J. Phycol. **39**, 1008–1018 (2003)

REVIEW

HEAVY METAL–INDUCED OXIDATIVE STRESS IN ALGAE[1]

Ernani Pinto

Projeto CEPEMA (Centro de Ensino e Pesquisa do Meio Ambiente), Escola Politécnica da Universidade de São Paulo and Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciencias Farmaceuticas, Universidade de São Paulo, São Paulo CEP 05508-900

Teresa C. S. Sigaud-Kutner, Maria A. S. Leitão, Oswaldo K. Okamoto

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 26077, CEP 05599-970, São Paulo, SP, Brazil

David Morse

Département de Sciences Biologiques, Université de Montréal, Canada

and

Pio Colepicolo[2]

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 26077, CEP 05599-970, São Paulo, SP, Brazil

Heavy metals, depending on their oxidation states, can be highly reactive and, as a consequence, toxic to most organisms. They are produced by an expanding variety of anthropogenic sources suggesting an increasingly important role for this form of pollution. The toxic effect of heavy metals appears to be related to production of reactive oxygen species (ROS) and the resulting unbalanced cellular redox status. Algae respond to heavy metals by induction of several antioxidants, including diverse enzymes such as superoxide dismutase, catalase, glutathione peroxidase and ascorbate peroxidase, and the synthesis of low molecular weight compounds such as carotenoids and glutathione. At high, or acute, levels of metal pollutants, damage to algal cells occurs because ROS levels exceed the capacity of the cell to cope. At lower, or chronic, levels algae accumulate heavy metals and can pass them on to organisms of other trophic levels such as mollusks, crustaceans, and fishes. We review here the evidence linking metal accumulation, cellular toxicity, and the generation of ROS in aquatic environments.

Key index words: **algae; antioxidant enzymes; heavy metals; oxidative stress**

Abbreviations: **APX, ascorbate peroxidase; GPX, glutathione peroxidase; GSH, reduced glutathione; PC, phytochelatin; ROS, reactive oxygen species; SOD, superoxide dismutase; MT, metallothionein**

[1] Received 13 December 2002. Accepted 13 July 2003.
[2] Author for correspondence: e-mail piocolep@quim.iq.usp.br.

HEAVY METALS IN THE ENVIRONMENT

Metals occur naturally, and several of them are essential components of global ecosystems. They are present in the environment with a wide range of oxidation states and coordination numbers, and these differences are related to their toxicity. Metals such as copper (Cu) and zinc (Zn) are essential to life, whereas others such as lead (Pb) and mercury (Hg) are not known to perform a useful biochemical function (Allan 1997). Environmental pollution by metals became extensive as mining and industrial activities increased in the late 19th and early 20th century. The current worldwide mine production (Table 1) of Cu, Cd, Pb, and Hg is considerable (Kennish 1996). These pollutants, ultimately derived from a growing number of diverse anthropogenic sources (industrial effluents and wastes, urban runoff, sewage treatment plants, boating activities, agricultural fungicide runoff, domestic garbage dumps, and mining operations), have progressively affected more and more different ecosystems (Macfarlane and Burchett 2001).

It appears that human activity is often closely linked with toxic pollution. In one study comparing a developed with an undeveloped seawater bay in Massachusetts (Pospelova et al. 2002), pollution in the developed area caused a drastic decline in phytoplankton species richness as determined from analyses of dinoflagellate cysts in the sediment. Interestingly, this study concluded that 72% of the variability in species richness could be attributed to increases in copper. A phytotoxicity test, evaluating the inhibition of photosynthesis in the green alga *Chlamydomonas* by heavy metals (Hg, Cu, Pb, and Cd) leaching from salt mine wastes, showed that inhibition varied from about 100% (Hg) and 80% (Cd) to almost 0% for Pb (Wundram et al. 1996).

TABLE 1. Current worldwide mine production of copper (Cu), cadmium (Cd), lead (Pb), and mercury (Hg); the annual discharge into the ocean; oceanic water concentration under normal and polluted conditions; the volume of seawater potentially contaminated by the annual discharge; and toxicity of metals to phytoplankton in marine environment.

Metal	Production ($tons \cdot year^{-1}$)	Discharge ($tons \cdot year^{-1}$)	Oceanic water ($ng \cdot mL^{-1}$)	Polluted seawater ($ng \cdot mL^{-1}$)	Potential volume of contaminated seawater (m^3)	Growth inhibition, EC^{50} ($ng \cdot mL^{-1}$)
Hg	2×10^3	30	0.001	>0.01	3×10^{12}	>0.4
Cd	1×10^4	60	0.02	>1	0.06×10^{12}	>25
Pb	3.5×10^3	2350	0.03	>5	0.5×10^{12}	>250
Cu	9×10^6	4500	0.10	>2	2×10^{12}	>10

Data from Davies 1978; Hollibaugh et al. 1980; Kennish 1996; Mason et al. 1996; Morel et al. 1998; Faganeli et al. 2003; Hylander and Meili 2003.

Heavy metals are usually present at low concentrations in oceanic surface waters and arrive there by atmospheric transport and upwelling (Table 1). Higher levels occur in coastal waters, however, because of river runoff. Close to urban centers, pollution is associated with sewage outlets (Wickfors and Ukeles 1982, Rebhun and Amotz 1984), but levels are also elevated near extensive areas of industry (Cotté-Krief et al. 2000, Bu-Olayan et al. 2001, Esser and Volpe 2002). Interestingly, nutrient availability (in particular, nitrogen) dramatically increases the ability of algae to accumulate heavy metals (Wang and Dei 2001a,b), suggesting that agricultural runoff into fresh water and coastal areas will greatly increase the entry of heavy metals into the food chain. The annual discharge into the ocean (Table 1) from different sources was estimated some years ago to be substantial (Davies 1978).

In the natural environment, organisms living in chronically polluted sites are exposed to low concentrations of metals for long periods. In other cases, organisms may be abruptly exposed to high levels of metals upon the outfall of a pollutant in coastal waters. Though toxic at high concentrations, Cu and Zn are micronutrients essential for the activity of many enzymes and part of molecules playing key roles in photosynthetic electron transport (Raven et al. 1999), as distinct from Hg and Pb, which are toxic but nonessential elements. The storage of metals by cellular detoxifying mechanisms makes them available for assimilation by the biota and biomagnification along the aquatic food chain, with the potential for producing adverse effects throughout the marine environment. In this context, standard biotoxicity tests are useful tools to determine the effects of pollutants on cell growth and viability. These tests are especially important for evaluating the potential impact of pollution on aquatic ecosystems, aiming to prevent possible injuries to the biota by establishing maximum tolerable levels of toxicants (Laube et al. 1980, Chapman et al. 1996).

The mechanism underlying toxicity of heavy metals is not always clear. In some cases, such as the inhibition of photosynthesis in the marine diatom *Phaeodactylum tricornutum* by Cd, inhibition of diatoxanthin epoxidation to diadinoxanthin in the xanthophyll cycle was suggested to be responsible (Bertrand et al. 2001). More generally, however, heavy metal toxicity is related, at least in part, to the oxidative stress induced in living systems (Quinlan et al. 1988, Robinson et al. 1994, Okamoto and Colepicolo 1998, Adonaylo and Oteiza 1999, Livingstone 2001, Wang and Shi 2001). Heavy metals can promote oxidative damage both by directly increasing the cellular concentration of reactive oxygen species (ROS) (Winterbourn 1982) and by reducing the cellular antioxidant capacity (Sies 1999). In contrast to higher plants, the antioxidant response to oxidative and environmental stress has not been extensively investigated in algae at the molecular level (Reed and Gada 1990, Rodriguez-Ariza et al. 1991, Holovskà et al. 1996, Okamoto et al. 1996).

In recent years it is interesting to note the development of programs using microorganisms for water and wastewater treatment, heavy metal control in natural waters and industrial waste streams, and even biological detoxification. For example, potential tools for bioremediation of Cr pollution using algae have been described (Cervantes et al. 2001), and it seems clear that different species of algae accumulate metals to various degrees (Jordanova et al. 1999). Many parameters affect the accumulation of heavy metals from solution by *Chlorella vulgaris* (Bajguz 2000, López-Suárez et al. 2000). Likewise, the biological concentration factor (defined as C_b/C_w, where C_b is the metal concentration in the biota expressed as μmol or $\mu g\, g^{-1}$ of dry weight and C_w is the metal concentration in water given in μmol or $\mu g\, mL^{-1}$) for Cu, Pb, Cd, and Hg in *Porphyra* spp. and *Enteromorpha* spp. changed seasonally in field conditions and was specific for each metal reproducibly over several years (Vasconcelos and Leal 2001). The accumulation of $^{65}Zn(II)$ from seawater in the gastropod *Haliotis diversicolor supertexta* (via the alga *Gracilaria tenuistipitata*) indicated that both species accumulate considerable amounts of this metal with the concentration in algae 170-fold greater than in seawater (Lin and Liao 1999). The biological concentration factor values for inorganic trace elements in 35 species of algae changed with the element tested but reached up to 6 orders of magnitude for elements that exist mainly as 3^+ or 4^+ valence and rare earth elements (Hou and Yan 1998).

The use of algae in bioremediation depends on their ability to survive potentially toxic treatments. However, algae have been used to identify areas of trace metal contamination because of the absorption and toxicity of heavy metals (Muse et al. 1999, Yu et al. 1999). To reconcile these observations, it must be noted that accumulation of heavy metals is not always related to their toxicity. Heavy metal tolerance has been demonstrated for the green algae *Chlorella* and *Scenedesmus*. In particular, *Scenedesmus acutus* strains tolerant to Cr were also tolerant to Cu but not to Zn (Abd-El-Monem et al. 1998). To understand tolerance and to maximize the potential application of algae to bioremediation, it is essential to understand why heavy metals are toxic and how algae can defend against them.

ROS AND OXIDATIVE STRESS

Generation of ROS by respiration and photosynthesis. A number of different ROS, including the superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), singlet oxygen (O_2 ($^1\Delta_g$)), and the hydroxyl radical ($^{\cdot}OH$), occur transiently in aerobic organisms. These species are normal byproducts of oxidative metabolism and pose a constant threat to all aerobic organisms. Although some of them may function as important signaling molecules that alter gene expression and modulate the activity of specific defense proteins, all ROS can be extremely harmful to organisms at high concentrations. ROS can oxidize proteins, lipids, and nucleic acids, often leading to alterations in cell structure and mutagenesis (Halliwell and Gutteridge 1999).

Production of ROS constitutes a particularly severe threat to photosynthetic organisms, because a common biological source of $O_2^{\cdot -}$ is the single-electron reduction of molecular oxygen by electron transport chains. Indeed, because of the intense electron flux in their microenvironment, which also contains elevated oxygen and high metal ion concentrations, the mitochondria and chloroplasts of photosynthetic organisms are cell compartments highly susceptible to oxidative injury. Paradoxically, trace metals play key roles in photosynthetic electron transport in thylakoids of O_2-evolving organisms, participating in enzymes that remove ROS such as ascorbate peroxidase (APX) (Fe), Fe-superoxide dismutase (SOD) (cyanobacteria, higher plants, and most algae), and Cu-Zn-SOD (some algae and higher plants). In addition, they are part of essential components to the photosystems (Fe) or mobile electron carriers such as the iron-containing cytochrome c_6 and the copper-containing plastocyanin (Raven et al. 1999).

The effect of ROS in photosynthetic organisms is exacerbated by excessive illumination. For instance, excessive light energy input may increase the levels of excited molecules such as triplet chl and singlet state O_2 ($^1\Delta_g$), the latter being highly electrophilic and capable of oxidizing many other molecules. Moreover, photochemical production of $O_2^{\cdot -}$, generated by oxygen reduction in PSI (the Mehler reaction) results in diffusion of $O_2^{\cdot -}$ into the stroma where it is dismutated to O_2 and H_2O_2. The reaction of H_2O_2 with reduced metal ions produces $^{\cdot}OH$, a strong oxidant that can react with and damage biomolecules (Takeda et al. 1995). To add to the problem, chloroplasts have a complex system of membranes rich in polyunsaturated fatty acids, which are potential targets for peroxidation (Halliwell and Gutteridge 1999). Thus, although many ROS-generating processes are slow under normal conditions, pollutant metals, xenobiotics, and environmental factors such as high light or UV exposure can accelerate them. Higher levels of chloroplastic antioxidants would be critical to withstand photooxidative stress elicited by a reduced energy-utilizing capacity, as a consequence of metal toxicity (Okamoto et al. 2001a).

Generation of ROS by heavy metals. Many environmental factors can induce oxidative stress in the cell by generation of $O_2^{\cdot -}$. Therefore, modulation of antioxidant levels constitutes an important adaptive response to withstanding adverse conditions. Indeed, maintenance of a high antioxidant capacity in cells has been linked to increased tolerance against different kinds of environmental stress (Pedrajas et al. 1993, Dat et al. 1998, Thomas et al. 1999).

The intoxication with pollutant metals induces oxidative stress because they are involved in several different types of ROS-generating mechanisms (Fig. 1) (Stohs and Bagchi 1995). For example, transition metals (such as Fe^{3+} and Cu^{2+}) participate in the well-known Haber-Weiss cycle, producing $^{\cdot}OH$ from $O_2^{\cdot -}$ and H_2O_2 (Winterbourn 1982). Metals without redox capacity (such as Cd^{2+}, Pb^{2+}, and Hg^{2+}) can enhance the pro-oxidant status by reducing the antioxidant glutathione (GSH) pool, activating calcium-dependent systems and affecting iron-mediated processes. These heavy metals can also disrupt the photosynthetic electron chain, leading to $O_2^{\cdot -}$ and O_2 ($^1\Delta_g$) production (Asada and Takahashi 1987). Finally, metals such as Cr(VI) have been shown to generate $^{\cdot}OH$ radicals from H_2O_2 via a Fenton-type mechanism (Shi and Dalal 1990). Thus, algal tolerance to heavy metal pollution in the environment is likely to depend heavily on defense responses that prevent oxidative insult.

Cellular defense mechanisms against ROS. Organisms have developed a wide range of protective mechanisms that serve to remove ROS before they can damage sensitive parts of the cellular machinery. These can be conveniently divided into low molecular weight compounds (Table 2), such as GSH, phenolics, ascorbate, flavonoids, tocopherols, and carotenoids, as well as enzymatic catalysts of high molecular weight (Table 3).

Carotenoids are widely distributed naturally occurring pigments found in bacteria, yeast, algae, plants, animals, and humans (Britton et al. 1995). Fucoxanthin, peridinin, astaxanthin, and β-carotene are the most abundant carotenoids found in the aquatic environment and are located principally in the chloroplasts of many phytoplankton (Fig. 2). The role

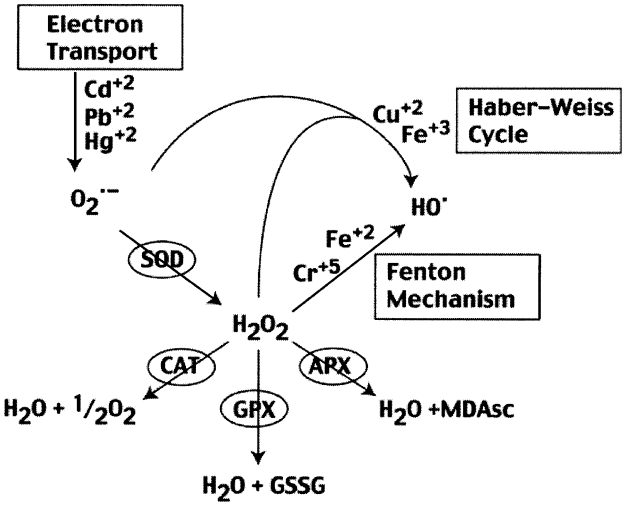

FIG. 1. Heavy metal stress induces cellular generation of ROS.

of these carotenoids is 2-fold: Not only do they aid in broadening the spectrum of PAR but they also protect the light-harvesting pigments in the antenna complexes against photochemical damage caused by excited triplet states (Krinsky 1989, Frank and Cogdell 1996, Pinto et al. 2003) and other ROS (Woodall et al. 1997). For example, the photoprotective role of ß-carotene as an O_2 ($^1\Delta_g$) quencher was established in the PSII reaction center isolated from *Pisum sativum* (Telfer et al. 1994). Peridinin (Fig. 2), an allenic oxicarotenoid that extends the range of absorption for light harvesting in the dinoflagellates, is associated with protein and chl *a* in a complex called peridinin-chlorophyll-protein (Larkum 1996). In the dinoflagellate *Amphidinium carterae*, each holoprotein in the soluble trimeric peridinin-chlorophyll-protein complex contains four peridinin molecules and one chl. The three-dimensional structure of this complex facilitates an efficient excitonic energy transfer from peridinin to chl (Hofmann et al. 1996, Hollnagel et al. 2002). Like ß-carotene, peridinin can also suppress electronically excited molecules such as O_2 ($^1\Delta_g$), which has been shown to be capable of inducing DNA damage and to be mutagenic (Di Mascio et al. 1990, Hollnagel et al. 1996). In general, quenching efficiency is directly proportional to the number of conjugated double bonds (Foote et al. 1970), and this holds true for peridinin whose quenching efficiency is roughly 10-fold lower than ß-carotene. However, HPLC analysis of pigments in the dinoflagellate *Lingulodinium polyedrum* indicates that peridinin is much more abundant than ß-carotene (Di Mascio et al. 1995), suggesting that despite its lower efficiency, it may contribute substantially to quenching. Fucoxanthin, a carotenoid with a structure similar to peridinin, is reported to be roughly 10 times less effective a quencher than ß-carotene and is therefore in the same range as peridinin (Pinto et al. 2000, Barros et al. 2001). Clearly, it is of great importance to understand the quenching ability of biological compounds, such as carotenoids, in the context in which they are normally found to adequately evaluate the role they may play in protection against chlorophyll-induced photosensitization.

TABLE 2. Cellular targets of natural low molecular weight antioxidants.

Compounds	Target
Ascorbate	O_2 ($^1\Delta_g$), $^{\cdot}OH$, $O_2^{\cdot-}$, $HO_2^{\cdot}$
β-Carotene	O_2 ($^1\Delta_g$), $RO_2^{\cdot}$
α-Tocopherol	$RO_2^{\cdot}$
Glutathione	Nonspecific
Urate	O_2 ($^1\Delta_g$), metal chelator
Metallothionein	$^{\cdot}OH$, metal chelator
Flavonoid	Plant antioxidant of $^{\cdot}OH$ and HOCl
Phytochelatin	Metal chelator

Other low molecular weight compounds include α-tocopherol (vitamin E), flavonoids of various types, ascorbate, and GSH (Table 2). Flavonoids are widely distributed in higher plants and, by directly scavenging $^{\cdot}OH$, ONOOH, and HOCl, are associated with strong inhibition of lipid peroxidation. The scavenging efficiency of flavonoids is directly proportional to the number of hydroxyl groups bound to the phenols because this allows the flavonoids to bind metal ions. Ascorbate is of particular interest as an electron donor for hydroxyl radicals (for which there are no enzymatically catalyzed detoxification mechanisms) and as a substrate for APX (see below). The reductant GSH, a tripeptide composed of glutamate, cysteine, and glycine, is nonspecific and is also both a general reductant and a substrate for enzymatically catalyzed reactions. It serves as a cofactor for some enzymatic reactions and as an aid in the rearrangement of protein disulfide bonds. The role of GSH as a reductant is extremely important, particularly in the highly oxidizing environment of photosynthetic cells. The sulfhydryl of GSH can be used to reduce peroxides formed during partial reduction of oxygen. The resulting oxidized form of GSH consists of two molecules disulfide-bonded together (abbreviated GSSG). The enzyme GSH reductase uses NADPH as a cofactor to reduce GSSG back to two molecules of GSH.

With regard to the high molecular weight compounds, aerobic organisms express a battery of enzymes that contribute to the control of cellular ROS levels (Table 3). SOD, catalase, glutathione peroxidase (GPX), APX, lipid peroxidase glutathione reductase, thioredoxin, and peroxiredoxin are considered the main natural antioxidant enzymes (Fig. 1) (Rice-Evans et al. 1996, Asada 1999). Peroxiredoxin catalyses the breakdown of alkyl hydroperoxides into water and their corresponding alcohols (Rouhier and Jacquot 2002). Catalase and GPX catalyze the production of H_2O from the degradation of H_2O_2 and ROOH, respectively, whereas APX reduces H_2O_2 to H_2O using ascorbate as electron donor (Fridovich 1997).

SOD, which catalyzes the disproportionation of $O_2^{\cdot-}$ to O_2 and H_2O_2, has been called the cell's first line of

TABLE 3. Cellular antioxidant enzymes.

Enzyme	Reaction catalyzed
Superoxide dismutase	$2\ O_2^{\cdot -} + 2\ H^+ \rightarrow H_2O_2 + O_2$
Catalase	$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
Glutathione peroxidase	H_2O_2 or ROOH + 2 GSH → 2 H_2O or ROH + GSSG
Ascorbate peroxidase	H_2O_2 + Ascorbate → H_2O + Monodehydroascorbate
Thioredoxin	Prot-S_2 + Prot'$(SH)_2$ → Prot$(SH)_2$ + Prot'-S_2
Peroxiredoxin	ROOH + R'$(SH)_2$ → ROH + R'S_2 + H_2O
Glutathione reductase	GSSG + NAD(P)H + H^+ → 2 GSH + $NAD(P)^+$

defense against ROS (Hassan and Scandalios 1990). This is because $O_2^{\cdot -}$ is a precursor to several other highly reactive species, so that control over the steady-state $O_2^{\cdot -}$ concentration by SOD constitutes an important protective mechanism (Fridovich 1997). Three major SOD isoforms have been described in eukaryotic photosynthetic organisms (Asada 1999): a CuZnSOD located in the thylakoid membranes and cytosol of higher plants, certain dinoflagellates, and charophycean green algae; a MnSOD isoform found within mitochondria; and an FeSOD isoform in the chloroplast stroma. Indeed, FeSOD is considered to be the major $O_2^{\cdot -}$ scavenger in chloroplasts, whereas MnSOD is the most active scavenger in mitochondria (Fridovich 1997). Interestingly, SOD is induced by its substrate (Colepicolo et al. 1992, Allen and Tresini 2000), and thus activation of specific SOD isoforms can serve as an indicator of the cell compartment experiencing pollutant-induced $O_2^{\cdot -}$ levels. Although SOD genes have been isolated from many different species, the FeSOD isoform has been reported from only a few. Recently, Okamoto et al. (2001b) reported the isolation and molecular cloning of the FeSOD isoform from the marine dinoflagellate *L. polyedrum*, and the changes in expression of this enzyme during algal growth have been described (Sigaud-Kutner et al. 2002). Finally, as might be expected on the basis of the involvement of SOD in mitigating the effects of light, SOD activity increases after exposure of *Gracilariopsis tenuifrons* to visible light (Rossa et al. 2002).

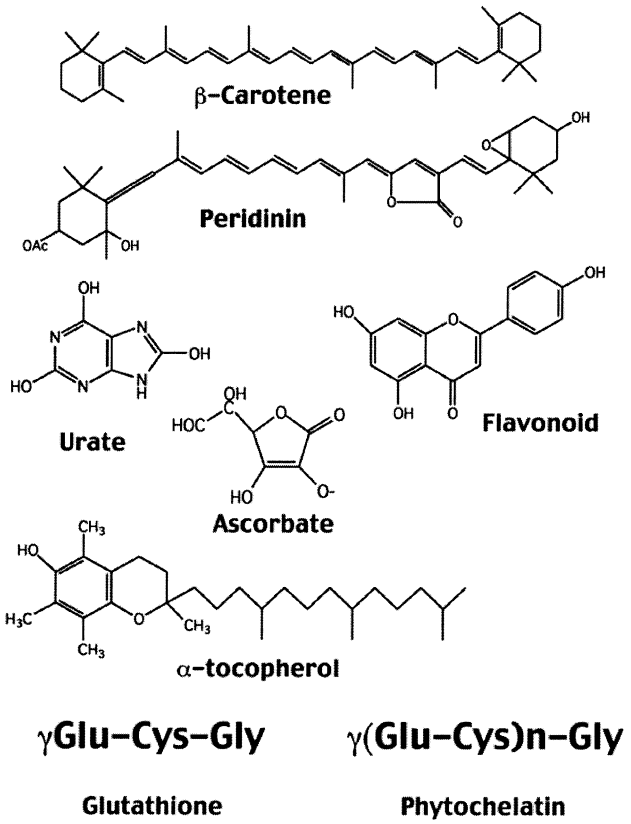

FIG. 2. The most common low molecular weight antioxidants.

ALGAL RESPONSES TO HEAVY METALS

Entry of heavy metals into cells. Toxic metal ions are able to cross membranes, and several possible mechanisms have been suggested to account for their transport (Van Ho et al. 2002, Zalups and Ahmad 2003). One type of mechanism is described as molecular mimicry, whereby metals either compete for binding to multivalent ion carriers (such as Ca^{2+} channels) or, after binding to low molecular weight thiols (such as cysteine), enter the cell by active transport (e.g. using amino acid transporters). In another type of mechanism, metals bound to chelating proteins (such as metallothioneins; see below) may enter the cell by endocytosis. The heavy metals can cause membrane depolarization and acidification of the cytoplasm (Cumming and Gregory 1990, Cardozo et al. 2002, Conner and Schimid 2003), and in fact, membrane injury is one important effect of metal ions that may lead to disruption of cellular homeostasis. Thus, cellular adaptations such as exudation of chelating compounds and active efflux of metal ions by primary ATPase pumps can provide some degree of metal tolerance (Cumming and Gregory 1990, Rosen 1996).

As an alternative to keeping metals outside the cell, cells can also induce the synthesis of protective proteins. In addition to the antioxidant proteins described above, there are also a number of metal chelators such as metallothioneins (MTs) and phytochelatins (PCs) (Cobbett and Goldsbrough 2002). Both are cysteine-rich polypeptides and owe their chelating activity to their ability to coordinate metals using the sulfhydryl groups on the protein. However, PCs are small, generally from 5 to 11 amino acids long, and are

formed by condensation of glutamate and cysteine via a pathway also involving GSH. In contrast, MTs are synthesized by translation of mRNA and can be up to several hundred amino acids long. The induction mechanisms also differ for the two classes of chelators (Steffens 1990). Increased rates of PC synthesis require the formation of a complex between GSH and a heavy metal, generally Cd^{+2}. Thus, new PC synthesis does not appear to require new synthesis of GSH metabolizing enzymes. Interestingly, metal binding by PC is relatively specific for Cd, at least in plants, because PC-deficient mutants are sensitive to Cd but not to other metals such as Cu, Hg, Zn, or Ni (Ha et al. 1999). In contrast to the PCs, induction of MT involves transcriptional control mechanisms. For example, Cu has been observed to induce MT gene expression in the seagrass *Posidonia oceanica* (Giordani et al. 2000) and the brown alga *Fucus vesiculosis* (Morris et al 1999). It is also possible that heat shock proteins may play a role in the cellular defense, although this does not seem to be due to a metal chelating activity (Vierling 1990).

The relationship between ROS and heavy metal toxicity. The effects of heavy metal on ROS metabolism in algae are varied (Adonaylo and Oteiza 1999). In the marine dinoflagellate *L. polyedrum*, heavy metals cause increased oxidation of proteins and lipids; increased levels of SOD, APX, and ß-carotene; and a decreased GSH content (Okamoto and Colepicolo 1998, Okamoto et al. 2001a). Interestingly, pollutant metal treatment of this organism induces the MnSOD and FeSOD isoforms, whereas the activity of the cytosolic CuZnSOD isoform is not significantly altered. These cellular responses may involve transcriptional control, because exposure of *L. polyedrum* to toxic metals results in an increased level of the FeSOD mRNA (Okamoto et al 2001b). In any event, the induction of both organellar isoforms suggests that both mitochondrial and chloroplast electron transport systems are affected by heavy metals. Increased SOD activity after treatment with Cd was noted both in the prasinophycean *Tetraselmis gracilis* (Okamoto et al. 1996) and in the diatom *Ditylum brightwellii* (Rijstenbil et al. 1994), but cellular responses can differ in other algae. In the green unicellular alga *Selenastrum capricornutum*, increased APX activity has been observed (Sauser et al. 1997), whereas a decreased GSH redox ratio has been reported in the green macroalga *Enteromorpha prolifera* (Rijstenbil et al. 1998) and in the freshwater macrophyte *Ceratophyllum demersum* (Devi and Prasad 1998). Nagalakshmi and Prasad (2001) observed increases in APX, SOD, and GPX activities in *Scenedesmus bijugatus* exposed to different copper concentrations (0–100 µM). Moreover, they also observed a progressive depletion of GSH content in the cells with increasing concentrations of Cu. The alteration of the equilibrium between synthesis and utilization of GSH was attributed to its antioxidant role or its use as precursor in the synthesis of phytochelatins (Nagalakshmi and Prasad 2001). Clearly, the general theme is an increase in antioxidant defense mechanisms, although the particular players involved may vary in different organisms.

Regarding the effects of metals on macroalgae, Segot et al. (1983) found significant reductions in growth rates at 0.31 ppm Cd^{2+} and 0.092 ppm Cu^{2+} in the red algal species *Asparagopsis armata*. In outdoor pond cultures of the red alga *Gracillaria tenuistipitata*, additions of Cu^{2+} have been used to decrease epiphytism by the green macroalga *Enteromorpha intestinalis*, because *G. tenuistipitata* is more resistant to Cu^{2+} than the green alga (Haglund et al. 1996). Similarly, Cu^{2+} additions have been used in *Gracilaria gracilis* cultures to reduce infestations of the brown alga *Ectocarpus siliculosus* (VanHeerden et al. 1997). In the red algae *Mastocarpus stellatus* and *Chondrus crispus*, a correlation was found between the general level of ROS metabolism and oxidative- and general-stress tolerance (Collen and Davison 1999). Although toxic at high concentrations, the effects of Cu^{2+} could be attenuated under chronic conditions because it is a micronutrient essential for the activity of several enzymes, including SOD. The bioaccumulation of Cd^{2+} by the red alga *Porphyra umbilicalis* and by several seaweeds has also been detected (McLean and Williamson 1977, Hu et al. 1996).

The metals Cu^{2+} and Cd^{2+} have received much attention because of their toxic effects on plants and other organisms. Copper plays a dual role in the metabolism of photosynthetic organism. It is both a micronutrient, for example, as an important part of oxidases (e.g. cytochrome oxidase and amino oxidases) and in electron transport chain components (e.g. plastocyanin), but it is also highly toxic (Fernandes and Henriques 1991). This duality extends to the generation of ROS as well. Not only is Cu^{2+} an important part of the reactive oxygen scavenging system (e.g. CuZnSOD), but it is also able to cause oxidative stress through increased production of ROS via its toxic effects on photosynthesis. Cadmium has no known metabolic function in macroalgae and, in contrast to Cu^{2+}, cannot contribute to $^{\cdot}OH$ formation in the Fenton reaction (Halliwell and Gutteridge 1999). However, Cd^{2+} has been shown to be a co-factor in a carbonic anhydrase of the diatom *Thalassiosira weissflogii* (Lane and Morel 2000), whereas in higher plants Cd^{2+} causes disturbances in growth, photosynthesis, ion- and water transport, and general decreases in enzyme activities due to reactions of Cd^{2+} with thiol groups (Prasad 1995). Although probably not essential for growth of macroalgae, Cd^{2+} is readily taken up. The uptake mechanism is not known but is partially light dependent (Hu et al. 1996) and requires protein synthesis (McLean and Williamson 1977).

When the levels of ROS formed exceed the ability of the antioxidant system to cope with them, damage to cellular compounds occurs. Thus, increased levels of oxidized proteins and lipids are indicative of a state of oxidative stress. Comparisons between the biological effects of Cu^{2+} and Cd^{2+} indicate that the former is proportionally more efficient in causing oxidative stress than the latter, whose effects probably lean more

toward nonoxidative stress-invoking reactions. It has been suggested that Cu^{2+} (as well as Pb^{2+} and Zn^{2+}) interacts directly with the thylakoid membranes in the chloroplast, whereas Cd^{2+} (and Ni^{2+}) interferes with other metabolic processes in plants (Szalontai et al. 1999). Because Cu^{2+} primarily triggers oxidative stress in the chloroplasts, an increase in chloroplastic APX and SOD levels is probably an efficient way to avoid the detrimental effects of this metal. An increase in the activity of these antioxidant enzymes reduces the concentrations of $O_2^{\cdot -}$ and H_2O_2 formed in the chloroplast, thereby reducing the risk of $^{\cdot}OH$ formation through cycling between Cu^{+} and Cu^{2+}. Indeed, increases in peroxidase activity are regarded as a reliable indicator of stress or potential phytotoxicity of heavy metals, the increases in peroxidase activity being a response to an increase in peroxides, disruption of the plasma membrane by lipid peroxidation, and the ROS produced by heavy metal accumulation (Macfarlane and Burchett 2001).

The induction of antioxidants in response to enhanced ROS production is generally proportional to the duration and severity of the stress applied to algal cultures (Okamoto et al. 1996). There are also distinct changes in the antioxidant response to chronic or acute treatment with metals, suggesting a different oxidative status for these two types of metal stress (Okamoto et al. 2001a). For example, enhanced levels of cellular antioxidants could allow cells to acclimatize to increased steady-state concentrations of ROS during chronic stress. In contrast, the abrupt generation of high levels of ROS over a short period of acute stress will usually exceed the total antioxidant capacity. It seems likely that overloading of antioxidant capacity of the low molecular weight compounds, such as GSH, NADPH, and ascorbate, will occur first. These are all related, because no active transport of ascorbate has been reported in chloroplasts and reduced ascorbate must be regenerated by the ascorbate/GSH cycle, in which GSH and NADPH both participate. Acute stress, which lowers both the GSH pool and depletes NADPH levels, will thus provoke rapid oxidation of the ascorbate pool. A decrease in ascorbate availability will as a result limit not only APX but also all peroxidase activity. To investigate this further, the O_2 uptake and the GSH pool in metal-treated cells can be monitored. A useful estimate of oxidative stress is the content of GSH relative to its oxidized form GSSG (Sies 1999). Increased O_2 uptake is also an useful index of oxidative stress because it is often associated with formation of $O_2^{\cdot -}$, H_2O_2, and peroxy radicals, during reduction of O_2 by components of electron-transport chains (Halliwell and Gutteridge 1999).

Interestingly, some dinoflagellate algae have developed an important defense mechanism: encystment. This diverse group of unicellular organisms constitutes a major fraction of marine phytoplankton, and their contribution to the ocean's primary production can be considerable (Lignell et al. 1993, Rao and Pan 1993). These organisms differ from other algae in many respects, such as photosynthetic capacity (Chan 1980), growth rate (Tang 1996), cell division, and ultrastructural and biochemical properties of the nucleus (Taylor 1987, Rizzo 1991). Dinoflagellates are responsible for "red tides," a serious concern for ecology and for humans if the species concerned produce toxins. The red tides are algal blooms whose high localized concentrations are thought to be related to their ability to produce resting cysts that act as dispersal agents and as a means to last overwinter. These same cysts are important to cell survival under adverse conditions (Anderson et al. 1984). For instance, nitrogen deficiency or low temperatures are able to trigger encystment of the freshwater dinoflagellates *Peridinium cinctum* and *Peridinium willey* (Chapman and Pfiester 1995). When exposed to pollutant metals (Hg^{2+}, Cd^{2+}, Pb^{2+}, and Cu^{2+}), the dinoflagellate *L. polyedrum* may exhibit cell death or cyst formation depending on the dose (Okamoto et al. 1999, Okamoto and Colepicolo 2001). Indeed, it seems likely that survival of this group of algae at high metal concentrations might be due to cyst formation. This idea is supported by the fact that three species of dinoflagellates, *Amphidinium carterae, L. polyedrum*, and *Prorocentrum micans*, can recover after metal-triggered encystment (Lage et al. 1994, Okamoto et al. 1999).

In summary, the regulation and induction of antioxidants takes place as a response to different kinds of pollutant stress. In many cases, a prompt induction of antioxidant enzymes is critical to control the steady-state levels of ROS and thus avoid the ensuing oxidative damage. This type of defense mechanism is especially important within subcellular sites highly prone to oxidative stress such as chloroplasts and mitochondria.

CONCLUSION

Algae are the basis of the food web in all aquatic ecosystems. Among the major primary producers, marine microalgae are responsible for about half of the O_2 production and most of the DMSO released to the atmosphere (Gibson et al. 1990, Stefels and van Baekel 1993) and constitute the main food source for bivalve mollusks in all their growth stages, for zooplankton (rotifers, copepods, and brine shrimps), and for larval stages of some crustacean and fish species. The nutritional value of an alga species is dependent on diverse characteristics including shape, size, digestibility, and toxicity. However, the primary determinant in establishing the food quality transferred to the other trophic levels of the food web appears to be the biochemical composition of the algae (fatty acids, sterols, amino acids, sugars, minerals, and vitamins) (Brown and Miller 1992). Clearly, stress treatments that affect an algal cell's biochemical composition will have a major impact on its food value. A second important consideration is the concentration of heavy metals in algae at the basis of the food chain.

This concentration may occur through the action of chelators, which directly store the metal ions, as well as by induction of defense mechanisms that allow the cell to reduce the metals' toxic effects.

Pollutant metals disturb the oxidative balance in algae, and thus an important palliative measure is the induction of antioxidants. The particular antioxidant responses will of course depend on the particular toxic compound and whether the stress is chronic or acute. Chronic conditions provoke increased activities of antioxidant enzymes, such as SOD, GPX, and APX. It appears that metal-induced disruption of the oxidative balance of chloroplasts depends on both the severity of the stress and the properties of each metal. Under these adverse conditions, modulation of chloroplastic antioxidants seems to be a particularly important strategy, allowing the algae to acclimate to the environment stress. Under acute conditions, however, the toxic effects of the pollutants may overwhelm the antioxidant defenses. Excessive damage to proteins under pollutant treatment could result from an attack by lipid peroxidation intermediates such as alkyl peroxyl and alkoxyl radicals as well as reactive aldehyde products. This may result in cell death or the shut down of all cellular machinery, such as seen with the induction of cyst formation in dinoflagellates. Interestingly, this latter may suggest that ROS have a possible role as signals in this cellular event and possibly in the mechanism of encystment itself.

The different aspects covered in this review show that the first steps have been taken toward elucidating the biochemical pathways involved in adaptive mechanisms to toxicant-induced oxidative stress in algae. Growth inhibition and chlorosis are common symptoms of metal phytotoxicity in several organisms, in which photosynthesis is probably the metabolic process most affected. A hyperoxidative status and the increased oxidative damage described in several studies suggest a correlation between metal treatment and oxidative stress. Acute exposure to metals is highly damaging, presumably because it exceeds the antioxidant defense. Nevertheless, increased activities of antioxidant enzymes and high GSH pools seem to be important in attenuating oxidative damage to chloroplasts. Antioxidant responses at the particular subcellular sites where oxidative stress is triggered could contribute to the overall tolerance of algae during conditions of pollutant stress. An exciting future lies ahead in exploring the role of ROS in algal signal transduction and the exploitation of algae strains for the large-scale production of natural antioxidants.

This work was financed by the Fundação para o Amparo e a Pesquisa do Estado de São Paulo (FAPESP, Brazil) and the Swedish Foundation for International Cooperation in Research and Higher Education (STINT, Sweden). The authors thank Professor John A. Raven (University of Dundee, UK) for helpful comments and suggestions.

Abd-El-Monem, H. M., Corradi, M. G. & Gorbi, G. 1998. Toxicity of copper and zinc to two strains of *Scenedesmus acutus* having different sensitivity to chromium. *Environ. Exp. Bot.* 40:59–66.

Adonaylo, V. N. & Oteiza, P. I. 1999. Lead intoxication: antioxidant defenses and oxidative damage in rat brain. *Toxicology* 135: 77–85.

Allan, R. 1997. Introduction: mining and metals in the environment. *J. Geochem. Expl.* 58:95–100.

Allen, R. G. & Tresini, M. 2000. Oxidative stress and gene regulation. *Free Radic. Biol. Med.* 28:463–99.

Anderson, D. M., Kulis, D. M. & Binder, B. J. 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: Cyst yield in batch cultures. *J. Phycol.* 20:418–25.

Asada, K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50:601–39.

Asada, K. & Takahashi, M. 1987. Production and scavenging of active oxygen in photosynthesis. *In* Kyle, D. J., Osmond, C. & Arntzen, C. J. [Eds.] *Photoinhibition*. Elsevier, New York, pp. 227–97.

Bajguz, A. 2000. Blockade of heavy metals accumulation in *Chlorella vulgaris* cells by 24-epibrassinolide. *Plant Physiol. Biochem.* 38:797–801.

Barros, M. P., Pinto, E., Colepicolo, P. & Pedersen, M. 2001. Astaxanthin and peridinin inhibit oxidative damage in Fe^{2+}-loaded liposomes: scavenging oxyradicals or changing membrane permeability? *Biochem. Biophys. Res. Commun.* 288:225–32.

Bertrand, M., Schoefs, B., Siffel, P., Rohacek, K. & Molnar, I. 2001. Cadmium inhibits epoxidation of diatoxanthin to diadinoxanthin in the xanthophyll cycle of the marine diatom *Phaeodactylum tricornutum*. *FEBS Lett.* 508:153–6.

Britton, G., Liaaen-Jensen, S. & Pfander, H. 1995. Carotenoids today and challenges for the future. *In* Britton, G., Liaaen-Jensen, S. & Pfander, H. [Eds.] *Carotenoids, Vol 1A: Isolation and Analysis*. Birkhöuser, Basel, pp. 13–7.

Brown, M. R. & Miller, K. A. 1992. The ascorbic acid content of eleven species of microalgae used in marine culture. *J. App. Phycol.* 4:205–15.

Bu-Olayan, A. H., Al-Hassan, R., Thomas, B. V. & Subrahmanyam, M. N. V. 2001. Impact of trace metals and nutrient levels on phytoplankton from the Kuwait coast. *Environ. Int.* 26: 199–203.

Cardozo, K. H. M., Oliveira, M. A. L., Tavares, M. F. M., Colepicolo, P. & Pinto, E. 2002. Daily oscillation of fatty acids and malondialdehyde in the dinoflagellate *Lingulodinium polyedrum*. *Biol. Rhythm Res.* 33:371–81.

Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J. C. & Moreno-Sánchez, R. 2001. Interactions of chromium with microorganisms and plants. *FEMS Microb. Rev.* 25:335–47.

Chan, A. 1980. Comparative physiology study of marine diatoms and dinoflagellates in relation to irradiance and cell size. II. Relationship between photosynthesis, growth, and carbon/chlorophyll *a* ratio. *J. Phycol.* 16:428–32.

Chapman, A. D. & Pfiester, L. A. 1995. The effects of temperature and nitrogen on the encystment and growth of the fresh water dinoflagellates *Peridinium cinctum* and *P. willey* in culture (Dinophyceae). *J. Phycol.* 31:355–9.

Chapman, P. M., Allen, H. E., Godtfredsen, K. & Zí Graggen, M. N. 1996. Evaluation of bioaccumulation factors in regulating metals. *Environ. Sci. Technol.* 30:448–42.

Cobbett, C. & Goldsbrough, P. 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.* 53:159–82.

Colepicolo, P., Camarero, V. C. P. C. & Hastings, J. W. 1992. A circadian rhythm activity of superoxide dismutase in photosynthetic alga *Gonyaulax polyedra*. *Chronobiol. Int.* 9:266–8.

Collen, J. & Davison, I. R. 1999. Stress tolerance and reactive oxygen metabolism in the intertidal red seaweeds *Mastocarpus stellatus* and *Chondrus crispus*. *Plant Cell Environ.* 22:1143–51.

Conner, S. D. & Schimid, S. L. 2003. Regulated portals of entry into the cell. *Nature* 422:37–44.

Cotté-Krief, M.-C., Guieu, C., Thomas, A. J. & Martin, J.-M. 2000. Sources of Cd, Cu, Ni and Zn in Portuguese coastal waters. *Mar. Chem.* 71:199–214.

Cumming, J. R. & Gregory, J. T. 1990. Mechanisms of metal tolerance in plants: physiological adaptions for exclusion of metal ions from the cytoplasm. *In* Alscher, R. G. & Cumming, J. R. [Eds.] *Stress Responses in Plants: Adaptation and Acclimatation Mechanisms*. Wiley-Liss, New York, pp. 338–55.

Dat, J. F., Foyer, C. H. & Scott, I. 1998. Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. *Plant Physiol.* 118:1455–61.

Davies, A. G. 1978. Pollution studies with marine plankton. Part II. Heavy metals. *Adv. Mar. Biol.* 15:381–508.

Devi, S. R. & Prasad, M. N. V. 1998. Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a free floating macrophyte: response of antioxidant enzymes and antioxidants. *Plant Sci.* 138: 157–65.

Di Mascio, P., Menck, C. F. M., Nigro, R. G., Sarasin, A. & Sies, H. 1990. Singlet molecular oxygen induced mutagenicity in a mammalian SV40-based shuttle vector. *Photochem. Photobiol.* 51:293–8.

Di Mascio, P., Hollnagel, H. C., Sperança, M. A. & Colepicolo, P. 1995. Diurnal rhythm of carotenoids in the photosynthetic algae *Gonyaulax polyedra*. *Biol. Chem. Hoppe-Seyler* 376:297–301.

Esser, B. K. & Volpe, A. 2002. At-sea high-resolution trace element mapping: San Diego bay and its plume in the adjacent coastal ocean. *Environ. Sci. Technol.* 36:2826–32.

Faganeli, J., Horvat, M., Covelli, S., Fajon, V., Logar, M., Lipej, L. & Cermelj, B. 2003. Mercury and methylmercury in the Gulf of Trieste (northern Adriatic Sea). *Sci. Total Environ.* 304:315–26.

Fernandes, J. C. & Henriques, F. S. 1991. Biochemical, physiological, and structural effects of excess copper in plants. *Bot. Rev.* 57:246–73.

Foote, C. S., Denny, R. N., Weaver, L., Chang, Y. & Peters, J. 1970. Quenching of singlet oxygen. *Ann. N. Y. Acad. Sci.* 171:139–48.

Frank, H. A. & Cogdell, R. J. 1996. Carotenoids in photosynthesis. *Photochem. Photobiol.* 63:257–64.

Fridovich, I. 1997. Superoxide anion radical, superoxide dismutases, and related matters. *J. Biol. Chem.* 250:18515–17.

Gibson, J. A. E., Garrick, R.C, Burton, H. R. & McTaggart, A. R. 1990. Dimethyl sulfide and the alga *Phaeocystis pouchettii* in antarctic coastal waters. *Mar. Biol.* 104:339–46.

Giordani, T., Natali, L., Maserti, B. E., Taddei, S. & Cavallini, A. 2000. Characterization and expression of DNA sequences encoding putative type-II metallothioneins in the seagrass *Posidonia oceanica*. *Plant Physiol.* 123:1571–81.

Ha, S. B., Smith, A. P., Howden, R., Dietrich, W. M., Bugg, S., O'Connell, M. J., Goldsbrough, P. B. & Cobbett, C. S. 1999. Phytochelatin synthase genes from *Arabidopsis* and the yeast *Schizosaccharomyces pombe*. *Plant Cell* 11:1153–64.

Haglund, K., Björklund, M., Gunnare, S., Sandberg, A., Olander, U. & Pedersen, M. 1996. New method for toxicity assessment in marine and brackish environments using the macroalga *Gracilaria tenuistipitata* (Gracilariales, Rhodophyta). *Hydrobiologia* 326:317–25.

Halliwell, B. & Gutteridge, J. M. C. 1999. *Free Radicals in Biology and Medicine*. 3rd ed. Oxford University Press, New York, 936 pp.

Hassan, H. M. & Scandalios, J. M. 1990. Superoxide dismutases in aerobic organisms. *In* Alscher, R. G. & Cumming, J. R. [Eds.] *Stress Responses in Plants: Adaptation and Acclimatation Mechanisms*. Wiley-Liss, New York, pp. 175–99.

Hofmann, E., Wrench, P. M., Sharples, F. P., Hiller, R. G., Welte, W. & Diederichs, K. 1996. Structural basis of light harvesting by carotenoids: peridinin-chlorophyll-protein from *Amphidinium carterae*. *Science* 272:1788–91.

Hollibaugh, J. T., Seibert, D. L. R. & Thomas, W. H. 1980. A comparison of the acute toxicities of ten heavy metals to phytoplankton from Saanich Inlet, BC, Canada. *Estuar. Coast Mar. Sci.* 10:93–105.

Hollnagel, H. C., Di Mascio, P., Asano, C. S., Okamoto, O. K., Stringher, C. G., Oliveira, M. C. & Colepicolo, P. 1996. The effect of light on the biosynthesis of β-carotene and superoxide dismutase activity in the photosynthetic alga *Gonyaulax polyedra*. *Braz. J. Med. Biol. Res.* 29:105–11.

Hollnagel, H. C., Pinto, E., Morse, D. & Colepicolo, P. 2002. The oscillation of photosynthetic capacity in *Lingulodinium polyedrum* is not related to differences in rubisco, reridinin or chlorophyll *a* amounts. *Biol. Rhythm Res.* 33:443–57.

Holovská, K., Lenártová, V., Pedrajas, J. R., Peinado, J., Lopéz-Barea, J., Rosival, I. & Legáth, J. 1996. Superoxide dismutase, glutathione peroxidase, and glutathione reductase in sheep organs. *Comp. Biochem. Physiol.* 115B:451–6.

Hou, X. & Yan, X. 1998. Study on the concentration and seasonal variation of inorganic elements in 35 species of marine algae. *Sci. Total Environ.* 222:141–56.

Hu, S., Tang, C. H. & Wu, M. 1996. Cadmium accumulation by several seaweeds. *Sci. Total Environ.* 187:65–71.

Hylander, L. D. & Meili, M. 2003. 500 years of mercury production: global annual inventory by region until 2000 and associated emissions. *Sci. Total Environ.* 304:13–27.

Jordanova, A., Strezov, A., Ayranov, M., Petkov, N. & Stoilova, T. 1999. Heavy metal assessment in algae, sediments and water from the Bulgarian Black Sea Coast. *Water Sci. Tech.* 39:207–12.

Kennish, M. J. 1996. *Practical Handbook of Estuarine and Marine Pollution*. CRC Press, New York, 535 pp.

Krinsky, N. I. 1989. Antioxidant functions of carotenoids. *Free Radic. Biol. Med.* 7:617–35.

Lage, O. M., Parente, A. M., Soares, H. M. V. M., Vasconselos, M. T. S. D. & Salema, R. 1994. Some effects of copper on the dinoflagellates *Amphidinium carterae* and *Prorocentrum micans* in batch culture. *Eur. J. Phycol.* 29:253–60.

Lane, T. W. & Morel, F. M. M. 2000. A biological function for cadmium in marine diatoms. *Proc. Natl. Acad. Sci. USA* 97:4627–31.

Larkum, T. 1996. How dinoflagellates make light work with peridinin. *Trends Plant Sci.* 1:247–8.

Laube, V. M., McKenzie, C. N. & Kushner, D. J. 1980. Strategies of response to copper, cadmium, and lead by a blue-green and a green alga. *Can. J. Microbiol.* 26:1300–11.

Lignell, R., Heiskanen, A. S., Kuosa, H., Gundersen, K., Kuuppoleinikki, P., Pajuniemi, R. & Uitto, A. 1993. Fate of a phytoplankton spring bloom—sedimentation and carbon flow in the planktonic food web in the northern Baltic. *Mar. Ecol. Progr. Ser.* 94:239–52.

Lin, M.-C. & Liao, C.-M. 1999. ^{65}Zn(II) accumulation in the soft tissue and shell of abalone *Haliotis diversicolor supertexta* via the alga *Gracilaria tenuistipitata* var. *liui* and the ambient water. *Aquaculture* 178:89–101.

Livingstone, D. R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull.* 42:656–66.

López-Suárez, C. E., Castro-Romero, J. M., González-Rodriguez, M. V., González-Soto, E., Pérez-Iglesias, J., Seco-Lago, H. M. & Fernández-Solís, J. M. 2000. Study of the parameters affecting the binding of metals in solution by *Chlorella vulgaris*. *Talanta* 50:1313–8.

Macfarlane, G. R. & Burchett, M. D. 2001. Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Mar. Pollut. Bull.* 42:233–40.

Mason, R.P, Reinfelder, J. R. & Morel, F. M. M. 1996. Uptake, toxicity and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* 30:1835–45.

McLean, M. W. & Williamson, F. B. 1977. Cadmium accumulation by the marine red alga *Porphyra umbilicalis*. *Physiol. Plantarum* 41:268–72.

Morel, F. M. M., Kraepiel, A. M. L. & Amyot, M. 1998. The chemical cycle and bioaccumulation of Mercury. *Annu. Rev. Ecol. Syst.* 29:543–66.

Morris, C. A., Nicolaus, B., Sampson, V., Harwood, J. L. & Kille, P. 1999. Identification and characterization of a recombinant metallothionein protein from a marine alga, *Fucus vesiculosis*. *Biochem. J.* 338:553–60.

Muse, J. O., Stripeikis, J. D., Fernández, F. M., d'Huicque, L., Tudino, M. B., Carducci, C. N. & Troccoli, O. E. 1999. Sea-

weeds in the assessment of heavy metal pollution in the Gulf San Jorge, Argentina. *Environ. Pollut.* 104:315–22.
Nagalakshmi, N. & Prasad, M. N. V. 2001. Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*. *Plant Sci.* 160:291–9.
Okamoto, O. K., Asano, C. S., Aidar, E. & Colepicolo, P. 1996. Effects of cadmium on growth and superoxide dismutase activity of the marine microalga *Tetraselmis gracilis*. *J. Phycol.* 32:74–9.
Okamoto, O. K. & Colepicolo, P. 1998. Response of superoxide dismutase to pollutant metal stress in the marine dinoflagellate *Gonyaulax polyedra*. *Comp. Biochem. Physiol. C* 119:67–73.
Okamoto, O. K. & Colepicolo, P. 2001. Circadian protection against reactive oxygen species involves daily synthesis of manganese- and iron-containing superoxide dismutase in *Gonyaulax polyedra*. *Biol. Rhythm Res.* 32:439–48.
Okamoto, O. K., Shao, L., Hastings, J. W. & Colepicolo, P. 1999. Acute and chronic effects of toxic metals on viability, encystment and bioluminescence in the dinoflagellate *Gonyaulax polyedra*. *Comp. Biochem. Physiol. C* 123:75–83.
Okamoto, O. K., Pinto, E., Latorre, L. R., Bechara, E. J. H. & Colepicolo, P. 2001a. Antioxidant modulation in response to metal-induced oxidative stress in algal chloroplasts. *Arch. Environ. Contam. Toxicol.* 40:18–24.
Okamoto, O. K., Robertson, D. L., Fagan, T. F., Hastings, J. W. & Colepicolo, P. 2001b. Different regulatory mechanisms modulate the expression of a dinoflagellate iron-superoxide dismutase. *J. Biol. Chem.* 276:19989–93.
Pedrajas, R. J., Peinado, J. & Lopez-Barea, J. 1993. Purification of Cu-Zn-superoxide dismutase isoenzymes from fish liver: appearance of new isoforms as a consequence of pollution. *Free Radic. Res. Commun.* 19:29–41.
Pinto, E., Catalani, L. H., Lopes, N. P., Di Mascio, P. & Colepicolo, P. 2000. Peridinin as the major biological carotenoid quencher of singlet oxygen in *Gonyaulax polyedra*. *Biochem. Biophys. Res. Commun.* 268:496–500.
Pinto, E., Nieuwerbugh, L. V., Barros, M. P., Pedérsen, M., Colepicolo, P. & Snoeijs, P. 2003. Density-dependent patterns of tiamine and pigments production in the diatom *Nitzschia microcephala*. *Phytochemistry* 63:155–63.
Pospelova, V., Chmura, G. L., Boothman, W. S. & Latimer, J. S. 2002. Dinoflagellate cyst records and human disturbance in two neighboring estuaries, New Bedford Harbor and Apponagansett Bay, Massachusetts. *Sci. Total Environ.* 298: 81–102.
Prasad, M. N. V. 1995. Cadmium toxicity and tolerance in vascular plants. *Environ. Exp. Bot.* 35:525–45.
Quinlan, G. J., Halliwell, B., Moorhouse, C. P. & Gutteridge, J. M. C. 1988. Action of lead (II) and aluminium (III) ions on iron-stimulated lipid peroxidation in liposomes, erythrocytes and rat liver microsomal fractions. *Biochim. Biophys. Acta* 962: 196–200.
Rao, D. V. S. & Pan, Y. L. 1993. Photosynthetic characteristics of *Dinophysis* Norvegica Claparede and Lachmann, a red-tide dinoflagellate. *J. Plankton Res.* 15:965–76.
Raven, J. A., Evans, M. C. W. & Korb, R. E. 1999. The role of trace metals in photosynthetic electron transport in O_2-evolving organisms. *Photosynth. Res.* 60:111–49.
Rebhun, S. & Amotz, A. B. 1984. The distribution of cadmium between the marine algae *Chlorella stigmatophora* and sea water medium. *Water Res.* 18:173–8.
Reed, R. H. & Gada, G. M. 1990. Metal tolerance in eukariotic and prokariotic algae. *In* Shaw, A. J. [Ed.] *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. CRC Press, Boca Raton, FL, pp. 105–18.
Rice-Evans, C. A., Miller, N. J. & Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 20:933–56.
Rijstenbil, J. W., Derksen, J. W. M., Gerringa, L. J. A., Poortvliet, T. C. W., Sandee, A., Van den Berg, M., Van Drie, J. & Wijnholds, J. A. 1994. Oxidative stress induced by copper: defense and damage in the marine planktonic diatom *Ditylum brightwellii* grown in continuous cultures with high and low zinc levels. *Mar. Biol.* 119:583–90.
Rijstenbil, J. W., Haritonidis, S., Malea, P., Seferlis, M. & Wijnholds, J. A. 1998. Thiol pools and glutathione redox ratios as possible indicators of copper toxicity in the green macroalgae *Enteromorpha* spp. from the Scheldt estuary (SW Netherlands, Belgium) and Thermaikos Gulf (Greece, N. Aegean Sea). *Hydrobiologia* 385:171–81.
Rizzo, P. J. 1991. The enigma of the dinoflagellate chromosome. *J. Protozool.* 38:246–52.
Robinson, N. J., Urwin, P. E., Robinson, P. J. & Jackson, P. J. 1994. Gene expression in relation to metal toxicity and tolerance. *In* Basra, A. J. [Ed.] *Stress Induced Gene Expression in Plants*. Harwood Academic Publishers, Amsterdam, pp. 209–48.
Rodriguez-Ariza, A., Dourado, G., Navas, J. I., Pueyo, C. & Lopez-Barea, J. 1991. Pro-mutagen activation by fish liver as a biomarker of littoral pollution. *Environ. Mol. Mutagen.* 24:116–23.
Rosen, B. 1996. Bacterial resistance to heavy metals and metalloids. *J. Biol. Inorg. Chem.* 1:273–7.
Rossa, M. M., Oliveira, M. C., Okamoto, O. K., Lopes, P. F. & Colepicolo, P. 2002. Effect of visible light on superoxide dismutase (SOD) activity in the red alga *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta). *J. App. Phycol.* 14:151–7.
Rouhier, N. & Jacquot, J.-P. 2002. Plant peroxiredoxins: alternative hydroperoxide scavenging enzymes. *Photosynth. Res.* 74:259–68.
Sauser, K. R., Liu, J. K. & Wong, T. Y. 1997. Identification of a copper-sensitive ascorbate peroxidase in the unicellular green alga *Selenastrum capricornutum*. *Biometals* 10:163–8.
Segot, M., Codomier, L. & Combaut, G. 1983. Action de quatre metaux lourds (cadmium, cuivre, mercure, plombs) sur la croissance de *Asparagopsis armata* en culture. *J. Exp. Mar. Biol. Ecol.* 66:41–8.
Shi, X. & Dalal, N. S. 1990. Evidence for a Fenton-type mechanism for the generation of OH radicals in the reduction of Cr(VI) in cellular media. *Arch. Biochem. Biophys.* 281:90–5.
Sies, H. 1999. Glutathione and its role in cellular functions. *Free Radic. Biol. Med.* 27:916–21.
Sigaud-Kutner, T. C. S., Pinto, E., Okamoto, O. K., Latorre, L. R. & Colepicolo, P. 2002. Changes in superoxide dismutase activity and photosynthetic pigment content during growth of marine phytoplankters in batch-cultures. *Physiol. Plantar.* 114:566–72.
Stefels, J. & van Boekel, W. H. M. 1993. Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species *Phaeocystis* sp. *Mar. Ecol. Prog. Ser.* 97:11–8.
Steffens, J. C. 1990. Heavy metals stress and the phytochelatin response. *In* Alscher, R. G. & Cumming, J. R. [Eds.] *Stress Responses in Plants: Adaptation and Acclimatation Mechanisms*. Wiley-Liss, New York, pp. 377–94.
Stohs, S. J. & Bagchi, D. 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* 18:321–36.
Szalontai, B., Horvath, L. I., Debreczeny, M., Droppa, M. & Horvath, G. 1999. Molecular rearrangements of thylakoids after heavy metal poisoning, as seen by Fourier transform infrared (FTIR) and electron spin resonance (eSR) spectroscopy. *Photosyn. Res.* 61:241–52.
Takeda, T., Yokota, A. & Shigeoka, S. 1995. Resistance of photosynthesis to hydrogen peroxide in algae. *Plant Cell Physiol.* 36:1089–5.
Tang, E. P. Y. 1996. Why do dinoflagellates have lower growth rates? *J. Phycol.* 32:80–4.
Taylor, F. J. R. 1987. *The Biology of Dinoflagellates*. Blackwell Scientific, Oxford, 785 pp.
Telfer, A., Dhami, S., Bishop, S. M., Philips, D. & Barber, J. 1994. Beta-carotene quenches singlet oxygen formed by isolates photosystem II reaction center. *Biochemistry* 33: 14469–74.
Thomas, D. J., Thomas, J. B., Prier, S. D., Nasso, N. E. & Herbert, S. K. 1999. Iron superoxide dismutase protects against chilling damage in the cyanobacterium *Synechococcus* species PCC7942. *Plant Physiol.* 120:275–82.
VanHeerden, P. D. R, Robertson, B. L. & DeKock, L. 1997. Inhibition of *Ectocarpus siliculosus* infestations with copper

chloride in tank cultures of *Gracilaria gracilis*. *J. Appl. Phycol.* 9:255–9.

Van Ho, A., Ward, D. M. & Kaplan, J. 2002. Transition metal transport in yeast. *Annu. Rev. Microbiol.* 56:237–61.

Vasconcelos, M. T. S. D. & Leal, M. F. C. 2001. Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Mar. Chem.* 74:65–85.

Vierling, E. 1990. Heat shock proteins function and expression in plants. *In* Alscher, R. G. & Cumming, J. R. [Eds.] *Stress Responses in Plants Adaptation and Acclimatation Mechanisms*. Wiley-Liss, New York, pp. 357–75.

Wang, S. & Shi, X. 2001. Molecular mechanisms of metal toxicity and carcinogenesis. *Mol. Cell Biochem.* 222:3–9.

Wang, W.-X. & Dei, R. C. H. 2001a. Metal uptake in a coastal diatom influenced by major nutrients (N, P and Si). *Water Res.* 35: 315–21.

Wang, W.-X. & Dei, R. C. H. 2001b. Effects of major nutrient additions on metal uptake in phytoplankton. *Environ. Poll.* 111: 233–40.

Wickfors, G. H. & Ukeles, R. 1982. Growth and adaptation of estuarine unicellular algae in media with excess copper, cadmium or zinc. *Mar. Ecol. Progr. Ser.* 7:191–206.

Winterbourn, C. C. 1982. Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts is a feasible source of hydroxyl radicals *in vivo*. *Biochem. J. Lett.* 205: 461–3.

Woodall, A. A., Britton, G. & Jackson, M. J. 1997. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: relationship and protective ability. *Biochem. Biophys. Acta* 7:617–35.

Wundram, M., Selmar, D. & Bahadir, M. 1996. The *Chlamydo monas* test: a new phytotoxicity test based on the inhibition of algal photosynthesis enables the assessment of hazardous leachates from waste disposals in salt mines. *Chemosphere* 32:1623–31.

Yu, Q., Matheickal, J. T., Yin, P. & Kaewsarn, P. 1999. Heavy metal capacities of common marine macro algal biomass. *Water Res.* 33:1534–7.

Zalups, R. K. & Ahmad, S. 2003. Molecular handling of cadmium in transporting epithelia. *Toxicol. Appl. Pharmacol.* 186:163–88.