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Biochemical biomarkers in algae and marine pollution: A review

Moacir A. Torres^{a,b}, Marcelo P. Barros^b, Sara C.G. Campos^a, Ernani Pinto^c, Satish Rajamani^d, Richard T. Sayre^d, Pio Colepicolo^{a,*}

^a Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, SP, Brazil

^b Centro de Ciências Biológicas e da Saúde (CCBS), Universidade Cruzeiro do Sul (UNICSUL), São Paulo, SP, Brazil

^c Depto. de análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brazil

^d Department of Plant Cellular and Molecular Biology, Ohio State University, Columbus, OH, USA

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ABSTRACT

Environmental pollution by organic compounds and metals became extensive as mining and industrial activities increased in the 19th century and have intensified since then. Environmental pollutants originating from diverse anthropogenic sources have been known to possess adverse values capable of degrading the ecological integrity of marine environment. The consequences of anthropogenic contamination of marine environments have been ignored or poorly characterized with the possible exception of coastal and estuarine waters close to sewage outlets. Monitoring the impact of pollutants on aquatic life forms is challenging due to the differential sensitivities of organisms to a given pollutant, and the inability to assess the long-term effects of persistent pollutants on the ecosystem as they are bio-accumulated at higher trophic levels. Marine microalgae are particularly promising indicator species for organic and inorganic pollutants since they are typically the most abundant life forms in aquatic environments and occupy the base of the food chain.

We review the effects of pollutants on the cellular biochemistry of microalgae and the biochemical mechanisms that microalgae use to detoxify or modify pollutants. In addition, we evaluate the potential uses of microalgae as bioindicator species as an early sentinel in polluted sites.

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1. Aquatic pollution: a global concern

Globally, more than 3 billion people live in proximity to the marine coast. Wastes from both industrial and domestic sources as well as habitat destruction have a substantial impact on the coastal environments (Moore et al., 2004). Internationally accepted procedures for environmental/ecological impact and risk assessment have been established to manage human impact on coastal environments (Rice, 2003). The oceans were previously considered to be a vast reservoir for the safe disposal of pollutants. Many chemical contaminants, including organochlorine compounds, herbicides, domestic and municipal wastes, petroleum products and heavy metals are now recognized to have adverse effects on ocean environments, even when released at low levels (Haynes and Johnson, 2000; Pinto et al., 2003). Little attention has been given to this problem until shortly before the 19th century. The adverse effects of environmental pollution have been well documented in recent years (His et al., 1999; Swaminathan, 2003).

The atmosphere plays an important role in pollutant transport over long distances. The presence of pesticide in remote areas of the earth confirms large-scale dispersion and deposition at sites far removed from the original site of application (Islam and Tanaka, 2004; Shen et al., 2005).

The relative contribution of various organisms to the biogeochemical cycling of environmental pollutants varies substantially (Morel and Price, 2003). In marine environments, the sedimentation of microalgae during algal blooms has been associated with substantial (20–75%) reductions in the level of suspended heavy metals, as well as heavy metal deposition (Luoma et al., 1998). Similarly, for organic xenobiotics, algae play an important role in the dispersal (Wang et al., 1998; Kowalewska, 1999), chemical transformation and bioaccumulation of many toxic compounds (Okay et al., 2000; Todd et al., 2002; Lei et al., 2002, 2007; Murray et al., 2003; Bopp and Lettieri, 2007).

Many toxic and bioaccumulative pollutants are found in only trace amounts in water, and often at elevated levels in sediments. Risk assessments based only on data derived from water analyses may be misleading. On the other hand, data from sediments may not be representative of pollutant concentrations in the overlying water column and cannot give information on patterns of contamination at the higher levels of the food chain (Binelli and Provini, 2003). For example, the uptake of xenobiotics by

* Corresponding author. Present address: Instituto de Química—USP, Av. Prof. Lineu Prestes, No. 749, bloco 9 sup., sala 970, CEP 05599970 São Paulo, SP, Brazil. Fax: +55 11 30912170.

E-mail address: piocolep@iq.usp.br (P. Colepicolo).

phytoplankton is the first step in the bioaccumulation in aquatic food webs. Macro- and microalgae also play an important role in the removal of polychlorinated biphenyls from the euphotic zone by direct sinking of the cells (Wang et al., 1998; Gerofke et al., 2005).

Evidence points to a coupling between microalgae uptake and air–water organic pollutant concentration (Fig. 1). Air–water exchange dynamics are influenced not only by physical parameters but also by phytoplankton biomass and growth rate (Dachs et al., 1999). Pollutants with low octanol–water partition coefficients ($\log K_{ow} < 5$) can be taken up and accumulated by aquatic organisms while more hydrophobic pollutants ($\log K_{ow} > 5$) may partition in lipid membranes of cells leading to their biomagnification (Binelli and Provini, 2003). Thus, microalgae may reduce pollutant exposure to organisms that do not consume them either directly or indirectly. Conversely, as a food-source, microalgae may facilitate the uptake of contaminants into higher organisms, increasing the possibility of toxicity (Okay et al., 2000).

1.1. Oil derivatives and organic compounds

1.1.1. Polycyclic aromatic hydrocarbons

Marine oil pollution has been receiving increasing attention since the middle of the 19th century with the intensification of tanker operations and oil use (Islam and Tanaka, 2004), marine tanker collisions (Owen, 1999), pollutant release from coastal refineries (Wake, 2005; Tolosa et al., 2005) and continuous operative discharges from ships (ESA, 1998; Carpenter and MacGill, 2001). Annually, 48% of the oil pollution in the oceans is due to fuels and 29% to crude oil. Tanker accidents contribute only 5% of all pollution entering into the sea (Brekke and Solberg, 2005). Despite this, an estimated 1.6 million tons of oil have spilled from tankers since 1965 (over 650,000 ton in Europe and Pacific Asia) (Wang and Fingas, 2003).

Polycyclic aromatic hydrocarbons (PAHs) is one of the biologically toxic, biopersistent chemical components accounting for ca. 20% of crude oil, and include a range of compounds with two or more condensed aromatic rings either with or without alkyl groups substituent (Neff, 1990). They are natural constituents of crude oil (Boehm et al., 2004), but are also released as a result of the combustion of petroleum-based fuels (Kowalewska, 1999). These compounds have low vapor pressures and $\log K_{ow} > 5$;

therefore, they are rapidly absorbed by particulate matter and by living organisms (Nielsen et al., 1997). The Integrated Risk Information System (IRIS) of the U.S. Environment Protection Agency (EPA) contains assessments of over 540 individual chemicals with potential human health effects (USEPA, 2003a). The EPA has identified 16 unsubstituted PAHs as priority pollutants (Rodríguez and Sanz, 2000). Some of these are considered to be human carcinogens including: benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c-d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene (Menzie et al., 1992). Their toxicity and widespread presence in the environment have elevated them to the top of the list of the most aggressive pollutants (Readman et al., 2002).

1.1.2. Persistent organic pollutants

Persistent organic pollutants (POPs) or hydrophobic organic compounds (HOCs) constitute a group of organic chemicals (carbon based) that contain bound chlorine or bromide atoms. The majority of these halocarbons and polybrominated diphenyl ethers emanates from anthropogenic sources and enter the environment through industrial and agricultural activities (Haynes and Johnson, 2000). Some POPs, like polychlorinated dibenzo-p-dioxins/furans, are residual or secondary products of agrochemical industries (Jones and Voogt, 1999). A major impetus for the Stockholm Convention on Persistent Organic pollution in 2001, was the finding of POP contamination in relatively pristine arctic regions thousands of miles from any known pollutant source. Tracing the movement of most POPs in the environment is a complex task due to the distribution and exchange dynamics of these compounds in different physical phases, e.g., in the gas phase or attached to airborne particles (USEPA, 2002a). POPs have a propensity to enter the gas phase under ambient temperatures and travel long distances before being re-deposited. The cycle of volatilization and deposition may be repeated many times, with the result that POPs accumulate in an area far removed from where they were initially used or emitted (Jones and Voogt, 1999). POPs may also bind to atmospheric particles in snow, rain, or mist or to animal carriers such as migratory species (USEPA, 2002a). Under the Stockholm Convention, 90 signatory countries have agreed to reduce and/or eliminate the production, use, and release of the 12 POPs (e.g., aldrin, chlordane, DDT, dieldrin, heptachlor, mirex, etc.) of greatest concern to the global community (USEPA, 2002b). In addition, envisioned to be a dynamic treaty by the

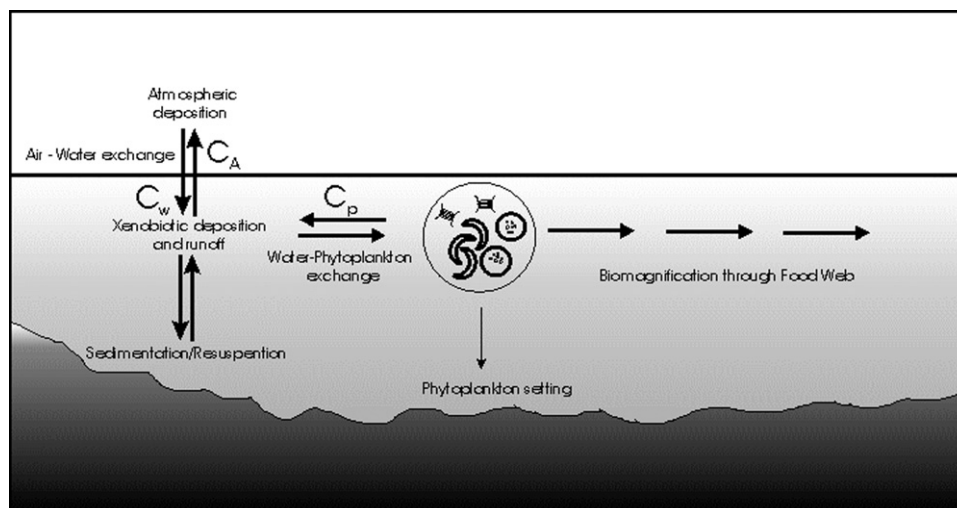


Fig. 1. Schematic of the air–water–phytoplankton exchange process. C_a , C_w , and C_p are the POP concentrations in the gas phase, water, and phytoplankton, respectively (Dachs et al., 1999 with mod.).

international community, the Stockholm Convention provides a rigorous scientific process through which new chemicals with POP characteristics can be added to the treaty. Several additional POP candidates await international attention (WWF, 2005).

1.1.3. Pesticides

The safest pesticides should not affect non-target species (usually in the soil zone) and not persist in the environment (Warren et al., 2003). In practice, however, most pesticides are often not rapidly degraded—rapid degradation might reduce their applicability (Islam and Tanaka, 2004; Bromilow et al., 2006; Navarro et al., 2007). Therefore, it is likely that a large volume of pesticide residues accumulates in the environment (Zi-Wei et al., 2002; Storelli et al., 2005). Paradoxically, the environmental persistence that enhances the efficacy of organochlorine pesticides (OCPs) also increases their potential for environmental destructiveness (Li and Macdonald, 2005). Moreover, pesticides do not always remain in the soil, but find their way into sedimentary systems through leaching, surface run-off, spray drift, soil erosion and volatilization (Warren et al., 2003). A complex range of factors determines the fate of pesticides applied to agricultural soils including: method of application, active ingredients, weather conditions, land topography, soil type, etc. These factors all influence the persistence and extent of contamination of non-target sites (Larson et al., 1995). Additionally, overuse of pesticides increases the probability of negative impacts on non-target organisms such as aquatic biota, terrestrial plants, mammals, and soil microorganisms (Tremolada et al., 2004).

1.1.4. Polychlorinated biphenyls

Another class of persistent environmental pollutants is the polychlorinated biphenyls (PCBs) marketed worldwide under trade names such as Aroclor, Askarel, Clophen, Therminol, etc. PCBs comprise mixtures of 209 possible synthetic organic chemicals (congeners), ranging from oily liquids to waxy solids (Borja et al., 2005). Because of their non-inflammable nature, chemical stability, and insulating properties, commercial PCB mixtures have been used in many industrial applications, especially in capacitors, transformers, and other electrical equipment (USEPA, 1996). Biodegradation processes, including dechlorination, can transform PCBs, effectively altering their potential toxicity. On the other hand, dechlorination reactions are usually slow, while altered PCB mixtures can persist in the environment for many years (Doick et al., 2005; Borja et al., 2005). Based on long-term persistence in the environment and their toxicity, the commercial production and use of PCBs is believed to have finally ceased in the mid-1980s in the USA and in Northern Europe (USEPA, 1996; HELCOM, 2001).

1.1.5. Dioxins

The terms “dioxin” or “dioxin-like” refers to a group of chemical compounds that share chemical similarities and mode-of-action (biological) characteristics. A total of 30 of these dioxin-like compounds belong to three closely related families: the polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and certain PCBs. PCDDs and PCDFs are typically generated as unwanted by-products of chemical syntheses, but can also be produced inadvertently in nature. Combustion, chlorine bleaching of pulp and paper, and other industrial processes can all create small quantities of dioxins (USEPA, 2003b).

The health effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related dioxin-like compounds (i.e. polychlorinated dibenzo-*p*-dioxins and dibenzofurans) on mammals has been the

object of extensive studies over the past 30 years (Beatty and Neal, 1978; Kociba et al., 1978; Rao et al., 1988; Wu and Whitlock, 1992; Hays et al., 1997; Cooney, 2001; Schwaneckamp et al., 2006). While toxicological and epidemiological research has yielded a robust database on TCDD toxicity in animals and humans, controversy has arisen with regard to its cancer hazard assessment (the degree of risk attributable to exposure to these compounds) (Cole et al., 2003). Significant differences of opinion persist among scientists and risk management agencies on the health effects of TCDD. The European Commission Scientific Committee on Foods (ECSCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that a daily TCDD dose of 1–4 pg/kg is likely to be harmless to human health. Conversely, the USEPA has suggested that TCDD doses in the range of 1 pg/kg-day may pose a significant health risk (Hays and Aylward, 2003). However, there is a broader concern about the cumulative effects of dioxin-like compounds and their synergistic properties (Schwarz and Appel, 2005).

1.2. Heavy metals

A number of trace metals are used by living organisms to stabilize protein structures, facilitate electron transfer reactions and catalyze enzymatic reactions (Ash and Stone, 2003). For example, copper (Cu), zinc (Zn), and iron (Fe) are essential as constituents of the catalytic sites of several enzymes (Allan, 1997). Other metals, however, such as lead (Pb), mercury (Hg), and cadmium (Cd) may displace or substitute for essential trace metals and interfere with proper functioning of enzymes and associated cofactors. Metals are usually present at low or very low concentrations in the oceans (Ash and Stone, 2003). In coastal waters, metals can occur at much higher concentrations, probably due to inputs from river systems (Morillo et al., 2004). Close to urban centers, metal pollution has been associated with sewage outlets (Chen et al., 2005; Wannaz et al., 2006). Although, there have been several successful programmes of phasing out lead in the developing world modeled on the programs of industrialized countries (Lovei, 1998; Singh and Singh, 2006), with important emission reducing by improved control to replace leaded petrol by unleaded petrol (AMAP, 1997, 2002). A major source of air contamination is the non-ferrous metals industry, which emits Cd, Pb, Ni, As, Cu, Se, and Zn (Liu et al., 2003; Lewtas, 2007; Blake et al., 2007). Coal burning is the major source of Hg, As, chromium (Cr), and Se (Zhuang et al., 2004; Keegan et al., 2006; Gujian et al., 2007), while combustion of oil is the most important source of Ni and vanadium (V) (USEPA, 2002c; Dundar, 2006).

2. Behavioral dynamics of pollutants

In the natural environment, organisms living in chronically polluted sites may be exposed to low concentrations of xenobiotics for long periods of time. In other cases, organisms may be abruptly exposed to high levels of toxic agents upon the outfall of a pollutant in coastal waters. Xenobiotics in the aquatic ecosystem can partition between land (1), sediment (2), sediment–water interface (3), interstitial waters (4), biota (5) and the air–water interface (6) (Fig. 2). Thus, the dynamic behavior of pollutants in the environment is hypothetically under the influence of water and atmospheric conditions and, biotic and abiotic (sediments) materials. Although the physical–chemical sorption of xenobiotics onto solid phases is subject to a vast range of factors, sediments may also be the most substantive source of environmental pollutants (Pérez-Ruzafa et al., 2000).

Correlations and functional relationships must be established between abiotic and biotic levels of pollution exposure in order to make early and realistic environmental risk assessments (ERA).

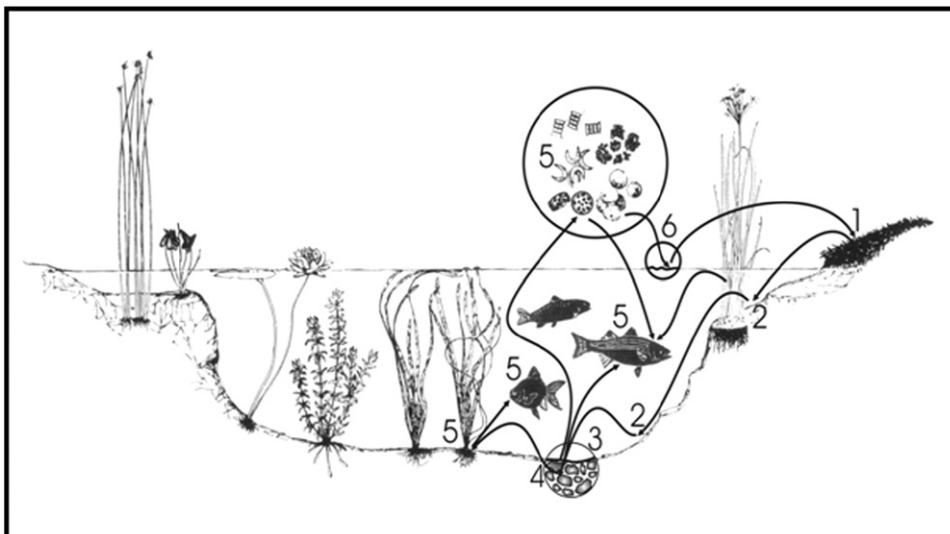


Fig. 2. Scheme showing connections between various segments of the ecosystem in the presence of xenobiotics. Phytoplankton (detail) is the basic trophic level in the food web and could signal the first damage in the aquatic ecosystem. (1) Land, (2) sediment, (3) sediment–water interface, (4) interstitial water, (5) biota, and (6) air–water interface.

Persistent hydrophobic chemicals and heavy metals may accumulate in aquatic organisms through different mechanisms: directly from water, via uptake of suspended particles, or by the consumption of lower trophic level organisms (Binelli and Proveni, 2003; Van der Oost et al., 2003). An essential point to consider during the application of an ERA program is the food chain structure, since bioaccumulation and biomagnification of xenobiotics in the organisms are critical factors in evaluating adverse effects on ecosystems. The study of physiological and biochemical alterations, as well as the identification and quantification of pollutants in basal-level trophic organisms are an essential diagnostic tool (Van Gestel and Van Brummelen, 1996; Handy and Depledge, 1999; Handy et al., 2003). The presence of chemical compounds in isolated sediments does not, by itself, indicate injurious effects to organisms (Wang et al., 1998), as bioavailability of these materials should also be taken into account. On the other hand, the detection of pollutants (quantitative analysis) (Moy and Walday, 1996; Baum et al., 2004) or their effects (biochemical biomarkers) (Warszawsky et al., 1995; Thies et al., 1996; Okamoto et al., 2001a; Lewis et al., 2001; Aksmann and Tukaj, 2004; Geoffroy et al., 2004) in photosynthetic organisms such as micro and macroalgae are early and timely indicators of potential hazard in aquatic systems. Biochemical approaches for the detection of environmental pollutants in microalgae—the most important of the Earth's biomass producers—should be seriously considered in any environmental assessment program (Kowalewska, 1999; Okay et al., 2000).

3. Biomonitoring as a *soft-path* to marine environments in jeopardy

The United Nations Environment Program (UNEP) has defined monitoring as a repetitive observation (for defined purposes) of one or more chemical or biological elements according to a prearranged schedule over time and space, using comparable and standardized methods (van der Oost et al., 2003). In the early phase of environmental monitoring of coastal areas, most programs consisted of the measurement of physical and chemical variables, and lacked important information about biological organisms. Such programs provided useful information on levels of contamination but, did not supply report concerning the

pollutant effects on biota (Rivera and Riccardi, 1997; Lam and Gray, 2003). In this context, quantitative structure activity relationships (QSAR) are recognized to be a powerful tool in ecotoxicology for predicting the potential toxic effects of pollutants based on the physical and chemical properties of compounds (Bradbury et al., 2003; Dearden, 2003; Perkins et al., 2003). The so-called quantitative inter-specific chemical activity relationships (QUICAR) represent a different approach. Such relationships make it possible to predict toxic effects on a particular organism, for which experimental values are unavailable, based on data from a different but related species (Tremolada et al., 2004).

In the 1960s, concerns arose as to the effects of organochlorine chemicals on the marine environment (e.g., DDT and PCBs) and attention turned to monitoring the biological impacts of the pollutants (Cairns and van der Schalie, 1980) rather than contaminant monitoring (Lam and Gray, 2003). In order to assess the risks of contaminants to organisms and to classify the environmental quality of ecosystems, at least five environmental monitoring methods (Fig. 3) should be performed: chemical monitoring (CM), bioaccumulation monitoring (BAM), biological effect monitoring (BEM), health monitoring (HM), and ecosystem monitoring (EM). Living organisms are used in BAM, BEM, HM, and EM methods in order to evaluate environmental changes and, for this reason these systems are collectively designated biomonitoring (van der Oost et al., 2003).

Biological monitoring or biomonitoring can be defined as the systematic use of biological responses to evaluate changes in the environment, with the intent of establishing a quality control program (Cairns and van der Schalie, 1980). When carried out with regularity, the systematic employment of organisms in the biomonitoring process offers the opportunity to assess the impact of pollutants on the aquatic environment more realistically (Cairns, 1982). Bivalve mollusks, particularly mussels, have been elected as “sentinel” organisms in international environmental monitoring programs as part of the MUSSEL WATCH PROGRAM (Goldberg, 1975; Tavares et al., 1988; Claisse, 1989; Tripp et al., 1992; Tanabe, 1994). Many other organisms have also been used as regionally important tools in environmental programs, e.g., mangrove mussels in South Brazil (Torres et al., 2002), crabs in South Africa (Thawley et al., 2004), polychaetes in Spain and France (Gesteira and Dauvin, 2000), fish in Australia, Asia, and

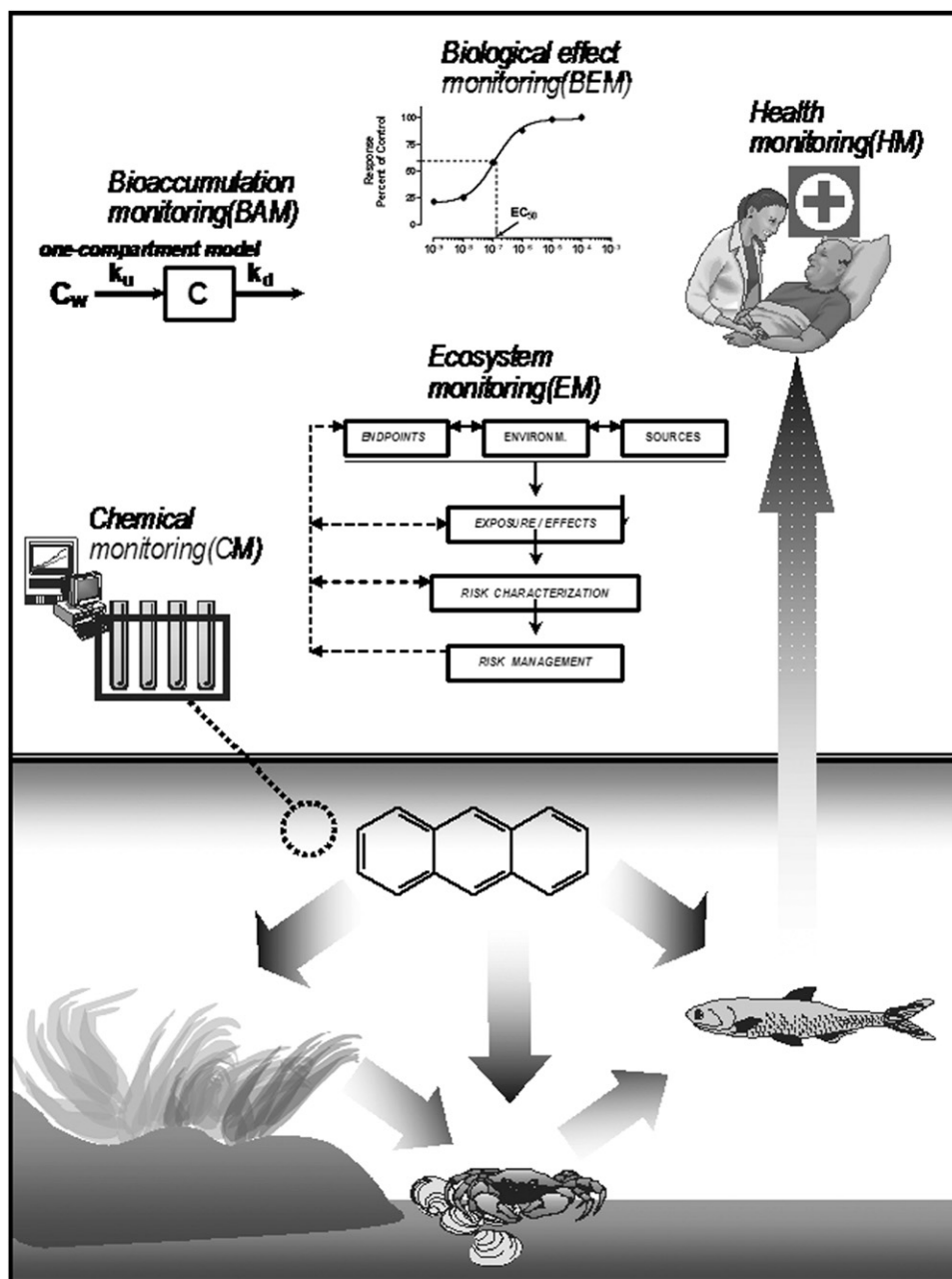


Fig. 3. The relationship among the different approaches for environmental monitoring, biomonitoring, and risk assessment characterization. Concentration of chemical in water (C_w), uptake constant (k_u), concentration of chemical in biota (C), and ratio between excretion and metabolism constant (k_d) (Van der Oost et al., 2003 with mod.).

America (Edwards et al., 2001; Ueno et al., 2005; Carrasco-Letelier et al., 2006, respectively).

Despite the massive use of marine animals in biomonitoring programs, photosynthetic organisms like algae (seaweeds) have increasingly been used as biotectors to monitor xenobiotics in marine environments (Levine, 1984; Stewart, 1995; Whitton and Kelly, 1995; Jayasekera and Rossbach, 1996; Ali et al., 1999; Volterra and Conti, 2000; Sánchez-Rodríguez et al., 2001; Barreiro et al., 2002; Conti and Cecchetti, 2003; Conti et al., 2007). Because of its natural and widespread occurrence along worldwide seashores, photosynthesizing organisms could be useful for a time-integrated picture of the ecosystem response to exposure to toxic compounds. Both, macroalgae (Fytianos et al., 1999; Sánchez-Rodríguez et al., 2001; Conti and Cecchetti, 2003) and microalgae (Rijstenbil et al., 1994; Luoma et al., 1998; Randhawa et al., 2001; Siripornadulsil et al., 2002; Nishikawa et al., 2003;

Pinto et al., 2003; Mallick, 2004; Tripathi et al., 2006) are important tools to monitor physiological changes in the presence of heavy metals. In addition, biochemical and physiological responses of these organisms exposed to POPs (Conner, 1981; Mayer et al., 1998; Wang et al., 1998; Dachs et al., 1999; Montone et al., 2001; Leitao et al., 2003; Gerofke et al., 2005), PHAs (Cody et al., 1984; Warshawsky et al., 1990, 1995; Kirso and Irha, 1998; Pflugmacher et al., 1999; Aksmann and Tukaj, 2004; Djomo et al., 2004; Lei et al., 2007), and pesticides (Traunspurger et al., 1996; Thies et al., 1996; Saenz et al., 1997; Wei et al., 1998; Pflugmacher and Sandermann, 1998a; Nyström et al., 2002; Geoffroy et al., 2002, 2003; Ma and Chen, 2005; Cai et al., 2007) have been reported for the last two decades.

As a result of their substantial biomass and comparatively large surface-to-volume ratio, microalgae play a major role in the biogeochemical cycling of nutrients and pollutants in the oceans

(Van Gestel and Van Brummelen, 1996; Okamoto and Colepicolo, 1998). They have been referred to as a “green liver” of the oceans, acting as important sinks for environmental chemical compounds (Sandermann, 1992). Given their importance in the global cycling of pollutants, the monitoring of the effect of xenobiotics on microalgae is of pivotal importance (Wang et al., 1998; Kowalewska, 1999; Sørensen et al., 2000; Nyström et al., 2002). The challenge, however, is that the low levels of pollutants regularly present in individual cells may not be sufficient to induce significant biochemical adaptations in microalgae, whereas biomagnification/bioaccumulation through the food web may cause drastic impacts on organisms at higher levels.

4. Algae and biomarkers of aquatic hazards

It is clearly recognized that stress-induced changes at the ecosystem level are of eminent concern. However, such changes are generally too complex and are often omitted from the list of indicators used for early detection and prediction of environmental stress (Depledge et al., 1993). A probable solution to this problem lies in the effective characterization of “distress signals” at the molecular and cellular levels that can provide “early warning prognostics” of reduced performance with possible linkage to the higher ecological levels (Moore et al., 2004). Typically, biomarkers are defined as quantitative measures of changes in the biological system that can be related to exposure to the toxic effects of environmental chemicals (WHO, 1993; Peakall and Walker, 1994). Although not explicitly contained in most definitions, the use of the term “biomarker” or “biomarker response” is often restricted to cellular, biochemical, molecular, or physiological changes that are measured in cells, body fluids, tissues, or organs within an organism that are indicative of xenobiotic exposure (Van Gestel and Van Brummelen, 1996; van der Oost et al., 2003; Lam and Gray, 2003).

4.1. Metal chelators as an algal response to heavy metals

Some metals (e.g., Cu) are essential at certain concentrations and toxic at others (Speisky et al., 2003). However, essential and toxic trace metals in the open oceans are often extremely low (e.g., Fe concentration in open ocean may be 10^{-10} M), to be productive in these metal “deserts,” special strategies are employed by planktonic organisms to assimilate them (Ash and Stone, 2003; Morel and Price, 2003). Essential metals are often transported actively across membranes (Tamás and Wysocki, 2001; Van Ho et al., 2002). And, these metal transporters can also be gateways for the entry of toxic metals into phytoplankton. One such mechanism is molecular mimicry, in which a metal ion binds to an essential metal chelator and is transported into the cell (Zalups and Ahmad, 2003; Bridges and Zalups, 2005).

Microalgae employ a variety of biochemical strategies (Fig. 4) to reduce the toxicity of non-essential trace metals (e.g., Hg and Cd) and to metal homeostasis in the cytoplasm (Hall, 2002; Cobbett and Goldsbrough, 2002; Perales-Vela et al., 2006).

The amino acid cysteine (Cys) contains a sulfhydryl (thiol group, $-SH$) which is the site of metal binding. Cys-containing peptides such as glutathione (GSH) are responsible for metal sequestration in living cells. A Cd-tolerant strain of the alga *Chlamydomonas reinhardtii* has significantly higher levels of Cys than the Cd-sensitive strain (Hu et al., 2001) and similarly a Cu-tolerant strain of the lichen photobiont *Trebouxia erici* synthesizes significantly higher Cys levels than wild *T. erici* even in a control culture medium (Backor et al., 2007). A Cys pool may thus be critical to algal defense against metal toxicity. Alternative mechanisms may involve sequestering toxic metals by heavy metal-binding Cys-rich proteins such as class II metallothioneins (MTII) (Klaassen et al., 1999; Romero-Isart and Vasak, 2002; Vasak, 2005) and non-translationally synthesized polypeptides some times described as class III metallothioneins (phytochelatins—PCs or MTIII) (Grill et al., 1985; Rauser, 1990; Steffens, 1990; Zenk, 1996; Hirata et al., 2001; Mendoza-Cózatl et al., 2005;

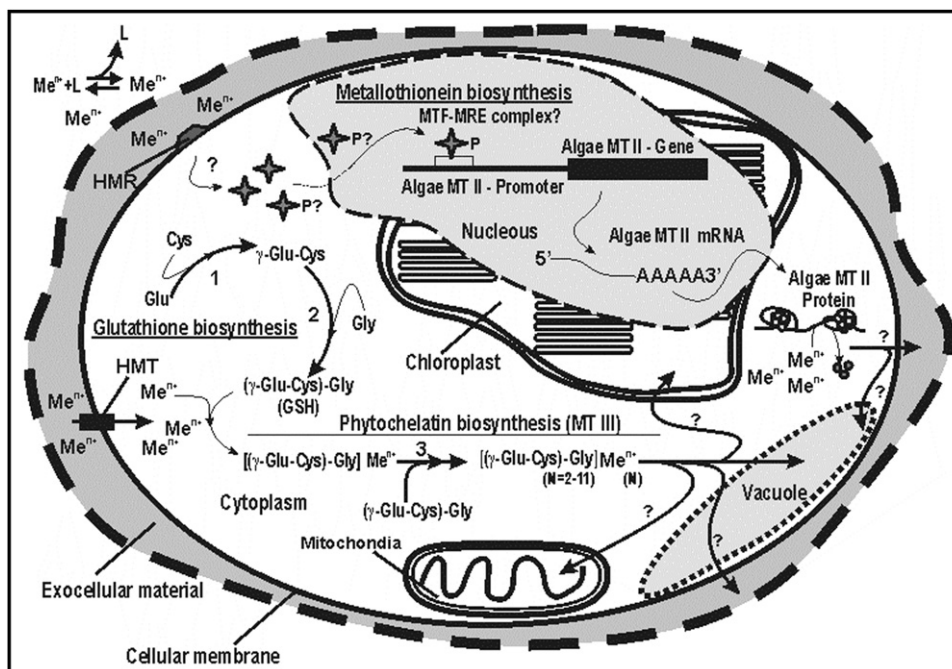


Fig. 4. General scheme of heavy metal detoxification and metal homeostasis in microalgae mediated by PCs and MTs. Heavy metal receptor (HMR), heavy metal transporter (HMT), MT transcription factor (MTF), metal regulatory element (MRE), metal complex in solution ($Me^{n+}L$), free metal ion (Me^{n+}). (1) γ -Glu-Cys synthetase, (2) glutathione synthetase, (3) PC synthase, (?) Little is known about the algae, only plant/animal-based MT expression and/or PC heavy metal complex metabolism (Vallee, 1995; Cobbett and Goldsbrough, 2002; Romero-Isart and Vasak, 2002; Perales-Vela et al., 2006 with mod.).

Perales-Vela et al., 2006). In plants and algae, the zone of competence of MTII and PCs is still unclear, and some evidences point to the selection of these mechanisms are related to the age of the organism, sensitivity of enzymes and type of heavy metals (Perales-Vela et al., 2006).

PCs have a general chain structure (γ -Glu-Cys) n -Gly ($n = 2$ –11) with molecular weight ranges from 2 to 10 kDa, is synthesized by the constitutive enzyme named phytochelatin synthase (Perales-Vela et al., 2006). They are structurally related to GSH (Cobbett, 2000). In fact, studies of PCs regulation have shown that the enzymes related to the GSH synthesis could confirm the hypothesis that GSH is the primary peptide involved in binding heavy metals and the substrate for the non-ribosomal PCs synthesis (Rauser, 1995; Zenk, 1996; Mendoza-Cózatl et al., 2005). *In vivo* studies have shown that PCs synthesis can be induced by a range of metal ions (Cobbett, 2000; Scarano and Morelli, 2002). Synthesis of PCs is increased in the lichen unicellular alga *T. erici* in response to excess Cd or Cu. However, Cd was a more potent activator of PCs synthesis under identical experimental conditions and even able to induce synthesis of PCs with longer (more stable) chains, up to PC5 (Backor et al., 2007). Moreover, kinetic studies demonstrated that PCs synthesis occurs in minutes independent of *de novo* protein synthesis (Cobbett and Goldsbrough, 2002). However, in contrast to early models (Grill et al., 1985; Rauser, 1990; Steffens, 1990) for the activation of PC synthase, Vatamaniuk et al. (2000) demonstrated, in this way, that any metal ions may have the capacity to activate PCs biosynthesis only forming firstly a thiolate bonds with GSH (e.g., Cd.GS₂) (Vatamaniuk et al., 2000).

Metallothioneins proteins (MTs), like in PCs, are cysteine-rich and metal-binding proteins. However, MTs are characterized as low molecular weight (6–7 kDa) and they are products of mRNA translation and its enzymatic synthesis distinguishes them from PCs synthesis (Romero-Isart and Vasak, 2002; Cobbett and Goldsbrough, 2002). MTs are classified based on the arrangement of cysteine (Cys) residues (Vasak, 2005). In vertebrates, MTs class I proteins (MTI) contains 20 highly conserved Cys residues. However, MTs class II (MTII) does not present this strict arrangement of Cys and they are constituted in fungi and photosynthetic organisms. Besides, there are four additional categories of plant MTII based on amino acid sequences (Types 1–4) (Cobbett and Goldsbrough, 2002). Despite of scarce information of MTII on nonflowering plant species, an MT-encoding gene, with several dissimilarities into any of the four plant types has also been isolated from algae (Morris et al., 1999). This diversity of the photosynthetic organisms MTs gene family could point to possible different functions (Cobbett and Goldsbrough, 2002).

Proline (Pro) has been shown to play an important role in heavy metal stress in some but not all algal species. Transgenic microalgae *C. reinhardtii* expressing a mothbean Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) gene showed higher free-Pro levels than wild-type cells, grew to higher densities than wild-type cells in the presence of toxic concentrations of Cd, and sequestered four times more Cd per cell than wild-type cultures (Siripornadulsil et al., 2002). Accumulations of this amino acid may permit osmotic adjustment and provide protection for enzymes, biological membranes and polyribosomes by forming stable complexes with free radicals that could otherwise prove toxic. Proline may also play a role in maintaining NAD(P)⁺/NAD(P)H ratios during stress at values similar to those characteristic of normal growing conditions (Hare and Cress, 1997). Free Pro accumulates in response to Cu stress in axenic cultures of wild and Cu-tolerant strains of the lichen alga *T. erici* (Backor et al., 2004). As a result of short-term exposure, a Cu-tolerant strain exhibits significantly more intracellular Pro than a wild type. Proline inhibition of metal-induced loss of potassium in the Cu-tolerant strain is

similar to that in the free-living alga *Chlorella vulgaris* (Mehta and Gaur, 1999).

4.2. Stress proteins in algae

As with other biomarkers, stress proteins (or heat shock proteins—HSPs) share the characteristic over analytical procedures of estimating the effective concentration of xenobiotic that could provoke metabolic alterations in an organism (Bierkens et al., 1998). Metals and extremes of other environmental factors such as temperature and oxygen trigger changes in the transcript levels of numerous genes encoding proteins. Heat shock proteins are a group of chaperones, which specifically deliver metal ions to cell organelles and metal-requiring proteins; HSPs are highly conserved and involved in maintenance of protein homeostasis within cells. Ubiquitous in nature, HSPs, under normal conditions, are found at constitutive levels and conserved in species from bacteria to humans (Burdon, 1986). Forming a group divided according to their molecular weight, we can found four major stress protein families (HSP 90, 70, 60, and small HSP [SHSP] with 16–24 kDa) (Feder and Hofmann, 1999).

Among others protection abilities, HSPs increase tolerance to heavy metal stress by preventing membrane damage (Spijkerman et al., 2007). HSP 60 specifically, respond to redox-stress caused by metal toxicity (Lewis et al., 2001). Heavy metals can interfere in the photosynthetic activity by increased photoinhibition from excess of light (Heckathorn et al., 2004). *C. reinhardtii* respond to heavy metal exposure expressing nuclear HSP 70 genes (involved with a chloroplast-localized chaperone) (Schroda et al., 1999) and several SHSP (Downs et al., 1999). The expression of HSP 70 in axenic cultures of alga *T. erici* during short-term exposure to excess Cd and Cu displayed that Cu-treated cells maintained a relatively constant amount of HSP 70 over all tested concentrations, up to 10 nM, but Cd caused an increase in HSP70 expressions, especially at the lowest concentration (1.0 nM) (Backor et al., 2006). This phenomenon has been observed previously in the marine macroalgae *Fucus serratus* and aquatic plant *Lemna minor* in response to Cd stress (Ireland et al., 2004). The levels of stress proteins probably cannot continuously increase because the cost of HSP expression will outweigh its benefits (Pyza et al., 1997).

The use of chemical dispersants for oil spill clean-up reduces the injury of oil in the shorelines (Tiehm, 1994). However, the bioavailable oil fraction could increase through solubilization or emulsification process in the water column and alter the interactions between dispersant, oil, and biological membranes increasing bioaccumulation and changing biotransformation process, resulting in toxicity via food chain (Wolfe et al., 1999). Researches, using chemical dispersants for oil spill remediation have shown that the HSPs response in algae may enhance tolerance to crude oil (Wolfe et al., 1998).

4.3. Defense mechanisms against oxidative stress

Both animal and plant cells are capable of generating—via multiple sources—a number of different reactive oxygen species (ROS), including the superoxide anion (O₂^{•−}), hydrogen peroxide (H₂O₂), singlet oxygen [O₂ (¹Δ_g)], and by Fenton reaction, the hydroxyl radical (•OH) (Halliwell and Gutteridge, 2007). These species occur transiently and are regular products of oxidative metabolism. Although some ROS may function as important signaling molecules that alter gene expression and modulate the activity of specific defense proteins, all ROS are harmful to organisms at high concentrations (Apel and Hirt, 2004). The rate of ROS production in photosynthetic organisms constitutes part of

It is well established that, among the cellular defenses (Fig. 5) against ROS, carotenoids (Car) quench electronically excited-state molecules (the quenching efficiency is directly proportional to the number of conjugated double bonds (Cantrell et al., 2003)) such as O₂ (¹Δ_g), which have been shown to be capable of inducing DNA damage and to be mutagenic (Di Mascio et al., 1990; Barros et al., 2001; Bohm et al., 2001; Murthy et al., 2005; Sthal et al., 2006). Flavonoids and polyphenols are other low molecular weight compounds widely distributed in higher plants and algae that are able to scavenge oxy-radicals in biological systems (Rajendran et al., 2004). Additionally, ascorbate (Asc) is of particular interest as an electron donor for the aggressive [•]OH and as a substrate for ascorbate peroxidase producing dehydroascorbate (DHAsc) (Perricone et al., 1999; Raven, 2000; Nagata et al., 2003). Finally,

the tripeptide polymer composed of γ -glutamate, cysteine, and glycine (GSH), among its several metal detoxification functions, is both a non-specific general reductant and a substrate for enzyme catalyzed reactions (e.g., for recycling of DHAsC by dehydroascorbate reductase (DHAsCR) or aids 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 resulting oxidized form of GSH consists of the corresponding disulfide (GSSG). The enzyme glutathione reductase (GR) utilizes NADPH as a cofactor to reduce GSSG back to two molecules of GSH (Ogawa, 2005). With regard to high molecular weight compounds, aerobic organisms express a battery of enzymes that contribute to the control of cellular ROS levels. Superoxide dismutase (SOD), the cell's first line of defense against ROS, catalyzes the disproportionation of $O_2^{\cdot -}$ to O_2 and H_2O_2 (Ken et al., 2005). Since $O_2^{\cdot -}$ is a precursor to several other highly reactive species, control of this free radical concentration by SOD constitutes an important protective mechanism (Fridovich, 1997). The activation of specific SOD isoforms (FeSOD in chloroplasts, MnSOD in mitochondria, and CuZnSOD in the cytosol) can serve as a biomarker for cells that are experiencing pollutant-induced increases in $O_2^{\cdot -}$ levels (Barros et al., 2005; Murthy et al., 2005). Although SOD genes have been isolated from many different species, the FeSOD isoform has been reported from only a few microalgae (Okamoto et al., 2001b). Subsequently, the enzyme catalase (CAT) catalyzes the production of H_2O from H_2O_2 , while ascorbate peroxidase (APX) reduces peroxides (H_2O_2 and organic hydroperoxides) to H_2O or the corresponding alcohols, respectively, using ascorbate as electron donor (Tripathi et al., 2006). Other auxiliary H_2O_2 -removing mechanism is the production of volatile halocarbons (i.e., bromoform, chloroform, and trichloroethylene) from vanadium-bromoperoxidases (VBPx) in cell walls of seaweeds affected by both biotic and abiotic stresses (Mtolera et al., 1996; Dring, 2006). Addition of external H_2O_2 also provoked a 3-fold increase in the production of brominated halocarbons in the red alga *Meristiella gelidium*, confirming that H_2O_2 is a substrate for the brominating activity in red seaweeds

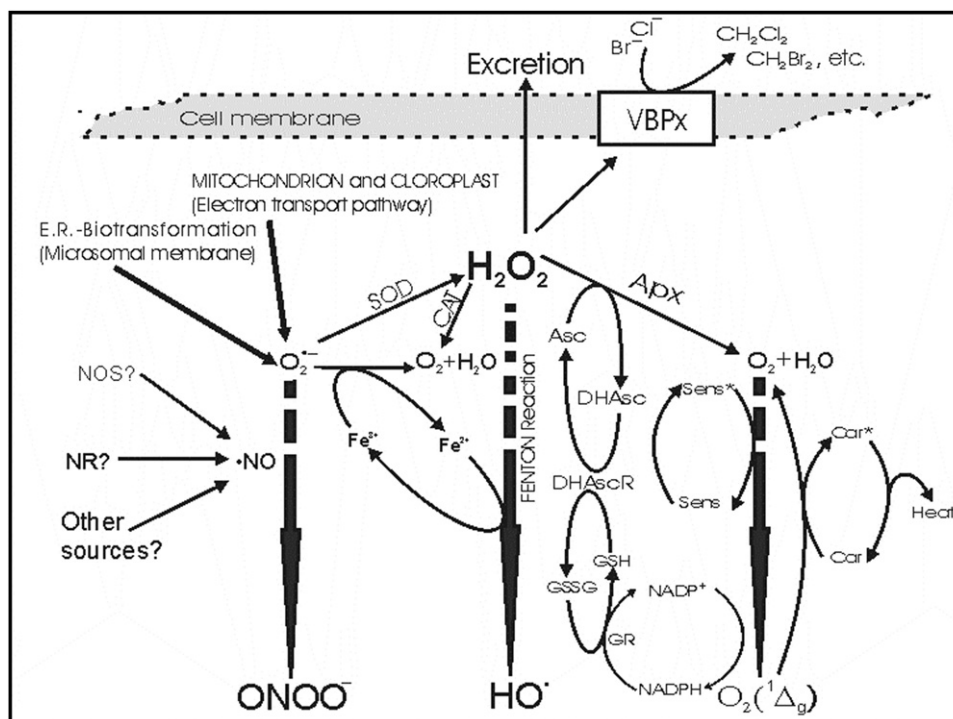


Fig. 5. Scheme showing the relationship between antioxidant defenses and free radical productions in photosynthetic organism.

(Collen et al., 1994). The H_2O_2 -dependent production of volatile halocarbons is considered to be part of alga chemical defenses against occasional epiphyte attack, as well as protection from grazing fish and invertebrates (Abrahamsson et al., 2003).

Research on reactive nitrogen species (RNS), such as nitric oxide ($\cdot\text{NO}$), peroxyxynitrite (ONOO^-) in photosynthetic organisms has gained considerable attention in recent years, testifying to the role of these substances (particularly $\cdot\text{NO}$). Inside specific compartments of plant and algal cell such as cytosol, chloroplasts and peroxisomes, $\cdot\text{NO}$ from NO synthase (NOS) or from nitrate reductase (NR) can react with O_2^- radicals to form the powerful oxidizing agent ONOO^- (Barros et al., 2005).

4.4. Xenobiotic detoxification systems

In a simplified conceptualization of bioaccumulation in aquatic environments, the contaminant uptake from water (directly or from adsorption/accumulation in food) is counterbalanced by endogenous enzymatic biotransformations and elimination processes. Hydrologic (e.g., water flow, hydrodynamic properties of the aquifer), geochemical (e.g., sediment granulometry and composition), and environmental conditions (e.g., temperature, pH and salinity) can also strongly affect bioaccumulation of a particular contaminant (either metal or organic) by interfering with its bioavailability in the aqueous phase (Guha, 2004). In most of the cases, biotransformation can lead to enhanced elimination, detoxification and redistribution within an organism, although (bio) activation processes can occasionally increase the toxicity of a contaminant. Bioactivation processes are of particular interest in evaluating ecotoxicological events in the whole aquatic biological system (Vlckova et al., 1999). An illustrative study of inter-trophic bioactivation is focused on the toxicity of organic compounds in sediment–alga–zooplankton systems. Given sufficient time, 2,6-dinitrotoluene present in marine sediments is biotransformed by

the red macroalgae *Ulva fasciata* to 2-amino-6-nitrotoluene, a metabolite harmless to algal zoospores (Nipper et al., 2004). On the other hand, selenium toxicity in aquatic systems results in storage of selenite (SeO_3^{2-}), calcium and phosphate in starch grains of *C. reinhardtii*, resulting in more granulous and less-dense stroma, severely inhibiting essential chloroplast processes such as photosynthesis (Morlon et al., 2005). Resistance of algal species to Se(IV) toxicity is apparently dependent on algal ability to biotransform SeO_3^{2-} to the insoluble Se(0) form (Li et al., 2003). Thus, selenium exposure dramatically affects the species composition of algal communities in aquatic environments, as organisms less adapted to biotransform Se(IV) to Se(0) tend to succumb earlier (Morlon et al., 2005).

Metabolism of xenobiotics (biotransformation) (Fig. 6) proceeds in photosynthetic organisms (Thies and Grimme, 1994; Warshawsky et al., 1995; Kirso and Irha, 1998; Pflugmacher et al., 1999; DellaGreca et al., 2003) in three phases.

In the first phase (Phase I) characterized by adding reactive functional groups (transformation) involves oxidations, reductions, or hydrolysis catalyzed by microsomal monooxygenase (MO) enzymes or mixed-function oxidases (MFO) (i.e., cytochrome P-450 (Cyt P450), cytochrome b5 (Cyt b5), and NADPH cytochrome P450 reductase (P45OR) (Thies et al., 1996; Pflugmacher and Sandermann, 1998a; Barque et al., 2002). The cytochrome P450 MOs are membrane bound proteins, which are predominantly located in the endoplasmic reticulum. The most important feature of the MFO system is its ability to facilitate the excretion of certain compounds, by transforming lipophilic xenobiotics to more water-soluble compounds (Zangar et al., 2004). Xenobiotic phase I biotransformations via MO system follows a reaction cycle which can be divided into various steps: In the first step, the substrate binds to the prosthetic heme ferric iron (Fe^{3+}) group of the enzyme. Following substrate binding, the iron is reduced by flavoprotein P450Reductase. Subsequently, O_2 is bound with possible generation of free radicals. The next step

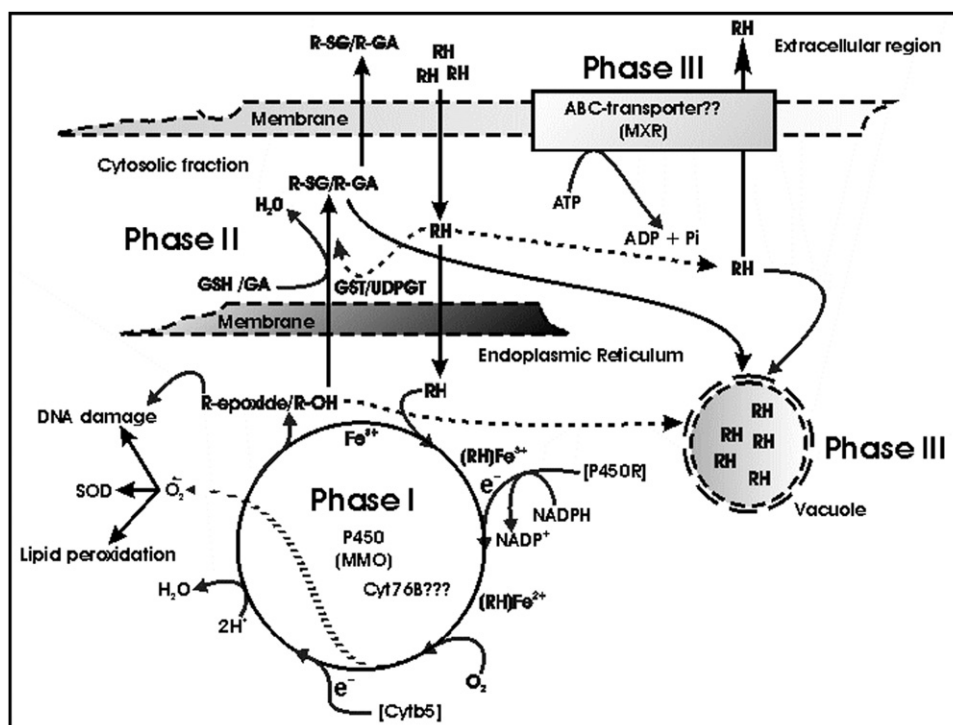


Fig. 6. This figure illustrates the complex interactions that are thought to occur between biochemical systems involved in detoxification (or toxification) of chemical compounds in aquatic photosynthesized organism cells. The dot line represents bay-pass reactions. Some hypothesis like ABC-transporters and Cyt76B need more researches to be confirmed in these organisms.

involves the addition of a second electron, via Cyt b_5 and the formation of a peroxide, followed by cleavage of the O–O bond, the formation of a substrate radical and the release of the product (Stegeman and Hahn, 1994). In animals, the class of CytP450 isozymes which is responsible for biotransformation of xenobiotic compounds is CYP1A subfamily (van der Oost et al., 2003). However, in plants, P450s (CYP76B) with high xenobiotic-metabolizing capacities actively catalyzes the NADPH-dependent O-dealkylation of 7-ethoxycoumarin (ECOD activity) and xenobiotics (Batard et al., 1998; Robineau et al., 1998; Werck-Reichhart et al., 2000). Despite evidences for xenobiotic biotransformation processes in microalgae (Pflugmacher et al., 1999; DellaGrecia et al., 2003), there is no reported CYP76B subfamily in these organisms. The so-called phase II of xenobiotic metabolism (conjugation) is characterized by addition reactions in which large and often polar compounds (e.g., GSH and glucuronic acid (GA)) are covalently added to xenobiotic compounds facilitating the excretion (Warshawsky et al., 1990; Pflugmacher and Sandermann, 1998b). Some xenobiotic compounds possess the requisite functional groups (e.g., –COOH, –OH or –NH $_2$) for direct metabolism by conjugative phase II enzyme systems (e.g., glutathione *S*-transferases (GSTs) and UDP-glucuronyl transferases (UDPGTs) (Pflugmacher et al., 2000), while others, are metabolized by an integrative process involving prior action of the phase I enzymes. In photosynthetic organisms, phase III is often characterized by compartmentation of the exported xenobiotic in the cell wall fraction or in the vacuole (Avery et al., 1995; Jabusch and Swackhamer, 2004; Alivés et al., 2005). Alternatively, the multixenobiotic resistance (MXR) *P*-glycoprotein transporter (ABC) may be induced in aquatic animals in environments containing high-level pollutants leading to the export of xenobiotic conjugates from the cell (Bard et al., 2002; Smital et al., 2003). There is currently no evidence for the existence of MXR into phytoplankton or macroalgae.

The first steps (phases I and II) of these detoxication pathways bear similarities to those of the mammalian liver, hence microalgae could function as “green livers” acting as important sinks for environmental chemicals (Sandermann, 1992, 1994, 2004; Pflugmacher and Sandermann, 1998a; Pflugmacher et al., 1999). There is little information available on the activities of these enzymes in algae and more detailed knowledge is required. These organisms represent the largest biomass component of aquatic systems and could therefore act as a significant tool in ecotoxicological studies.

5. Role of algae in biodegradation and bioremediation process

Bioaccumulation is the result of the net accumulation of a contaminant in living organisms. Undoubtedly, the consequences of xenobiotic bioaccumulation in a biological system are revealed at multiple hierarchical levels: from single organism effects (physiological and/or biochemical) to cross-linked trophic connections in the ecosystem as a whole (Newman and Unger, 2003). As a general consensus, most anthropogenic organic chemicals can be naturally biodegraded within aquatic environments as a result of multiple processes performed by auto- and heterotrophic organisms within the biological system (Singer et al., 2004). Through the process of evolution, organisms accumulated a variety of biodegrading enzymes to cope with hundreds of thousands of different allelochemicals, synthesized to attract, defend, antagonize, monitor, and misdirect one another. Recent scientific researches in pesticide and herbicide technologies have been designed to study ecological impacts based on allelochemical interactions, with emphasis on linking terrestrial and aquatic organisms (Fritz and Braun, 2006).

To monitor changes in toxicity during bioremediation processes, bioassays are often recommended as complements to chemical analyses. Among several available bioassays, endpoints of genotoxicity tests, such as chromosome/chromatid aberrations and micronuclei, can be monitored in fast-dividing microbial cells (Migid et al., 2005). Undeniably, rational bioremediation programs should take the diversity of biodegradation enzymes into account, following multiple trophic level analyses. The use of microalgae in bioremediation programs is a growing field of research in environmental microbiology (Semple et al., 1999; Juhasz and Naidu, 2000; Gourlay et al., 2005).

6. Conclusion and trends

Although there abound uncertainty regarding xenobiotic detoxification metabolism in photosynthetic organisms, several studies involving the utilization of algae as bioindicator are available (Pinto et al., 2003; Gerofke et al., 2005; Tripathi et al., 2006; Conti et al., 2007). Moreover, the vast information available from other organisms (fish (Carrasco-Letelier et al., 2006), mussels (Torres et al., 2002), crabs (Thawley et al., 2004), etc.) vis-à-vis the biotransformation processes could be interplayed with algal detoxification data in order to improve the knowledge about risk assessment in the aquatic environment.

Algae are able to absorb pollutants from the aquatic environment and biotransform organic compounds and immobilize inorganic elements to make them less toxic (Pflugmacher et al., 1999; Sánchez-Rodríguez et al., 2001). Besides, it is well known that they are at the basis of pollutant biomagnification and the transfer to upper levels of the food web have been considered (Sandermann, 1992; Nyström et al., 2002).

Algae have been suggested and used as potential bioindicators of aquatic pollution and its metabolic response to xenobiotic could point to important biomarkers (Witton and Kelly, 1995; Ali et al., 1999; Volterra and Conti, 2000). The presence of metals in algae induces the synthesis of several proteins, including metallothionein (Romero-Isart and Vasak, 2002; Vasak, 2005), phytochelatins (Cobbett and Goldsbrough, 2002; Perales-Vela et al., 2006), and HSPs (Wolfe et al., 1999; Spijkerman et al., 2007). However, the gene regulation and the preferential way of these detoxification systems are still unclear.

Possibly the enzymatic system (Phase I) to organic compounds and oil derivatives detoxification in algae is via cytochrome P450 (Pflugmacher and Sandermann, 1998a). Despite of some evidences in plants showing specific enzymes CYP 450 family activities (Robineau et al., 1998; Werck-Reichhart et al., 2000), the mechanisms related to the biotransformation phase I of organic compounds in algae are also unknown. Similarly unclear in algae are the MXR transporters which marine animals (Smital et al., 2003) use to transport xenobiotics outside the cell (Bard et al., 2002).

On the other hand, it has been shown that the presence of pollutants can induce oxidative and nitrosative stress and therefore, since algae has important antioxidant system, they can be used as powerful biomarker tools for pollution exposure (Pinto et al., 2003).

It has been confirmed that inhibition of growth and photosynthesis are the basic reflex of the toxic effects of pollutants on microalgae (Franqueira et al., 2000). Moreover, toxicity tests based on algae have been used in conjunction with other organisms to assess associated environmental effects of pollutants and the integrity of aquatic ecosystems (Cid et al., 1996; Blaise and Menard, 1998; Stauber et al., 2002). However, the algal multi-species studies are limited using algal-growth inhibition standard due to difficulty of distinguishing multispecies populations and

the detection of toxicity endpoints on the targeted species (Yu et al., 2007). Although it is widely used in medical and oceanography applications, flow cytometry has only recently been applied to ecotoxicological studies (Stauber et al., 2002). This kind of approach is enabling to separate each microalgal population on the basis of its characteristic fluorescence signal, so that effects of contaminants can be assessed in multispecies bioassays (Yu et al., 2007).

Increased consciousness of the necessity to safeguard aquatic environments has prompted a search for alternative technologies to remove toxic compounds from the aqueous fraction (Paquin et al., 2003). The most common algal-based biotechnologies used for inorganic contamination removal are high rate algal ponds (HRAP) and the patented algal turf scrubber (ATS), which employs suspended biomass of microalgae, cyanobacteria or consortia of both (Andrade et al., 2004; Perales-Vela et al., 2006). Additionally, an algal-bacterial consortium has been tested for the treatment of heavy metal ions and organic pollutants (Munoz et al., 2006). Water recycling systems, in tandem with biofilters, also reduce the amount of water discharge from aquaculture operations, thus limiting eutrophication events in aquifers (Gutierrez-Wing and Malone, 2006; Avnimelech, 2006).

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