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Oxidative stress and its biomarkers in microalgal ecotoxicology

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Abstract

The article reviews oxidative stress metabolites in plants and their use as biomarkers in ecotoxicology. Environmental pollutants increase the intracellular formation of reactive oxygen species (ROS) that in turn trigger various defense systems against oxidative stress. The ROS, oxidative damage to cells, and activity of antioxidants can be used as biomarkers. An overview of the use of microalgal oxidative stress biomarkers for the study of toxicity mechanisms of common xenobiotics is given.

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1. Introduction

Oxidative stress is an important indicator of hazardous properties of xenobiotics and widely used for monitoring of environmental quality. During the processes of cell respiration and photosynthesis, the flow of electrons constantly form free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Living organisms use free radicals for many complex biological processes. There are many experimental confirmations of the use of superoxide anion radical ($O_2^{\bullet -}$), singlet oxygen O_2 ($^1\Delta_g$), hydrogen peroxide (H_2O_2), peroxyl radicals ($ROO^{\bullet}$), hydroxyl radical ($^{\bullet}OH$), nitrogen oxide ($NO^{\bullet}$), and other radicals by living organisms [1]. However, an excess of these highly reactive molecules can lead to damage of DNA, proteins, and lipids. In normal conditions, overflow of these molecules is neutralized by an antioxidant defense system. The imbalance between the production of ROS and RNS and their neutralization is called oxidative stress [2].

Xenobiotics influence various metabolic mechanisms and stimulate intracellular production of ROS and RNS, causing oxidative damage [3]. Antioxidant defense can be used only in the early stages of xenobiotics exposure until depletion of appropriate antioxidants [4]. In addition, living organisms have enzymatic systems that are responsible for the restoration and removal of damaged proteins, lipids, and DNA and can adapt to fluctuations in the level of oxidative stress, synthesizing additional antioxidants [5,6].

By using molecular biomarkers, such as increase in ROS/RNS production and activation of antioxidative systems, it is possible to determine the level of oxidative damage to the organism in response of xenobiotics exposure. However, the diversity of antioxidants formed under the influence of different chemicals, the different sensitivity of test organisms to different xenobiotics, and dependence of environmental conditions cause difficulties in using oxidative stress as a tool for ecotoxicology. Thus, the presence of many significant oxidative stress factors and combinations of these factors require strict systematization.

2. Oxidative stress in plants and mechanisms of defense

Photosynthetic organisms depend on free radicals that are formed as intermediates during photosynthesis, photorespiration, lipid metabolism, and cell signaling [7–9]. However, constant flux of electrons coming from mitochondria and chloroplasts and high concentration of polyunsaturated lipids in the envelopes/membranes of these organelles make the cells of photosynthetic organisms susceptible to oxidative damage [10].

Plant cells use a variety of enzymatic and nonenzymatic antioxidants to prevent the negative effects of ROS and

Table 1 Plant antioxidative protection mechanisms.

Antioxidant	Mediators of oxidative stress	Mechanism of protection	Reference
Nonenzymatic antioxidants			
Carotenoids (Car)	Singlet oxygen O_2 ($^1\Delta_g$) and peroxyls ($RO_2^{\bullet}$)	Protection of the light-harvesting pigments by quenching of free radicals; the effectiveness of quenching is proportional to the number of conjugated double bonds in the carotenoid molecule	[12,13]
Flavonoids and phenols	Not specific	ROS and RNS scavenging	[14]
Ascorbate (Asc)	Hydroxyl radical ($^{\bullet}OH$), singlet oxygen O_2 ($^1\Delta_g$), and superoxide ($O_2^{\bullet-}$)	Radical scavenger; serves as an electron donor for ascorbate peroxidase (APX)	[15–17];
Anthocyanin	Superoxide ($O_2^{\bullet-}$)	Radical scavenger	[15]
Tocopherols and tocotrienols	Lipid peroxy radicals and singlet oxygen O_2 ($^1\Delta_g$)	Free-radical scavengers due to the ability to donate phenolic hydrogen to lipid free radicals; singlet oxygen quenchers by electron donation as well as scavengers by chemical reaction	[18]
Mycosporine-like amino acids (MAA)	UV radiation, hydroxyl radical ($^{\bullet}OH$), and singlet oxygen O_2 ($^1\Delta_g$)	Absorption of UV wavelengths from sunlight; protection from photooxidative stress	[19]
Glutathione (GSH)	Not specific	Substrate for enzyme-catalyzed reactions; rearrangement of protein disulfide bonds; after oxidation forms two disulfide molecules bonded together (GSSG)	[20]
Phytochelatin (PC)	Heavy metals	Synthesized from GSH; carries out metal chelation (creates complexes with metals) and transport of metals	[21]
Enzymatic antioxidants			
Superoxide dismutase (SOD)	Superoxide ($O_2^{\bullet}$)	Catalyzes conversion of excess $O_2^{\bullet-}$ to O_2 and H_2O_2, preventing the formation of other highly reactive species; three isoforms in eukaryotic photosynthetic organisms: CuZnSOD — in membranes of thylakoids and cytosol, MnSOD — in mitochondria, and FeSOD — in chloroplast stroma	[22,23]
Enzyme catalase (CAT)	Hydrogen peroxide (H_2O_2)	Catalyzes conversion of H_2O_2 to H_2O	[24]
Glutathione peroxidase (GPX)	Hydrogen peroxide (H_2O_2) and hydroperoxides (ROOH)	Catalyzes conversion of H_2O_2 to H_2O or ROOH to corresponding alcohols	[12,22]
Glutathione reductase (GR)	Hydroxyl radical ($^{\bullet}OH$) and singlet oxygen O_2 ($^1\Delta_g$)	Separates GSSG into two molecules of GSH with uptake of a molecule of NADPH	[20]
Ascorbate peroxidase (APX)	Hydrogen peroxide (H_2O_2) and ROOH	Decays peroxides to H_2O or ROOH to corresponding alcohols, using Asc as electron donor	[16]
Peroxiredoxins (PRX)	Hydrogen peroxide (H_2O_2), hydroperoxides (ROOH), and peroxynitrite ($ONOO^-$)	Catalyzes H_2O from H_2O_2, corresponding alcohols from ROOH or $NO_2^{\bullet}$ from $ONOO^-$	[25]
Vanadium-bromoperoxidases (VBPx)	Hydrogen peroxide (H_2O_2)	Produces volatile halocarbons (i.e. bromoform, chloroform, and trichlorethylene) using H_2O_2	[26]

RNS [11,12]. Plant protection mechanisms from ROS and RNS are presented in Table 1.

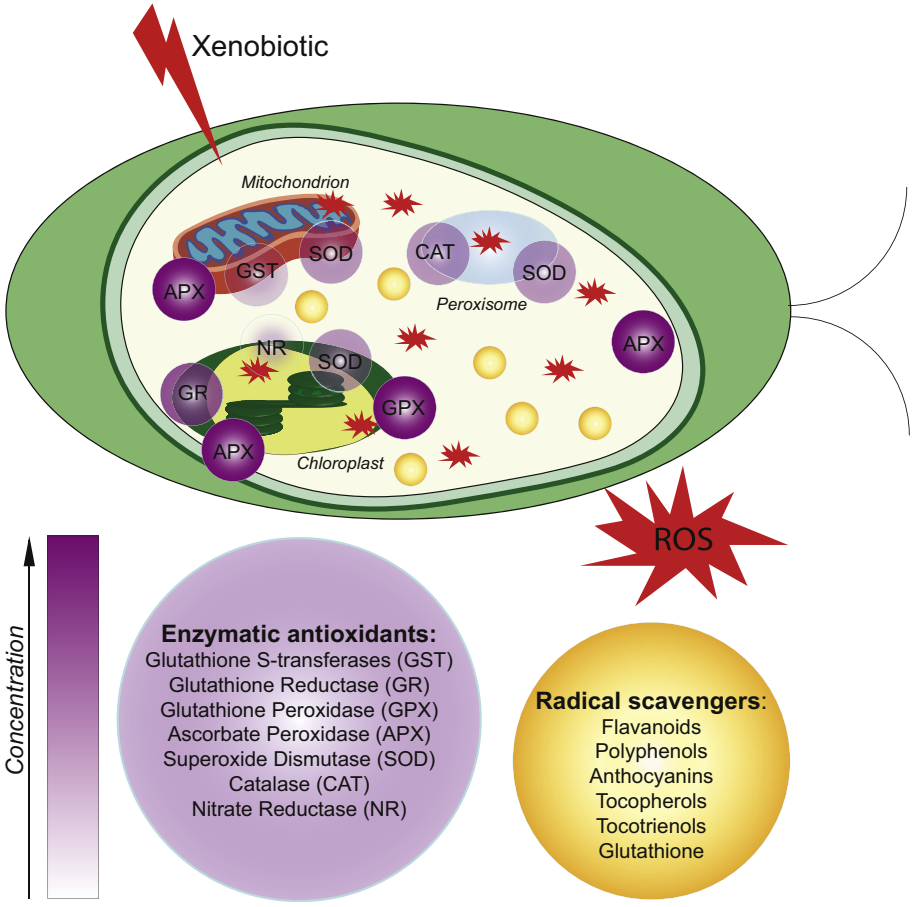
An important role in preventing oxidative damage and providing photosynthetic capacity of plants is played by nitric oxide ($NO^{\bullet}$). $NO^{\bullet}$ performs a signaling function (similar to ROS); it regulates the internal ion balance providing the basic functions of the cell, facilitates an appropriate response to oxidative stress, and changes gene expression patterns in response to abiotic factors [27].

3. Molecular biomarkers of oxidative stress in microalgae

Aquatic organisms are widely used in toxicology and ecotoxicology due to their sensitivity, ubiquity, and simplicity of cultivation. Microalgae can be highlighted among aquatic test organisms as a key link that forms the initial trophic level of food chains that are more widespread in the ocean than any other group [28]. Microalgae are described as a group of unicellular microorganisms that have chloroplasts, that is, intracellular structures possessing their own photosynthetic mechanism [29]. Microalgae are responsible for more than half of the photosynthetic activity on the planet [30], they are food producers for more than 70% of the world's biomass [31], and any processes and substances that affect their population can cause consequences for higher trophic levels.

To evaluate the anthropogenic impact or to determine the mechanisms of toxicity, biomarkers are often used.

Figure 1



Scheme of using algal antioxidants as xenobiotic stress biomarkers. ROS, reactive oxygen species.

There are biomarkers of exposure that characterize exposure at a suborganism level of biological organization and biomarkers of effect that include biochemical, physiological, or ecological structures or processes that have been correlated or causally linked to biological effects measured at one or more levels of biological organization [32–34].

As toxicity toward plants at the biochemical level can be identified at the earliest stages, biochemical parameters can be operative (may occur until visible changes appear) and more sensitive indicators of xenobiotic stress. Even a small impact can affect the photosynthetic apparatus of plants, which includes numerous finely tuned biochemical pathways, depending on the constant flow of electrons. Such a change will increase the formation of ROS and, accordingly, protective antioxidants. The pigment content of chloroplasts and the efficiency of photosynthesis may also change, leading to change in growth rate. Thus, toxic effects can be potentially measured by the change in the activities of individual biochemical indicators. The principle of using algae antioxidants as xenobiotic biomarkers is presented in Figure 1.

An overview of oxidative stress biomarkers' response to common xenobiotics in microalgae is presented in Table 2.

Table 2 Biomarkers used to evaluate xenobiotic stress by algae species.

Biomarkers	Xenobiotic	Species	Biomarker response	Reference
Glutathione S -transferases (GST)	Pyrene	*Chlorella vulgaris*	No change in GST activity at 0.1–1 mg/L	[35]
		Scenedesmus quadricauda	Decrease in GST activity by 80% and 30% after 4- and 7-d exposure to 0.6–1.0 mg/L	[35]
		Scenedesmus platydiscus	A linear increase in GST activity after 4- and 7-d exposure to 0.1–1.0 mg/L. Fivefold increase at 1.0 mg/L	[35]
		Selenastrum capricornutum	A linear increase in GST activity after 1-, 4-, and 7-d exposure to 0.1–1.0 mg/L. Twofold increase at 1.0 mg/L	[35]
	Oxyfluorfen	*Scenedesmus obliquus*	22.5 μg L^{-1} of oxyfluorfen increases GST activity at 76% compared to control for 24 h; 15 μg L^{-1} — at 53%	[36]
	Erythromycin lactobionate (ETM)	*Raphidocelis subcapitata*	At 0.3 mg L^{-1}, total glutathione decreased to 64.8%	[37]
	Ciprofloxacin hydrochloride (CPF)	*Raphidocelis subcapitata*	Increased at 2.5 mg L^{-1} of CPF	[37]
	Sulfamethoxazole (SMZ)	*Raphidocelis subcapitata*	Increased at 2.5 mg L^{-1} SMZ	[37]
	Zinc	*Chlorella sorokiniana*	At concentration 1.0 mM of Zn, GST activity increased threefold compared to control	[38]
Glutathione reductase (GR)	CuO NP	*Chlamydomonas reinhardtii*	Increase in activity under CuO NP concentrations above 10 mg L^{-1} to 67% compared to control	[39]
	Zinc	*Chlorella sorokiniana*	At concentration 1.0 mM of Zn, GR activity increased 1.8-fold compared to control	[38]
	Oxyfluorfen	*Scenedesmus obliquus*	15 μg L^{-1} of oxyfluorfen increased GR activity at 47% compared to control for 24 h; 15 μg L^{-1} — at 53%	[36]
	Polybrominated diphenyl ethers (PBDEs)	*Alexandrium minutum*	The activity of GR increased 348%, 393%, and 74% at 0.1, 0.5, and 1 mg L^{-1} BDE-47, respectively, after 96 h of exposure compared to control	[40]
		Dunaliella salina	The activity of GR increased 35%, 118%, and 94% at 0.1, 0.5, and 1 mg L^{-1} BDE-47, respectively, after 96 h of exposure compared to control	[40]
	The herbicide S-metolachlor (S-MET)	*Parachlorella kessleri*	The increase in GR activity was observed starting from 48 h after treatment. The enhancement of GR activity persisted up to 72 h of treatment in cells exposed to 50, 100, and 200 μg/L S-MET	[41]
Glutathione peroxidase (GPX)	Diesel fuel (Water accommodated fraction, WAF)	*Raphidocelis subcapitata*	At 2–6.5 mg L^{-1}, GPX activity increased to 67–187% over control; GPX activity was observed at concentrations from 8 to 12.5 mg L^{-1}	[42]
		Chlorella sp.	No effect up to 0.5 mg L^{-1}; from 1.25 to 3.75 mg L^{-1}, the enzyme activity was increased and completely inhibited at 5 mg L^{-1}	[42]
	Polybrominated diphenyl ethers (PBDEs)	*Alexandrium minutum*	The activity of GPX increased 68%, 71%, and 126% at 0.1, 0.5, and 1 mg L^{-1}, respectively, after 96 h of BDE-47 exposure	[40]
		Dunaliella salina	The activity of GPX decreased by 16%, 50%, and 53% at 0.1, 0.5, and 1 mg L^{-1} BDE-47, respectively, after 96 h of exposure compared to control	[40]
Ascorbate peroxidase (APX)	Copper oxide nanoparticles (CuO NP)	*Chlamydomonas reinhardtii*	Increase in activity under CuO NP concentrations above 10 mg L^{-1} to 136% compared to control	[39]
	Oxyfluorfen	*Scenedesmus obliquus*	Stimulated by 15 and 22:5 μg L^{-1} of oxyfluorfen at 19% and 29%, respectively, compared to control	[36]
	ETM	*Raphidocelis subcapitata*	At 0.3 mg L^{-1}, total ascorbate decreased to 47.8%	[37]
	CPF	*Raphidocelis subcapitata*	At 1.5 mg L^{-1}, activities increased to 187% of the control	[37]
	SMZ	*Raphidocelis subcapitata*	No effect up to 2.5 mg L^{-1}	[37]
	The herbicide S-metolachlor (S-MET)	*Parachlorella kessleri*	The APX activity increased at 50 and 100 μg/L of S-MET after 4 h of treatment. The APX activity returned to control values by the end of the experiment (72 h)	[41]
Superoxide dismutase (SOD)	Diesel fuel (WAF)	*Raphidocelis subcapitata*	No effect up to 2 mg L^{-1} diesel, SOD activity increased significantly up to 6.5 mg L^{-1}, and was completely inhibited at 8 mg L^{-1}	[42]
		Chlorella sp.	No effect up to 1.25 mg L^{-1} diesel, 2.5 and 3.75 mg L^{-1} concentrations caused an increase in enzyme activity, activity was completely inhibited at 5 mg L^{-1}	[42]

(*continued on next page*)

Table 2 (*continued*)

Biomarkers	Xenobiotic	Species	Biomarker response	Reference
	Zinc	*Chlorella sorokiniana*	At concentration 1.0 mM of Zn, SOD activity increased 2.2-fold compared to control	[38]
	Polybrominated diphenyl ethers (PBDEs)	*Alexandrium minutum*	The activity of SOD increased 75%, 108%, and 131% at 0.1, 0.5, and 1 mg L^{-1} BDE-47, respectively, after 96 h of exposure compared to control	[40]
		Dunaliella salina	The activity of SOD increased 64% and 75% at 0.1 and 0.5 mg L^{-1} BDE-47, respectively, and decreased 49% at 1 mg L^{-1} after 96 h of exposure compared to control	[40]
	Cu^{2+}, $Cr_2O_7^{2+}$, Cd^{2+}, and Hg^{2+} ions	*Chlamydomonas reinhardtii*	In heavy metal−stressed cultures, the transcription level of gene MSD1, which encodes MnSOD, increased approximately 8- and 10-fold in Cu^{2+}- and Hg^{2+}-treated cultures and threefold and twofold in $Cr_2O_7^{2+}$- and Cd^{2+}-treated cultures, respectively	[43]
Catalase (CAT)	Diesel fuel (WAF)	*Raphidocelis subcapitata*	No effect up to 5 mg L^{-1}; 6.5 mg L^{-1} enhanced the enzyme activity by 46% over control; 8−12.5 mg L^{-1} resulted in complete inhibition of CAT activity	[42]
		Chlorella sp.	Increase in the activity of CAT up to 3.75 mg L^{-1} of diesel, but 5 mg L^{-1} concentration resulted in complete loss of activity	[42]
	CuO NP	*Chlamydomonas reinhardtii*	Increase at 139% compared to control to the concentration of 1 mg L^{-1} CuO NP and then quick decline of activity	[39]
	The herbicide S-metolachlor (S-MET)	*Parachlorella kessleri*	The activity was decreased in all treatments at 4 h and after 24 h in 50 and 200 μg/L S-MET concentrations. On the contrary, the significant enhancement was observed in longer treatments. At 48 h, the activity was significantly higher in cells treated with 2 and 50 μg/L S-MET, whereas longer exposure (72 h) increased CAT activity in all treatment groups	[41]
Nitrate reductase (NR)	Atrazine	*Chlamydomonas reinhardtii*	0.1 and 0.25 μM of atrazine provoked a decrease in NR activity > 40%; and NR dropped > 80% with atrazine concentrations of 0.5 μM. At 1 and 2 μM, NR was completely inhibited	[44]
	Glufosinate	*Phaeodactylum tricornutm*	Decrease of NR to 26% of the control after 96 h of exposure under 4 mg L^{-1} glufosinate	[45]
Flavonoids	Zinc	*Chlorella sorokiniana*	At concentration of 1 mM, the content of flavonoids increased by 1.9-fold compared to control	[38]
Polyphenols	Zinc	*Chlorella sorokiniana*	At concentration of 1 mM, the content of polyphenols increased by 2.5-fold compared to control	[38]
Reactive oxygen species (ROS)	Zinc	*Chlorella sorokiniana*	At concentration of 1.0 mM, the content of H_2O_2 increased by 2.1-fold compared to the nonstressed control	[38]
		Scenedesmus acuminatus	At concentration of 0.6 mM, the content of H_2O_2 increased by 1.7-fold compared to the nonstressed control	[38]
	Cr_2O_3 NP	*Chlamydomonas reinhardtii*	ROS level increased by 38.5%, 63.39%, and 66.81% at Cr_2O_3 NP concentration of 0.1, 1.0, and 10.0 g L^{-1}, respectively, compared to control. The intracellular ROS increased by 160.24% at a Cr_2O3 NP concentration of 10 g L^{-1} after 24 h of exposure. However, at the same concentration, the intracellular ROS levels decreased to 59.91% after 72 h of exposure	[46]
	Lead	*Chlorella* sp.	At concentration of 0.145 10^{-9} M, ROS increased by 1.7-fold from 5 to 24 h of lead exposure; in the long-term treatment, ROS levels increased gradually at 0.87 10^{-9} M lead and then sharply declined after 8 d of exposure	[47]
		Scenedesmus acutus	At concentration of 0.034 10^{-9} M, ROS production increased 1.49-fold; at 0.87 10^{-9} M, ROS production increased 4.57-fold compared to control, after 3 d of lead exposure. In the short-term lead treatments (within the range allowing growth), there was an increase of ROS and significant decrease of ROS production over time during long-term treatment (8 d)	[47]
	the herbicide S-metolachlor (S-MET)	*Parachlorella kessleri*	ROS content increases up to 24 h of exposure and a gradual decrease by the end of the experiment (72 h). The highest ROS levels were observed at 4 h (100 and 200 μg/L S-MET). At 48 h, only 50 μg/L treatment significantly increased the ROS levels compared to control. After 72 h, cell ROS levels were close to control	[41]

Table 2 (*continued*)

Biomarkers	Xenobiotic	Species	Biomarker response	Reference
	CdSe/ZnS quantum dots/Cadmium	*Chlamydomonas reinhardtii*	Continuous hydrogen peroxide measurement shows that ROS/ antioxidant balance is reestablished after 60 min of Cd exposure; consecutive exposures induce enhanced H_2O_2 generation.	[51]
Malondialdehyde (MDA)	TiO_2 NP	*Chlamydomonas reinhardtii*	MDA content in all treatment groups (0.1, 1, 10, 20, and 100 mg/l) increased after 4 h of treatment compared to control	[48]
Stress proteins	Zinc	*Raphidocelis subcapitata*	Increase in Hsp70 content; LOEC = 3.6 μM	[49]
	Selenium	*Raphidocelis subcapitata*	Increase in Hsp70 content; LOEC = 1.6 μM	[49]
	Sodium dodecyl sulfate	*Raphidocelis subcapitata*	Increase in Hsp70 content; LOEC = 83 μM	[49]
	Carbaryl	*Raphidocelis subcapitata*	Increase in Hsp70 content; LOEC = 10.3 μM	[49]
	Lindane	*Raphidocelis subcapitata*	Increase in Hsp70 content; LOEC = 9 μM	[49]
Photosynthetic pigments	CuO NP	*Chlamydomonas reinhardtii*	Decrease of total chlorophyll (Chl tot) (1.88 μg mL^{-1} on control and 0.49 μg mL^{-1} on 1000 mg L^{-1} of CuO NP); reduction of carotenoid levels in response to increasing of CuO NP concentration (0.71 μg mL^{-1} on control and 0.13 μg mL^{-1} on 1000 mg L^{-1} of CuO NP); increase in the 'Chl tot/Carotenoids' ratio (2.64 on control and 3.64 on 1000 mg L^{-1} of CuO NP)	[39]
	The herbicide S-metolachlor (S-MET)	*Parachlorella kessleri*	A significant decrease in Chl *a* content per cell up to 24 h of treatment, whereas at 48 and 72 h, Chl *a* concentration was significantly decreased for 50, 100, and 200 μg/L of S-MET compared to control; the Chl *b* content decreased significantly compared to control in all S-MET concentrations tested at every time point; Chl *a*/Chl *b* ratio was mainly uniform throughout the experiment	[41]
	$Cr_2O_7^{2+}$, Cd^{2+}., Cu^{2+}, Ag^{+}, and Hg^{2+} ions	*Chlamydomonas reinhardtii*	There was observed dose-dependent inhibition of Chl synthesis for all ions; the Chl *a*/*b* ratio was similar to control in $HgCl_2$- and $AgNO_3$- treated cultures. The Car/Chl ratio in the cultures exposed to chronic heavy metal–induced stress was also similar to control, except series with $K_2Cr_2O_7$, where a slight increase was observed	[50]

NP, nanoparticles; MSD1, gene manganese superoxide dismutase 1; LOEC, Lowest Observed Effect Concentration.

4. Conclusion

Oxidative stress and diverse mechanisms of its regulation in plants create a fertile field for tracing and preventing the influence of xenobiotics on the environment. The most frequently used indicators in ecotoxicology, such as growth rate, biomass buildup, and morphological changes, are insufficient to understand toxicity mechanisms of xenobiotics. The biomarkers associated with oxidative stress that have been reviewed in this article are more promising in that respect. However, the determination of chemical hazard and environmental risk assessment requires an integrated approach that includes measurements both at the level of ecosystems and populations, as well as at the level of cells and molecules.

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