

# Effects of Pesticides on Freshwater Diatoms

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## Contents

1	Introduction . . . . .	87
2	Effects on Cytology and Cell Ultrastructure . . . . .	89
2.1	The Cytoskeleton . . . . .	89
2.2	The Nucleus and DNA . . . . .	89
2.3	The Siliceous Cell Wall . . . . .	90
3	Effects on Cell Metabolism . . . . .	91
3.1	Photosynthesis . . . . .	91
3.2	Synthesis of Protein, Lipids, and Carbohydrates . . . . .	92
3.3	Nutrient Absorption . . . . .	92
4	Effects on Diatom Growth . . . . .	93
4.1	Algal Biomass . . . . .	93
4.2	Diatom Cell Density . . . . .	94
5	Effects on Species Composition . . . . .	94
5.1	Diatom Species . . . . .	94
5.2	Algal Community . . . . .	96
6	Interferences in the Response of Diatoms to Pesticide Exposure . . . . .	96
7	Summary . . . . .	97
	References . . . . .	98

## 1 Introduction

Human activities have resulted in release of a large range of toxic contaminants into aquatic ecosystems. The assessment of environmental pesticide residue concentrations in Europe and North America has shown significant contamination of streams and groundwater in both agricultural and urban settings (Gilliom et al. 1999; IFEN

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2006; Devault et al. 2007; Gilliom 2007; Sprague and Nowell 2008). According to data recently available in France and the United States, water quality benchmarks for aquatic life were exceeded in half of the sites studied (IFEN 2006; Gilliom 2007). Gilliom et al. (2007) reported that the pollution of streams was relatively concentrated in time with frequent short pulses of higher pesticide concentrations. Numerous publications have shown that pesticide concentrations exceeded 10  $\mu\text{g/L}$ , and even reached values approaching 700  $\mu\text{g/L}$  in samples collected after heavy rains or during high river flow (Spalding and Snow 1989; Carder and Hoagland 1998; Schulz 2001; Ferenczi et al. 2002). Such events have elevated concerns about environmental risk.

Enforcement of new legislation in Europe (Water Framework Directive 2000/60) has been undertaken to restore the quality of the most contaminated water resources and to meet “good ecological status” by 2015. The main purpose of these rules was to establish levels of pesticide pollution that may have noxious effects on aquatic ecosystems. The majority of ecotoxicity data are only available for single pesticides, and have not been adapted for use in estimating the collective toxicity of pesticides in stream water. The combined toxicity of such pollution is much more complex, given the large range of toxic molecules (pesticides and their metabolites) that may exist in streams and other water bodies (Gilliom et al. 1999; Gilliom 2007). It has been reported that the toxicity of pesticide mixtures is greater than that of individual pesticides (Lydy et al. 2004; Belden et al. 2007). Therefore, pesticide pollution may constitute a major threat to the health and productivity of aquatic ecosystems. At the base of the trophic food chain, primary producers such as diatoms, which represent a source of food for numerous other organisms, may be seriously affected by pesticide exposure. Moreover, such pollutants may significantly disturb the equilibrium of the trophic food chain (Stevenson and Pan 1999).

When rapid water flow limits the development of other algae, diatoms are often the dominant remaining primary producers (Ghosh and Gaur 1998). Therefore, these microalgae are particularly relevant when the harmful effects of pesticide pollution are investigated in aquatic ecosystems. In addition, herbicides (e.g., isoproturon, diuron, atrazine, and *s*-metolachlor), which are particularly toxic to algae, represent the major pesticide class that contaminate rivers, owing primarily to the large amounts used in agriculture and on urban areas, and sometimes owing to their environmental persistence (Agence de l’eau Adour-Garonne 2006; Gilliom 2007). Numerous studies have been performed to study the effects of pesticides on individual species and on species communities. Indeed, if diatom communities are recognized as useful bioindicators of herbicide-contaminated water, their efficiency to detect such toxic pollutants has yet to be demonstrated (Dorigo et al. 2004).

The purpose of this chapter is to provide a broad bibliographical review of articles that address the effects of pesticides and certain other xenobiotics on diatoms. In this review, we emphasize the following targets of pesticide action: (i) cytology and cell ultrastructure, (ii) cell metabolism, and, finally, (iii) effects on community species composition.

## 2 Effects on Cytology and Cell Ultrastructure

Organelles are strongly interlinked in diatoms. A single alteration can seriously perturb all cellular organelles. The study of different intracellular-component responses to toxic agents may help to understand how diatoms respond when exposed to pesticides. Nevertheless, the literature that deals with intracellular toxic effects in diatoms is quite limited. In this review, we have chosen to focus attention on the effects to major organelles (intracellular cytoskeleton, nucleus, and cell wall) of diatoms that may be linked to pesticide exposure.

### 2.1 The Cytoskeleton

The cytoskeleton (microtubules, actin filaments, and microfilaments) is involved in cell division and manages the internal layout of the cell. Many herbicides have been developed that can or do affect the cytoskeleton. Disturbance of cytoskeleton organization may induce several types of cell injuries, especially during mitosis. Thus, Coss and Pickett-Heaps (1974) have shown that IPC (isopropyl *N*-phenyl carbamate), a carbamate herbicide, produced mitotic spindle effects in the green alga *Oedogonium cardiacum*. Similarly, diatoms and green algae, exposed to the cytoskeleton inhibitors colchicine and cytochalasin D, induced a disruption of mitotic spindles (Coombs et al. 1968; Pickett-Heaps and Spurck 1982; Edgar and Pickett-Heaps 1984; Pickett-Heaps et al. 1984; Cohn and Pickett-Heaps 1988; Puiseux-Dao 1989; Sampson and Pickett-Heaps 2001). Colchicine is known to block the binding of tubulins  $\alpha$  and  $\beta$ , and thus, the synthesis of microtubules (Puisseux-Dao 1989). According to Coss and Pickett-Heaps (1974), colchicine and IPC have similar mechanisms of action. Similarly, the work of Spurck and Pickett-Heaps (1994) demonstrated an abnormal arrangement of chromosomes during metaphase for two diatom species (*Surirella robusta*, *Hantzschia amphioxys*) caused by the drug, diazepam; the primary effect observed in these two species was disturbed organization of the mitotic spindle. However, in the case of a moderate exposure to diazepam, these authors reported a restoration of the cytoskeleton integrity.

### 2.2 The Nucleus and DNA

A few studies have been conducted to determine the toxic effects of chemicals on the diatom cellular nucleus. Cassoti et al. (2005) observed dispersion of DNA in cells of the marine diatom, *Thalassiosira weissflogii*, when these organisms were exposed to the aldehyde, 2-*trans*,4-*trans*-decadienal. Debenest et al. (2008) also observed DNA dispersion in diatom cells exposed to the plant growth regulator, maleic hydrazide. Multinuclear cells were reported to occur in the diatom *Navicula pelliculosa*, when these cells were exposed to colchicine (Coombs et al. 1968; Duke and Reimann 1977) or when green algae (*O. cardiacum*) were exposed to IPC

(Coss and Pickett-Heaps 1974). The well-known genotoxic plant growth regulator, maleic hydrazide, induced micronucleus and multinuclear cells in a multispecific diatom culture (Debenest et al. 2008). As previously observed by Cassoti et al. (2005), DNA fragmentation was observed for diatoms exposed to certain genotoxic agents (cadmium, 1-methyl-3-nitro-1-nitrosoguanidine and benzo[*a*]pyrene) (Aoyama et al. 2003; Desai et al. 2006). Similar genotoxic effects were also reported for diatoms exposed to UV radiation. Cells exposed to genotoxic agents show increases in thymine dimers; such dimers induce chromosome bridges and produce nuclear alterations (Buma et al. 1995, 1996; Holzinger and Lutz 2006). Two interpretations were proposed to explain the subsequent effects of these alterations on DNA. Rijstenbil (2001) suggested that oxidative stress, induced by genotoxic agents, may alter DNA integrity (clastogenic action). This toxic mechanism has been widely demonstrated to occur in microorganisms and higher plants exposed to genotoxic pesticides. As observed for higher plants, multinuclear cells may also be produced when the diatom cytoskeleton is affected by chemicals or UV light; such effects may result in abnormal migration of the chromosomes during cell division (Grant et al. 1992; El Hajjouji et al. 2007). Moreover, in diatoms exposed to microtubule inhibitors (colchicine, IPC), abnormal mitotic spindles were noted, as were multinuclear cells (Coombs et al. 1968; Coss and Pickett-Heaps 1974).

### ***2.3 The Siliceous Cell Wall***

The main character of diatoms is their siliceous cell wall (frustule). Many authors have identified abnormal morphology or disturbed ornamentation for some diatom cells, potentially as a result of exposure to xenobiotics (Feldt et al. 1973; Thomas et al. 1980; Adshead-Simonsen et al. 1981; Fisher et al. 1981; Yang and Duthie 1993; McFarland et al. 1997; Dickman 1998; Gomez and Licursi 2003; Cattaneo et al. 2004; Stoermer 2004; Debenest et al. 2008).

Numerous toxic agents have been reported as potential inducers of diatom cell wall (frustule) abnormalities. In situ studies identified abnormal frustules in samples contaminated by heavy metals (Feldt et al. 1973; McFarland et al. 1997; Dickman 1998). Abnormal forms were also observed within diatom communities exposed to cadmium, copper, mercury, and zinc (Thomas et al. 1980; Adshead-Simonsen et al. 1981; Fisher et al. 1981; Rijstenbil et al. 1994; Ruggiu et al. 1998; Gold et al. 2003; Gomez and Licursi 2003; Cattaneo et al. 2004).

Few publications exist, however, that describe xenobiotic induction of abnormal cell walls. Schmitt-Jansen and Altenburger (2005) reported abnormal forms of diatoms exposed to high concentrations (up to 312  $\mu\text{g/L}$ ) of isoproturon, a herbicide widely used in agriculture. The growth regulator, maleic hydrazide, a known genotoxin, has induced abnormal frustules, completely destroying the ornamentation of some (Debenest et al. 2008). Abnormal frustule morphology was also observed in diatoms exposed to microtubule inhibitors such as colchicine (Duke et al. 1977; Edgar and Pickett-Heaps 1984; Van Den Hoek et al. 1995).

Oxidative stress due to radiation or toxic exposure may also be implicated in the induction of frustule abnormalities (Rijstenbil et al. 1994; Rijstenbil 2001).

Environmental factors, such as nutrient deficiencies and pH, may also play a role in the development of abnormal frustules (Dickman 1998). In particular, severe silica deficiencies show evidence of inducing these abnormalities (Thomas et al. 1980; McFarland et al. 1997). Among other mentioned causes for induction of abnormal frustules are the mechanical effects of high cell density in crowded communities (Andresen and Tuchman 1991). According to Stoermer (1998), abnormal frustules may constitute clones of a single cell which suffered a genetic mutation which resulted in cell wall distortion.

The cellular mechanisms involved in the genesis of abnormal forms are still poorly understood. Many authors have suggested that silica deficiency is involved in the synthesis of abnormal frustules (Thomas et al. 1980; McFarland et al. 1997). Such deficiency may also result in different intracellular effects from contact with toxic agents. Many researchers believe that abnormal frustule induction may be linked to a disturbance in silica absorption by cells (Fisher et al. 1981; Rijstenbil et al. 1994; McFarland et al. 1997; Cattaneo et al. 2004). Pollutant-induced membrane alteration may occur and would reduce the absorption of silica. Rijstenbil et al. (1994) offered the hypothesis that copper may potentially produce lipid peroxidation effects on membranes (Rijstenbil et al. 1994).

Another scientific interpretation of silica deficiency would implicate the cytoskeleton (microtubules, actin filaments, and microfilaments), which is known to manage the migration of silica deposition vesicles (SDV) for cell wall synthesis (Pickett-Heaps et al. 1979; Round et al. 1990; Pickett-Heaps 1991; Lee and Li 1992; Van Den Hoek et al. 1995). Operational microtubules are important to the proper synthesis of the frustule (Edgar and Pickett-Heaps 1984). Thus, Debenest et al. (2008) suggested that disturbance of the SDV transporters would lead to poor silica supply and thereby induction of frustule abnormalities. Numerous authors have observed abnormal frustules in diatoms treated with microtubule inhibitors (Coombs et al. 1968; Duke and Reimann 1977; Lee and Li 1992).

### **3 Effects on Cell Metabolism**

The metabolism in diatom cells can be disturbed by toxic chemicals at three different levels: (i) photosynthesis, (ii) fatty and amino acids synthesis, and (iii) nutrients absorption.

#### ***3.1 Photosynthesis***

Numerous herbicides such as the *s*-triazines and substituted ureas (phenylureas and sulphonylureas) are widely used in agriculture, and exert their action by disrupting photosynthesis. The active constituents of these molecules bind with a protein called the D1 protein, block electron transfer, and thus inhibit a redox reaction: the

Hill reaction (Berard and Pelte 1996; Peres et al. 1996; Dorigo and Leboulanger 2001; Leboulanger et al. 2001; Berard et al. 2003b; Dorigo et al. 2004). A large body of research has been published that concerns the impact of such molecules on algae. It has been observed that atrazine, in a concentration range from 1 to 5  $\mu\text{g/L}$ , affected the photosynthesis of phytoplankton (De Noyelles et al. 1982). At higher concentrations (20, 25, and 500  $\mu\text{g/L}$ ), a more pronounced inhibition of photosynthesis was reported (De Noyelles et al. 1982; Weiner et al. 2007). Irgarol, another *s*-triazine that is used as an algicide in copper-based antifoulant paints for controlling fouling organisms on the hulls of marine vessels, was also confirmed to inhibit algal photosynthetic activity (Dahl and Blanck 1996; Nystrom et al. 2002; Berard et al. 2003b). Subchronic studies with this algicide produced photosynthesis inhibition at low concentrations (0.063–0.25  $\mu\text{g/L}$ ) (Dahl and Blanck 1996). Kasai and Hanazato (1995) reported reduced photosynthetic activity in phytoplankton communities exposed to another *s*-triazine chemical, simetryn, but the induced effects occurred at higher concentrations (0.1 and 1  $\text{mg/L}$ ) than for Irgarol (Kasai and Hanazato 1995). Effects were also produced when diatoms and green algae were exposed to other photosynthesis inhibitors (diquat, hexazinone, and the sulfonyleureas such as chlorsulfuron and metsulfuron) (Peterson et al. 1997; Nystrom et al. 1999). A dose-dependent inhibition of photosynthesis was also reported for periphytic algae exposed to high concentrations (8–1800  $\text{mg/L}$ ) of glyphosate (Goldsborough and Brown 1988).

### ***3.2 Synthesis of Protein, Lipids, and Carbohydrates***

Exposure of several species of diatoms to atrazine is known to significantly reduce protein synthesis, including proteins D1 and D2, which play an important role in photosynthesis (Weiner et al. 2007). Nicosulfuron was also reported to inhibit amino acid (valine and isoleucine) synthesis and thereby disrupt protein production in algae (Rimet et al. 1999). Atrazine exposure also induced lipid accumulation in cultured diatoms, this accumulation being also observed in diatoms cultured in nutrient-deficient conditions (Weiner et al. 2007).

Disturbances in carbohydrate synthesis may also occur and may cause loss of mobility in some diatom species (*Hantzschia* sp.) exposed to phenylurea herbicides such as linuron (Pipe and Cullimore 1984). Polysaccharides secreted by these microalgae are known to be involved in their normal mobility (Round et al. 1990; Van Den Hoek et al. 1995; Bertrand 1999). Cohn and McGuire (2000) have thus proposed to use mobility loss as an indicator of toxic exposure.

### ***3.3 Nutrient Absorption***

Herbicides also affect the absorption of nutrients ( $\text{NO}_3$ ,  $\text{NO}_2$ , and Si) by algae. Krieger et al. (1988) have observed that continuous exposure to high concentrations (134  $\mu\text{g/L}$ ) of atrazine reduced the absorption of nitrate, nitrite, and

silica by microalgae. It has also been reported that periphytic algae, exposed to atrazine, behaved as do algae cultured under nutrient-deficient conditions (Carder and Hoagland 1998). It appears that atrazine disrupts absorption of nutrients by cells. Nevertheless, temperature played a significant role in this cellular mechanism. Krieger et al. (1988) have noted disturbed absorption of nutrients at 10°C, but not at 25°C, in several periphytons exposed to peak concentrations of four herbicides (alachlor, atrazine, metolachlor, and metribuzin).

An increase of nitrite and nitrate concentrations in the medium was also noted during simetryn exposure of phytoplankton communities. Similar results were reported in other experimental systems when treated with photosynthesis-inhibiting herbicides (e.g., *s*-triazines) (Goldsborough and Robinson 1986; Herman et al. 1986; Gurney and Robinson 1989). Peres et al. (1996) related nitrite and nitrate concentration increases to inhibition of periphytons; such organisms not being able to consume and maintain these nutrients in the upper layers of sediment (Peres et al. 1996). Goldsborough and Robinson (1986) attributed this increase to dead cell degradation, which would release nutrients to the medium. But, these observations are limited to the community level and are thus difficult to extrapolate to the cellular level.

## 4 Effects on Diatom Growth

### 4.1 Algal Biomass

Algal biomass is measured by evaluating chlorophyll pigment concentrations, using either classical spectrophotometry or liquid chromatography. The measurement of chlorophyll *a* is one of the most widely used parameters to assess effects of pesticides on algae growth. Numerous authors have shown that exposing algae, including diatoms, to concentrations of atrazine that ranges from 10 to 1000 µg/L produced a decrease in chlorophyll *a* concentration (De Noyelles et al. 1982; Kosinski and Merkle 1984; Krieger et al. 1988; Jurgensen and Hoagland 1990; Berard 1996; Guasch et al. 1997, 1998; Tang et al. 1997; Carder and Hoagland 1998). Nevertheless, these results were not consistent with other research studies. Some studies failed to find an impact from exposure to atrazine at a concentration of 25 µg/L on algal biomass. Other studies actually demonstrated an increase in chlorophyll *a* concentration in algae communities exposed to concentration ranges from 10 to 32 µg/L of either atrazine or to atrazine mixed with nicosulfuron (30 µg/L for each molecule) (Lynch et al. 1985; Tang et al. 1997; Seguin et al. 2001a, b). These results are unexpected; the increase in chlorophyll *a* content may be related to moderate exposure to herbicides, cells being able to maintain sufficient photosynthetic activity (Seguin et al. 2001a). Other herbicides that target photosynthetic activity, such as simetryn or isoproturon, disrupted the development of algal biomass at high concentrations (100–1000 µg/L for simetryn and 40–312 µg/L for isoproturon) (Kasai and Hanazato 1995; Schmitt-Jansen and Altenburger 2005).

At a lower concentration (20  $\mu\text{g/L}$ ) of isoproturon Schmitt-Jansen and Altenburger (2005) did not report algal biomass inhibition, whereas Peres et al. (1996) observed effects on biomass with the same molecule at a lower concentration (5  $\mu\text{g/L}$ ). In this last study, results covered only diatoms, without taking into account other algal classes (cyanophytes and chlorophytes). Schmitt-Jansen and Altenburger (2005) observed development of green algae at an intermediate concentration (20  $\mu\text{g/L}$ ). These algae could be more tolerant to this herbicide than are diatoms. Therefore, no effects were observed on global biomass, increases in green algal biomass apparently compensating for any inhibition of diatom biomass growth.

## 4.2 Diatom Cell Density

Diatom cell density response patterns, following pesticide exposure, are generally difficult to discern. Many authors have shown that atrazine exposure may decrease cell density in some centric diatom species, and do just the opposite for some pennate diatom species (Tang et al. 1997; Berard and Benninghoff 2001; Berard et al. 2004). For one diatom species (*Cocconeis placentula*) exposed to another *s*-triazine herbicide (simetryn), Goldsborough et al. (1986) observed an increase in cell density, whereas Kasai et al. (1995) reported noxious effects of this herbicide on phytoplankton. Isoproturon, even at low concentrations, had a strong effect on diatom cell density (Peres et al. 1996).

## 5 Effects on Species Composition

An exposure to a single pesticide, or a mixture of pesticides, may selectively disturb some species more than others and thereby disturb the balance within the community. Toxic agents such as atrazine may reduce the ability of some species to develop, even if the effects are not readily visible, and thus may favor the more tolerant species in an ecosystem (Berard and Pelte 1996).

### 5.1 Diatom Species

Generally, herbicide exposure alters the diversity of diatom communities. A decrease in diversity in such communities was observed from exposures to atrazine (10–90  $\mu\text{g/L}$ ) (Hamala and Kollig 1985; Berard and Pelte 1996; Berard et al. 2004) and isoproturon (40–160  $\mu\text{g/L}$ ) (Peres et al. 1996; Schmitt-Jansen and Altenburger 2005). At a lower concentration (2  $\mu\text{g/L}$ ), Schmitt-Jansen and Altenburger (2005) reported an increase in diversity, probably from development of tolerance by certain species, and the presence of sensitive species that had not yet disappeared.



In benthic diatom communities, eutrophic species tend to be more tolerant to herbicides than certain others. Survival of species, e.g., *Achnanthes lanceolata frequentissima*, *Achnanthes minutissima*, *Asterionella formosa*, *C. placentula*, *Fragilaria capucina* var. *vaucheriae*, *Gomphonema parvulum*, *Nitzschia palea*, *Navicula lanceolata* and *Synedra acus*, was favored in communities exposed to *s*-triazine herbicides (atrazine and Irgarol), either under controlled (Hamala and Kollig 1985; Goldsborough and Robinson 1986; Berard 1996; Munoz et al. 2001; Berard et al. 2003a) or natural conditions (Guasch et al. 1998; Berard et al. 2003b). *A. lanceolata frequentissima* and *N. palea* were known for their tolerance to atrazine (Kosinski and Merkle 1984; Kasai 1999; Downing et al. 2004). Similar results were reported in communities exposed to isoproturon; eutrophic species such as *Navicula cryptocephala*, *N. Halophila*, *N. minima* and *G. parvulum* being more abundant (Peres et al. 1996; Schmitt-Jansen and Altenburger 2005).

Results from numerous studies performed under controlled conditions disclosed that exposure to a herbicide, such as atrazine or isoproturon, promoted the development of smaller organism size in the following species: *A. lanceolata frequentissima*, *A. minutissima*, *Achnanthidium minutissimum*, *C. placentula*, *Navicula minima*, and *Sellaphora seminulum* (Goldsborough and Robinson 1986; Peres et al. 1996; Munoz et al. 2001; Seguin et al. 2001a; Schmitt-Jansen and Altenburger 2005). Several interpretations have been proposed to explain this response. Numerous authors believe that small species are pioneers in colonizing pebbles and all surfaces which permit the development of benthic algae (Korte and Blinn 1983; Sekar et al. 2004). The species' survival potential is based on high growth rate, which allows them to colonize substrates (pebbles, rocks, etc.) and other natural surfaces earlier than certain other species, even under unfavorable conditions such as the presence of toxic pollution (Goldsborough and Robinson 1986; Peres et al. 1996). The colonization pattern of substrates continues to be under discussion in the scientific community (Acs and Kiss 1993). Another explanation could be related to the availability of cell defenses against oxidative stress among the smaller species. Rijstenbil (2001) reported that concentrations of gamma-glutamylcysteinylglycine (GSH), an antioxidant molecule, are 5–10 times higher in smaller than in larger species.

The trophic mode of diatom species also influences their sensitivity to herbicides. Some authors noticed that N-heterotrophic species such as *N. halophila* or *N. minima* were tolerant to photosynthesis inhibitors (simazine, terbutryn, and isoproturon) (Goldsborough and Robinson 1986; Peres et al. 1996). Similarly, Hamala and Kollig (1985) observed that periphytic algae exposed to another well-known photosynthetic inhibitor (atrazine) demonstrated an increase in heterotrophic activity. Some diatom species are able to switch their principal trophic modes (autotrophy) when the environmental conditions are unfavorable to photosynthesis (Hellebust and Lewin 1977). This character would explain their relative tolerance to photosynthesis inhibitors (Hamilton et al. 1988).

## 5.2 Algal Community

A large body of research has been performed that emphasizes the effects of herbicides such as atrazine on the species composition of algal communities. Berard and Benninghoff (2001) showed that exposure to 10  $\mu\text{g/L}$  of atrazine modified algae community structure. The different algae genera did not present the same sensitivity to this herbicide. Several studies, carried out on periphytic communities, highlighted a shift in the algae composition of communities in support of diatoms (Goldsborough and Robinson 1986; Guasch et al. 1997, 1998). Numerous publications have reported that these microalgae are more tolerant to photosystem II inhibitors (*s*-triazines and phenylurea) (Kosinski and Merkle 1984; Gurney and Robinson 1989; Molander and Blanck 1992; Hoagland et al. 1993; Kasai 1999; Dorigo et al. 2004). Some authors have reported that green algae (chlorophyceae and chrysophyceae) and cyanobacteria are between 4 and 10 times more sensitive than are diatoms to these herbicides (Hoagland et al. 1993; Tang et al. 1997; Guasch et al. 1998; Guasch and Sabater 1998; Navarro et al. 2002; Lockert et al. 2006). Similar observations were made on phytoplankton communities exposed over the long term to metazachlor (Mohr et al. 2008).

The higher tolerance of diatoms may be linked either with the capacity of some species to change trophic mode or with the nature of pigments in diatoms (Plumley and Davis 1980; Hamilton et al. 1988; Peres et al. 1996). Diatoms contain carotenoids and xanthophylls. Several authors reported that these pigments have antioxidant properties, which would enhance the potential tolerance to oxidative stress caused by pesticides (Rijstenbil et al. 1994; Pinto et al. 2003). In this context, herbicide effects on algae community depend on the species composition at the beginning of the exposure period (Herman et al. 1986).

## 6 Interferences in the Response of Diatoms to Pesticide Exposure

Many ecological and environmental parameters may interfere when diatoms come into contact with pesticides. Thus, under natural conditions, the potential ecological disturbances observed in a population are often difficult to relate to pesticide exposure, with certainty, because, the complex biological matrix (biofilm), where benthic diatoms evolve, may protect these algae against pesticide effects (Peres et al. 1996). Similarly, the dynamics of colonization and the evolution of biofilms have an impact on the response of benthic diatom communities to toxic agent exposures such as atrazine (Guasch et al. 1997). This protective layer may be disturbed by invertebrate or fish grazers, which also affect cell structure and promote toxic effects (Munoz et al. 2001). Nevertheless, the real impact of these grazers on diatom response to toxic exposure remains quite difficult to evaluate. Without protective layers, planktonic diatoms have also developed mechanisms to escape from a toxic environment by deep-diving (Rijstenbil 2001).

Environmental parameters (light exposure, nutrient concentrations, etc.) also interfere in the responses of algal communities to pesticides (Guasch et al. 1997; Guasch and Sabater 1998; Berard and Benninghoff 2001; Navarro et al. 2002). Thus, several scientific studies showed that diatoms were more sensitive to atrazine during light exposure (Guasch et al. 1997; Guasch and Sabater 1998). In the context of light, the response of algae depends on the season of study and on the site where samples were taken. Navarro et al. (2002) noted lower tolerance to atrazine for periphytic communities during the summer. The opposite results were obtained in phytoplankton communities exposed to the same herbicide (Berard and Pelte 1996). Nutrient concentrations also affect the sensitivity of algae to herbicides. Lin et al. (2005) reported that deficiencies in nitrogen and phosphorus may increase the sensitivity of algae (Lin et al. 2005). Under field conditions, the spatial and temporal variations of nutrient concentrations must interfere in the response of algae to toxic agents, especially in the case of agricultural watersheds, where high water pollution by nitrates occurs (Berard and Benninghoff 2001; Navarro et al. 2002).

## 7 Summary

The study of pesticide effects on algae, and diatoms in particular, was focused on photosynthesis and biomass growth disturbances. Few studies have been performed to investigate the effects of these toxic agents on intracellular structures of diatom cells. Nuclear alterations and cell wall abnormalities were reported for diatoms exposed to toxic compounds. Nevertheless, the cellular mechanisms implicated in the development of such alterations and abnormalities remain unclear. Sensitivity to pesticides is known to be quite different among different diatom species. Eutrophic and small species are recognized for their tolerance to pesticides exposure. More pronounced cell defenses against oxidative stress may explain this absence of sensitivity in species of smaller physical size. Notwithstanding, on the whole, explaining the rationale behind tolerance variations among species has been quite difficult, thus far. In this context, the understanding of intracellular toxicity in diatoms and the relation between these intracellular effects and the disturbance of species composition in communities represent a key target for further research.

The original community species structure determines the response of a diatom community to toxic agent exposure. Diatom communities that have species capable of switching from autotrophic to heterotrophic modes, when photosynthesis is inhibited (e.g., after pesticide exposure), can continue to grow, even in the presence of high pesticide pollution. How diatoms respond to toxic stress, and the degree to which they respond, also depends on cell and community health, on ecological interactions with other organisms, and on general environmental conditions. The general structural parameters of diatom communities (biomass, global cell density) are less sensitive to pesticide effects than are the specific structural parameters of the unicellular organisms themselves (cell density by species, species composition). For benthic species, biofilm development and grazing on this matrix as a source of food for invertebrates and fishes may also modify the response of diatom communities.

Environmental parameters (light exposure, nutrient concentrations, and hydraulic conditions) affect, and often interfere with, the response of diatoms to pesticides. Therefore, the complexity of aquatic ecosystems and the complexity of pesticide pollution in stream water (many molecules in interaction) do not permit researchers to easily detect the effects of such pollutants on diatoms. Clearly more research will be required to address this problem.

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