gibberellin, HM heavy metal, JA jasmonic acid, Pb lead, ROS reactive oxygen species, SA salicylic acid, SOD: superoxide dismutase

toxic metals (Pb/Cd) (Falkowska et al. 2011). GAs also induce FAs and lipid biosynthesis in three different algae (*Chlorella pyrenoidosa*, *Nannochloropsis oceanica* and *Aurantiochytrium* sp. (Yu et al. 2016; Du et al. 2017; Udayan et al. 2018), suggesting that FA and lipid pathways are regulated by GAs under HM stress.

## ROS and their detoxification are auxin-dependent components in algae

Auxin is directly involved in HM stress in higher plants ((Wang et al. 2015; Jalmi et al. 2018; Table 1). Although manipulation of auxin polar transport genes (*aux1-7* and *pin2-1*) had no effect on lateral root formation under Cd stress (Hu et al. 2013), exogenously applied auxin interacted with miRNA to regulate HMs (Srivastava et al. 2013). In algae, the auxin role is mainly associated with the ROS detoxification system under HM stress. Even without HM stress, auxin stimulates enzymatic (APX, CAT, SOD) and non-enzymatic (ascorbate, GSH) antioxidant systems and reduces peroxidation and hydrogen peroxide levels (Piotrowska-Niczyporuk and Bajguz 2014). These components of cellular redox systems have a prominent role under the regulation of auxin-dependent processes. HM treatment in the combination with auxin-related ROS detoxification forms the auxin-dependent processes in *C. vulgaris* (Zhao et al. 2019a). In this context, auxin reduces hydrogen peroxide and malondialdehyde (MDA) content, which is attributed to the improved activities of ROS detoxification components (SOD, CAT, and APX) and enhanced ascorbate (43–57%) and GSH (24–40%) levels, ultimately leading to the improved cell growth under Cu, Pb, and Cd stress (order of auxin stimulating properties: IAA (indole-3-acetic acid) > NAA (1-naphthaleneacetic acid) > PAA (phenylacetic acid) > IBA (indole-3-butyric acid) (Piotrowska-Niczyporuk et al. 2012). In algae *A. obliquus*, exogenous application of auxins (0.01 μM IAA, 0.1 μM IBA, 0.1 μM PAA) stimulated the coordinated activation of metal tolerance mechanisms, resulting in an increased in PC synthase activity and accumulation of PCs and their precursors, enhancing Pb sequestration (Piotrowska-Niczyporuk et al. 2020).

## Do ABA and BRs have synergistic function with other phytohormones in HM stress responses?

In consideration of *in planta* studies and potential cross-talk with other phytohormones under stress, ABA is a key player in plant HM stress responses. An elevated level of ABA under Zn, Pb, and As exposure occurs in higher plants (Atici et al. 2005; Srivastava et al. 2013). Exogenous supply of ABA increased antioxidant activities and reduced Pb-induced oxidative damage in the Chinese herb, *Atractylodes macrocephala* (Wang et al. 2013), whereas in poplar tree, ABA regulated Zn uptake and detoxification, reducing Zn concentration (Shi et al. 2015). In black mustard, ABA induced accumulation of miR159, which regulates GA signaling and ET biosynthesis, thus playing an important role in the As stress response (Srivastava et al. 2013). Thus, ABA can reverse the toxic effects of HMs and function as a central cross-talking agent among other plant hormone groups in higher plants. Metal-induced toxicity on higher plant and algal growth is concomitant with an increase in cellular ABA levels (Piotrowska-Niczyporuk et al. 2017). In *A. obliquus* cells, exposure to Pb stress increased ABA level by 111% as compared with control. The ameliorative effects of exogenous application of CKs (tZ, Kin, DPU), auxins (IAA, IBA, PAA), and BR (EBL- 24-epibrassinolide) on *A. obliquus* growth and tolerance upon Pb stress have been associated with a reduction in endogenous ABA level (Talarek-Karwel et al. 2020). Cellular events such as modification of carbohydrate, lipid, and amino acid fluxes are fundamental features in cell adjustment to effectively adapt to unfavorable conditions; thus, there is a redirection away from growth pathways and towards stress response investments (Signorelli et al. 2019). Moreover, modulation of lipid components plays a critical function in cell-adaptive responses during stress (Hou et al. 2016). In *Scenedesmus quadricauda*, ABA (2 μM) improved cell growth (2.1-fold increase in dry biomass yield after 24 h) while 5 μM concentration of ABA induced accumulation of saturated FA (12%) and decrease of unsaturated FA content (11%) under nitrogen (N) starvation (Sulochana and Arumugam 2016). A possible role of ABA in HM stress response in algae was further supported using ABA for inducing cell growth in *Chlorella saccharophila* (Contreras-Pool et al. 2016) and *E. gracilis* (Noble et al. 2014) and increasing lipid accumulation in *C. saccharophila* (Contreras-Pool et al. 2016). In hormonal cross-talk, ABA interacted synergistically with BRs (BL enhanced the ABA content) during short-term heat stress in *C. vulgaris* (Bajguz 2009). Exogenously applied tZ ($10^{-7}$ M) combined with ABA ($10^{-9}$ M) increased cell growth by 140% after 144 h in *E. gracilis* (Noble et al. 2014). Hence, ABA could be a promising candidate for manipulating HM stress response regulation in algae.

In higher plants, BRs upregulated gene expression of SOD, CAT, APX, and GR and thereby enhanced antioxidant systems and strengthen Cr stress tolerance ((Sharma et al. 2016; Table 1). Exogenous application of BRs also reduced Zn and Cd toxicity (Sharma and Bhardwaj 2007; Li et al. 2016). In algae, BRs have a synergistic effect with auxin and CKs on enhancing growth, metabolite content (proteins, chlorophylls and monosaccharides), and antioxidant responses in *C. vulgaris* (Bajguz and Piotrowska-Niczyporuk 2013, 2014). These data indicate that combinations of treatments with BRs and two other well-known HM stress alleviators (CKs and auxin) enhance algae HM tolerance. The study confirmed that EBL treatment increased BR, $GA_3$, auxin, and CK content under Pb stress and reduced unfavorable consequences of HM on *A. obliquus* growth (Talarek-Karwel et al. 2020).

## ET and SA potentially regulate HM stress through the enzymatic and non-enzymatic antioxidant systems

ET, SA, and JA have long been recognized for their roles in mediating the response to HM stress in plants mainly through the ROS detoxification system (Table 1). However, no reports have yet uncovered ET and SA roles in HM tolerance in algae. The decrease in cell number, chlorophyll, carotenoid, monosaccharide, soluble protein, ascorbate, and GSH content as well as antioxidant enzyme activity was a response to JA and HMs (Cu, Pb and Cd) in *C. vulgaris* (Piotrowska-Niczyporuk et al. 2012) suggesting that JA play a negative role in HM stress response in algae.

ET (200 μM ethephon) significantly improves metabolite profiles in *C. vulgaris*, especially that of proline content after 7-days treatment (Kim et al. 2016). Proline acts as an effective hydroxyl radical scavenger of ROS species under HM exposure (Smirnoff and Cumbes 1989). Similar to the case of sugars, proline has a critical role as a direct scavenger of ROS species in the protection of membranes, photosynthesis activities, and cellular homeostasis (Rejeb et al. 2014). 1-Aminocyclopropane-1-carboxylic acid (ACC), an ethylene precursor, also promoted astaxanthin accumulation (11 and 22% increase in 0.1 and 1 mmol $L^{-1}$ ACC-treated cultures, respectively) and upregulated transcriptional patterns of SOD, and CAT genes, which have an important function in HM stress response in *H. pluvialis* (Vo et al. 2016). Astaxanthin is a carotenoid which showed the greatest increase in concentration and antioxidant capacity under abiotic stress (Zhao et al. 2019b), and this process has been associated with metal cation chelating capacity (Kim et al. 2016). A similar effect to ET was observed for SA. The effect of SA has been well documented in *H. pluvialis*, where SA improved astaxanthin content, APX, and CAT enzyme activities and transcriptional expression patterns of eight carotenoid genes (Raman and Ravi 2011). Overall, the role of three hormones, ET, JA and SA, in algal HM tolerance ranges from forming ROS antioxidant enzymes and role in non-enzymatic antioxidant mechanisms (astaxanthin and proline) to minimizing HM stress-oxidative damage (Fig. 2).

## Phytohormone biosynthesis and signaling in algal HM stress response: a look beyond higher plants

The current evidence shows there are significant impacts of phytohormones on HM stress response in microalgae.

Although the response of endogenous phytohormones under HM stress remains largely unmeasured, the physiochemical effects of exogenous application and genome based-metabolic/signaling constructions suggest that phytohormones are functional and their signal transduction networks work in a manner similar to that of higher plants. The interactions between phytohormones and ROS detoxification system under HM stress occur in algae, where ROS detoxification seems to be a key player interacting with phytohormones as a part of HM tolerance (Fig. 2). The transcriptional activities of SOD and CAT genes were also regulated by ET precursor in *Haematococcus* (Vo et al. 2016). Thus, ET controls ROS transcriptional components, leading to the antioxidative defense. Less clear are the metabolic and signaling components of phytohormone metabolism in algae and their response to HM stress. Figure 3 integrates the available information on the current metabolomic and de novo transcriptomic studies involving the role of phytohormones. Based on the available information, phytohormone signaling pathways are proposed to be among the main mechanisms that control algae response to HMs. This summary will help direct our understanding of hormonal signal transduction in algae and how it can be manipulated to enhance HM tolerance.

Genes in biosynthesis pathways in some of the classic phytohormones (auxin, ABA, CKs, and ET) are structurally highly conserved in microalgae (Ju et al. 2015; Lu and Xu 2015). IPT genes, which are responsible for CK biosynthesis, form two functional classes in *Arabidopsis*: t-RNA degradation (*AtIPT02* and *AtIPT09*) and ATP/ADP pathway (*AtIPT1*, *AtIPT3–8*). The green microalgae *Micromonas* sp. RCC299 and *Ostreococcus tauri* have two IPT homologs, one of which is more closely related to ATP/ADP IPTs and the remaining putative algal IPT exhibits higher level of similarity to tRNA IPTs of *Arabidopsis* (Lu and Xu 2015). The unicellular red alga, *Porphyridium purpureum*, has homologs of the ET biosynthesis–related genes, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase enzyme and ACC oxidase (ACO) homologs (Ju et al. 2015). ET synthesis occurs in unicellular green algae *H. pluvialis* (Maillard et al. 1993), although the enzymes responsible for this synthesis are largely unknown.

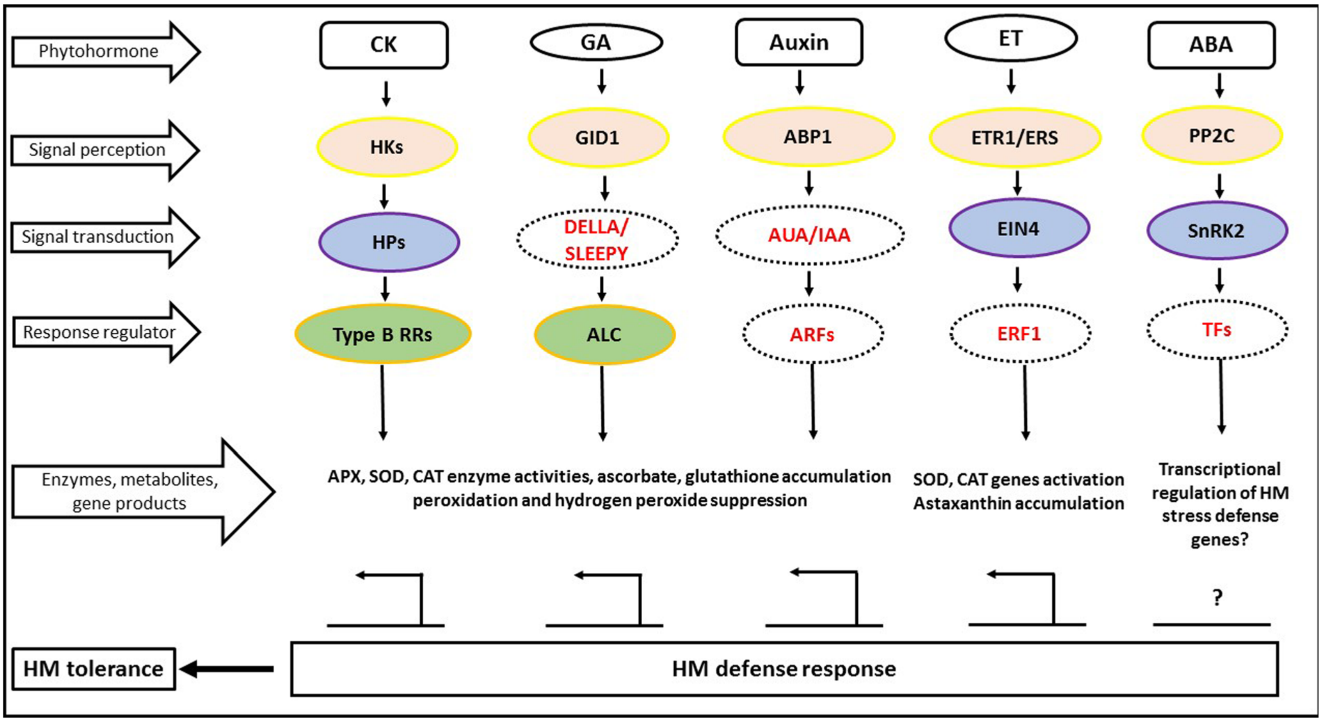

**Fig. 3** A schematic model of phytohormone signaling pathways involved in HM stress responses of algae. Dash-outlined oval shapes indicate proposed components that have not yet been identified in algae genomes. ABA abscisic acid, ABP1 auxin binding protein 1, ALC ALCATRAZ factors, APX ascorbate peroxidase, ARF AUXIN RESPONSE FACTOR, AUA/IAA auxin transcriptional repressors, CAT catalase, CK cytokinin, DELLA/SLEEPY DELLA-domain protein and the F-box protein SLEEPY1, EIN4 ETHYLENE INSENSITIVE4, ERF1 ETHYLENE RESPONSE FACTOR1, ET ethylene, ETR1/ERS ETHYLENE RESPONSE 1/ETHYLENE RESPONSE SENSOR, GA GIBBERELLIN, GID1 GIBBERELLIN INSENSITIVE DWARF1, HK HISTIDINE KINASE, HM heavy metal, HP HISTIDINE-CONTAINING PHOSPHOTRANSMITTER, PP2C PYRABACTIN RESISTANCE 1-like (PYL)–PROTEIN PHOSPHATASE 2C, SnRK2: SNF1-RELATED PROTEIN KINASE 2, SOD superoxide dismutase, TF ABA dependent/independent transcriptional factor, type B RR type B ARABIDOPSIS RESPONSE REGULATOR (Ju et al. 2015; Lu and Xu 2015; Sun et al. 2019)

Although known homologs of plant genes responsible for biosynthesis of phytohormones are highly conserved between higher plants and microalgae, this is not the case for signaling pathways. In the GA signaling pathway, orthologs encoding the GA receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) have been identified in microalgae. The remaining signaling components have been found in plants but not in microalgae. This includes the ALCATRAZ (ALC) response regulator and others which are involved in mediating the GA signaling such as the DELLA-domain proteins and the F-box protein SLEEPY1 (Lu and Xu 2015). Likewise, orthologs of the higher plant ET receptors, ETHYLENE RESPONSE 1/ETHYLENE RESPONSE SENSOR/ETHYLENE INSENSITIVE 4 (ETR1/ERS/EIN4), are widely present in microalgal lineages. However, there are no known orthologs for the remaining signaling components, such as ETHYLENE-INSENSITIVE 3 (EIN3) and the ERF1 response regulator (Ju et al. 2015). ABA signaling module PYRABACTIN RESISTANCE 1-like (PYL)–PROTEIN PHOSPHATASE 2C (PP2C)–SNF1-RELATED PROTEIN KINASE 2 (SnRK2) exists in the charophyte macroalgae (Sun et al. 2019). In microalgae, no ABA receptor has been identified, even though the downstream phosphatases from ABA signaling (SNF1-RELATED PROTEIN KINASE 2) are conserved (Lu and Xu 2015).

More composed than ET, ABA and GA signaling, CK signaling generally work as a two-component system that consists of sensor kinases (HKs), histidine phosphotransfer proteins (HPs), and response regulators (RRs) (To and Kieber 2008). Homologs of the CK receptor, ARABIDOPSIS HISTIDINE KINASE 1 (AHK1), are common in algal genomes. Homologs of the downstream CK signaling cascade (type B ARABIDOPSIS RESPONSE REGULATORS and HISTIDINE-CONTAINING PHOSPHOTRANSMITTER 1) also occur in green microalgae (Lu and Xu 2015). In auxin signaling, *C. variabilis* NC64A, *C. pyrenoidosa*, and *C. reinhardtii* genomes have putative orthologs of the auxin receptor AUXIN-BINDING-PROTEIN1 (ABP1) which are well characterized in higher plants (Lu and Xu 2015). Algae also share the conserved motif of Auxin Binding Protein 1 (ABP1) with land plants to form an auxin receptor.

Evidence has emerged that some phytohormone signaling components (likely not full sets except for CKs) of higher plants are present in microalgae. The current view is that phytohormones have a clear beneficial effect in HM stress alleviation in algae and their interplay with the ROS system is similar to the mechanisms known in higher plants. There is a possible existence of coordination signaling cascades between hormone signaling components and ROS detoxification genetic frameworks in microalgae under HM stress as proposed in this paper. However, these transcriptional trajectories need to be further explored (Fig. 3).

## Integration of omics approaches to study the function of phytohormones in HM stress response in algae

Hormonal application has potential to be a game-changing solution for HM bioremediation as well as global HM pollution challenges. Phytohormones can program algal cells to express specific metal binding proteins and strengthen antioxidant systems for enhanced HM tolerance (Table 1).

From unpredictable occurrences in HM contamination by global industries and agriculture, to ever-increasing health problems caused by the HM pollution, a thorough understanding of promising advances of algae and phytohormones provides an excellent remedy and proposes new forms of algae-based filtration methods for this scenario. Unraveling thus-far overlooked mechanisms of phytohormonal regulation of metal stress response will improve our knowledge of algae stress physiology, allowing for identification of new pathways for HM stress tolerance. Realizing these goals will require enormous efforts of researchers and industries to extend the utility and economic feasibility of phytohormone applications for HM stress alleviation in algae. These efforts should be directed towards studying (i) comprehensive inventories of exogenous application of phytohormones under HM stress, (ii) systematic characterization of hormone metabolism and signaling components under HM stress, and (iii) in algal characterization of potential molecular components of HM stress response.

The effect of phytohormones on HM stress response is dose dependent (Han et al. 2018). During stress, hormonal treatment generates economically valuable biomass associated with production of lipids, biofuels, and other value-added compounds (Zhao et al. 2019a). There is a need to apply a combination of suitable doses of phytohormones and omics-based approaches, such as metaproteomics and metabolomics, to illustrate mechanisms of bio-absorption/accumulation/chelation in HM stress controlled by phytohormones. More research on scale-up studies and technical aspects of phytohormone bioremediation systems are needed to effectively remove HMs and stimulate beneficial algae biomass production. While clear evidence exists to support the claim that phytohormones regulate HM stress responses via antioxidative systems and metal binders, an obvious question remains—can these aspects provide practical value for using microalgae in “phycoextraction” of HM-contaminated soils and water bodies on the industrial scale? Can algal phytohormone–based remediation be used for a wide range of HM bioremovals? Is it feasible to extract metal-binding compounds as well as algal bioactive compounds from HM algal filtration systems? Future work should provide further insight towards novel algae-based filtration techniques and methods to combat the global metal pollution.

Phytohormones work interactively through the complex signaling networks and they often cross-talk with each other. However, compared with higher plants, hormonal signaling networks and their response to HMs in algae is still in its infancy and there is a need to identify hormone-related HM stress–responsive algal genes using transcriptomic approaches. The next important step in this context will be in algal characterization, via overexpression, of potential phytohormone signaling components under HM exposure, to produce transgenic algae. In the view of successful results achieved previously using transgenic approaches for bioremediation, such as in *C. reinhardtii* (Cai et al. 1999; Siripornadulsil et al. 2002; Ibuot et al. 2017), algae bioremediation could benefit from genetic modification of the phytohormone biosynthesis components as well as the molecular signaling cascades. Hence, several issues need to be considered before the transgenic algae can be utilized in industrial application (Cheng et al. 2019). CRISPR/Cas technology for algal genome editing to induce knockout hormonal signaling components is also an option. Based on the successful results achieved using CK-deficient *Arabidopsis* for HM bioremediation (Mohan et al. 2016; Jiang et al. 2019), CK-deficient algae obtained through the editing of CK biosynthesis genes is a promising option. Furthermore, a new pathway of HM-binding compounds and hormone-associated components can be discovered based on these functional studies, thus opening new exciting areas in algal HM stress physiology and phytohormone studies.

## Conclusions and future needs

Over the last decade, significant efforts have been made to understand the biological functions of phytohormones in HM stress tolerance in higher plants. As discussed in this review, the available studies indicate that HM stress tolerance in microalgae can also be improved by phytohormones (CKs, Auxin, GAs, BRs, and ABA). Although the physiological roles of ET and SA remain largely unknown in HM stress response in microalgae, the essential and bioactive forms of these phytohormones have been detected in a wide range of algal lineages (Table 1). The biological insights derived from these data should act as significant drivers of translational innovation and lead to broadening the algal industry applications towards remediation strategies for HM pollution. For a more complete understanding of the potential of phytohormones in algal bioremediation systems, there is a need for a sustained collaborative endeavors on several fronts to generate a comprehensive picture from transcriptomic and metabolomic trajectory maps of the bioremediation mechanisms and to design scale-up studies that can be translated into algal biotechnology.

**Authors' contributions** Hai Ngoc Nguyen: conceptualization; visualization; writing, original draft; writing, review and editing. Anna B. Kisiala: writing, review and editing. R.J. Neil Emery: funding acquisition, supervision, writing, review and editing.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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