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Marine and Freshwater Microalgae as a Potential Source of Novel Herbicides

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Introduction

Despite the considerable contribution of synthetic herbicides to modern agriculture, lingering concerns remain - and, in some cases, have grown - with respect to most commonly used agents. In particular, concerns have included documented toxic effects on human, animal and ecosystem health, as well as associated potential for bioaccumulation, and growing evidence of acquired resistance (and cross-resistance) by target species with consequent implications for both cost and sustainability (Horrigan et al., 2002; Powles et al., 1996). As such, there is a continuing interest in the identification of novel herbicidal agents, specifically toward discovery of compounds that may address these concerns (e.g. reduced toxicity and/or potential for bioaccumulation, new molecular targets, high production capacity and low-cost of production).

In particular, numerous studies have explored the potential of naturally occurring secondary metabolites (i.e. "natural products"), particularly from plants and microorganisms, as a source of such compounds. This approach has included both purified and characterized metabolites, as well as mixtures (i.e. extracts or preparations), and "semi-synthetic" compounds based on natural product leads. Accordingly, in recent years, several good reviews have emerged on various topics related to bioactive natural products as a source of potential herbicides (e.g. Duke et al., 2002; Copping & Duke, 2007; Dayan et al., 2009; Lindell et al., 2009), and the reader would be directed to these resources for further information outside the scope of the current chapter.

This chapter focuses specifically on the marine and freshwater microalgae as a source of herbicidal compounds. As a group, the microalgae are recognized to produce a remarkable diversity of biologically active metabolites (see *Marine and Freshwater Microalgae: A Rich Source of Bioactive Compounds*, below). Furthermore, in addition to this recognized chemical diversity, a growing body of knowledge supports the notion that many of these bioactive metabolites from algae - as aquatic photoautotrophs - may specifically have evolved in the capacity of *allelopathy* providing a competitive advantage via interspecific, and particularly negative (i.e. inhibition), effects on growth, survival and reproduction of photoautotrophic competitor species (see *Algal Allelopathy*, below). Moreover, given the overlapping targets of action between allelopathic metabolites and herbicides (e.g. inhibition of photosynthesis), there is an emerging literature on the potential - including possible commercial potential - of algal metabolites as herbicides (as discussed in the final two sections of the chapter, below).

2. Marine and freshwater microalgae: a rich source of bioactive compounds

Algae, although not a taxonomically valid classification, encompass oxygenic photosynthetic organisms, exclusive of the true plants, spanning the prokaryotic and eukaryotic kingdoms of the Monera (bacteria) and Protista (protists). The group itself is frequently subdivided functionally into the so-called “microalgae” and “macroalgae” with the latter classification generally reserved for macrophytic representatives (i.e. “seaweeds”), and the former for strictly unicellular forms. Considerable overlap, however, can exist (e.g. colonial forms), as all algae are, strictly speaking, unicellular. Accordingly, the macroalgae classically include the protistan phyla of the Chlorophyta (“green algae”), Rhodophyta (“red algae”) and Phaeophyta (“brown algae”), whereas the microalgae include several photoautotrophic protistan taxa, particularly including the dinoflagellates, raphidophytes, diatoms (Bacilliarophyta), haptophytes and Chrysophyta (“golden algae”), as well as the prokaryotic cyanobacteria (“blue-green algae”). Alongside obvious morphological differences between the macroalgae and microalgae, there are notable differences in the secondary metabolites of the groups (see below) with the latter arguably representing a more chemically diverse repertoire. The current chapter will largely focus on the microalgae, however, references to relevant cases of herbicidal metabolites from macroalgae will be included as appropriate.

Equally diverse as their phylogeny is the ecology of the microalgae. Perhaps most conspicuous in aquatic systems, microalgae are, in fact, ubiquitous in their distribution, and include free-living, terrestrial representatives, as well as numerous symbiotic species (e.g. cyanobacterial symbionts of lichens, dinoflagellate zooxanthellae of corals, root nodule symbionts). Although the current chapter primarily focuses on marine and freshwater microalgae, examples of potentially herbicidal compounds from non-aquatic representatives will also be briefly mentioned.

In terms of bioactive compounds, the secondary metabolites of the microalgae include nearly every chemical class of natural product – ranging from fatty acids to alkaloids, and even a particularly interesting cabal of amino acids – but are particularly characterized by an array of metabolites with polyketide and non-ribosomal peptide biosynthetic origins (Dittman et al., 2001; Snyder et al., 2003; Jones et al., 2009). Respectively, the chemical repertoires of the cyanobacteria and eukaryotic microalgae are perhaps best typified by a myriad of peptides derived from the non-ribosomal peptide synthetase (NRPS), and polyketides derived from a pathway of sequential condensations (of malonyl- or acetyl-CoA units) orchestrated by the polyketide synthase (PKS) family of genes. Most notably, both biosynthetic pathways are specifically characterized by a family of modular genes (and associated gene products) such that nearly all biosynthetic steps, in both cases, are largely coordinated within so-called “modules” of a single, assembly-line like “megasyntases”. These megasyntases are responsible for several core biosynthetic steps (e.g. adenylation, condensation and peptide transfer in the case of NRPSs, and ketosynthesis and acyl transfer in the case of PKSs), as well as various secondary modification steps (e.g. ketone reduction, dehydration, methylation). In fact, metabolites presumably derived from both pathways are seen in prokaryotic and eukaryotic algae alike, and it is, moreover, becoming increasingly clear that many metabolites may be derived from mixed (PKS/NRPS) pathways (and mixed modular genes/enzymes). As an example, the mixed NRPS/PKS pathway (as proposed by Moffitt and Neilan, 2004) for the cyanobacterial metabolite, nodularin, is shown in Fig. 1.

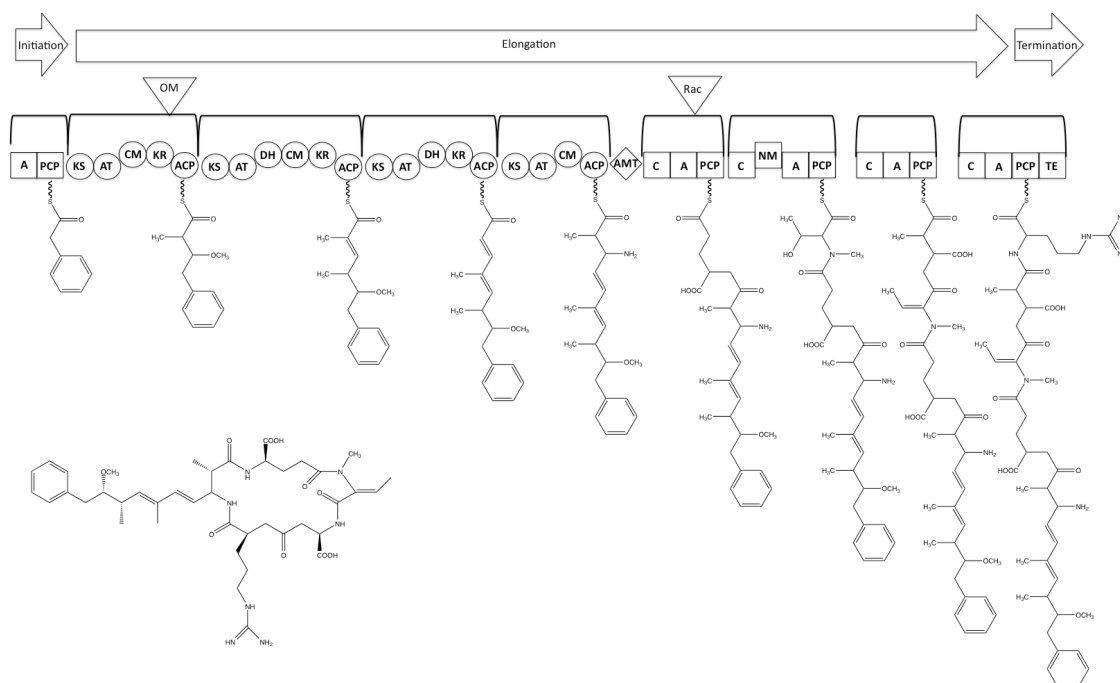


Fig. 1. Modular non-ribosomal peptide synthetase (NRPS)/polyketide synthase (PKS) biosynthetic pathway of the cyanobacterial toxin, nodularin. Modified from Moffitt and Neilan (2004). Each module, as indicated in the figure, contains essential domains - condensation (C), adenlyation (A) and peptide carrier protein (PCP) for NRPS, and ketosynthase (KS), acyltransferase (AT) and acyl carrier protein (ACP) for PKS - as well as additional domains for modifications including methylation (C-methyltransferase, CM; N-methyltransferase), ketoreductases (KR), dehydratases (DH). A thioesterase (TE), in the final NRPS module, releases and cyclizes the peptide. In addition to these NRPS and PKS modules, several other domains are incorporated, including O-methyltransferase (OM) for transfer of methyl to hydroxyl of C-9, racemase (Rac) which epimerizes the D-glutamate, aminotransferase (AMT).

This shared biosynthetic origin of algal secondary metabolites is, perhaps, what makes these organisms such particularly compelling candidates as a source of bioactive compounds. The modular - and presumably "interchangeable"- nature of these biosynthetic enzymes is likely, at its core, responsible for the high diversity of bioactive compounds found within the group. As simply put by Welker & von Döhren (2006), these biosynthetic pathways likely represent "nature's own combinatorial chemistry," resulting in an unequalled diversity of secondary metabolites, and presumably directed by largely unknown selective pressures (e.g. chemical defense against micrograzers, allelopathy). Moreover, from a compound discovery and development (and even sustainability) point-of-view, such a biosynthetic mechanism presents several exciting opportunities. For example, "semi-synthetic" modifications (i.e. via genetic modification of PKS/NRPS genes) can enable improvement of activity or other relevant parameters (e.g. reduced toxicity, increased bioavailability). Co-opting of biosynthetic genes, and subsequent heterologous expression, enables synthesis of relevant pharmacophores and scaled-up production of candidate compounds (discussed further in *Commercial Potential of Herbicides from Microalgae*).

Accordingly, the resulting diversity of toxic or otherwise bioactive metabolites has received considerable attention (see, for example, reviews by Codd et al., 1999; van Dolah, 2000; Gerwick et al., 2001; Cardozo et al., 2007; Tan, 2007; Berry et al., 2008) both as a leads to compounds with potential biomedical (i.e. drug discovery) or other commercially relevant (e.g. pesticides, herbicides) applications, as well as a growing relevance to public and environmental health (i.e. as “toxins”). In terms of the former, several bioactive compounds from microalgae, including both eukaryotic and prokaryotic representatives, are currently being investigated as potential “drug leads.” Several metabolites from cyanobacteria (e.g. cryptophycins, dolastatins, curacin A) have, for example, been investigated with respect to anticancer activities (i.e. inhibition of cell-division; Trimurtulu et al., 1994; Verdier-Pinard et al., 1998; Luesch et al., 2001). Dinoflagellate toxins (e.g. saxitoxins and brevetoxins) have recognized applications as biomedical research tools (i.e. inhibitors and activators, respectively, of voltage-gated sodium channels) and emerging applications as drugs (e.g. brevenal as an antagonist of bronchoconstriction and related pulmonary effects, such as those found in cystic fibrosis; Potera, 2007). On the other hand, as toxic compounds (see Fig. 2), and specifically in relation their role in “harmful algal blooms” (HABs), microalgal compounds have been well documented (Codd et al., 1999; van Dolah, 2000) to pose threats to human, animal and environmental health via contamination of drinking water, bioaccumulation in fish, shellfish and other seafood, and various other routes (e.g. recreational exposure).

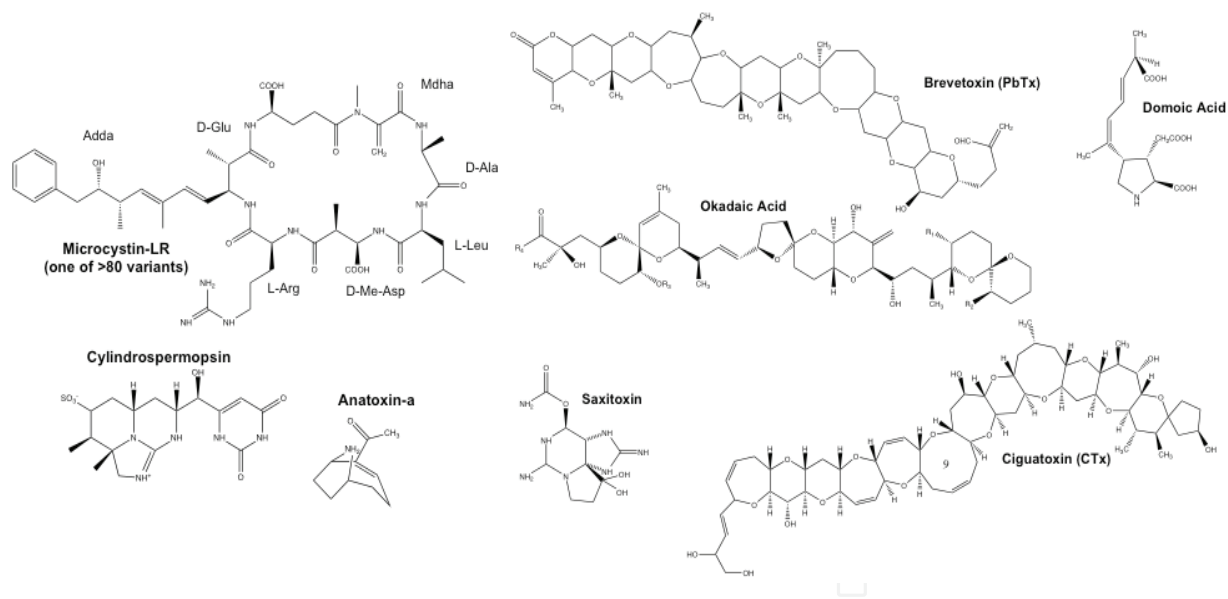


Fig. 2. Toxins from marine and freshwater microalgae with established concerns for human, animal and environmental health.

Interestingly, the role of these compounds in the life history of the algae remains largely unknown. However, it has been frequently proposed (e.g. Berry et al., 2008) that bioactive secondary metabolites of microalgae may be involved in the chemical ecology of these organisms, particularly including a role in allelopathy (discussed further below) and chemical defense (e.g. feeding deterrents, qualitative toxins) against potential micrograzers in aquatic habitats. Indeed, with respect to the current discussion, the specifically suited role of these compounds as allelochemicals (i.e. allelopathic inhibitors of competing

photoautotrophic plants and microbes), that makes them a particularly compelling candidate as a source of potential herbicidal agents.

3. Algal allelopathy

Given the likely intense competition for physical (e.g. space, light) and chemical (e.g. micro- and macronutrient) resources in aquatic habitats, it is not surprising that a growing number of studies have documented apparent allelopathy between photoautotrophic species - as well as phyla and kingdoms - in these systems. In fact, several good reviews on the topic (e.g. Irfanullah & Moss, 2005; Erhard, 2006; Graneli et al., 2008; Macias et al., 2007), specifically emphasizing aquatic plants, macroalgae and microalgae, have appeared over the past several years. Not only have these studies supported the possible role of these compounds as inhibitors of sympatric autotrophs, but have, in some cases, even provided convincing evidence that interplay of these interspecific inhibitors may support ecological succession in these habitats. Moreover, given an overlap - and presumptive evolutionary convergence or divergence - of targets shared by algae and true plants (e.g. oxygenic photosynthesis), the repertoire of such allelochemicals provides an especially rich source of compounds with activity directly relevant to possible development as herbicides and/or identification of novel herbicide targets.

Although the term "allelopathy" was not coined (Molisch, 1937) until the 1930s, scientific observation of apparent allelopathy in aquatic habitats dates back to the turn of the previous century. In particular, studies by Apstein (1896) and Pütter (1908) that detailed an apparent role of allelochemicals in determining community structure in these systems. A number of studies subsequently followed including, perhaps most notably, the seminal studies by Keating et al. (1977, 1978) which examined the role of extracellular metabolites from cyanobacteria with respect to interannual succession patterns of algal blooms in a closed aquatic system (i.e. Linsley Pond). These studies specifically demonstrated that filtrates prepared from axenic (or unialgal) cultures of "dominant" bloom strains (from a particular year) negatively affected (i.e. inhibited) growth of "predecessor" strains - but not "successor" strains on which filtrates, instead, exerted a positive or neutral effect - indicating an apparent role of extracellular metabolites in structuring the community from year to year. Interestingly, even these early studies (e.g. Keating, 1977) - although focused on ecological aspects - recognized the potential implications of these interactions as a means to control populations of nuisance algae. The subsequent decades witnessed numerous studies on the ecological role of allelopathy in both freshwater and marine habitats (see, for example, review by Gross, 2003). However, the vast majority of these studies examined extracts, filtrates or other crude preparations. With respect to the potential applications for novel herbicides (and/or algicides), identification and subsequent chemical characterization is an obvious necessity.

Indeed, numerous algal metabolites (Fig. 3) with algicidal activity, and accordingly suggested roles in allelopathy, have been isolated and characterized. The reader is directed to previous reviews of these compounds (Smith and Doan, 1999; Berry et al., 2008). The first such compound, cyanobacterin, was reported by Mason et al. (1982). The compound was isolated from cellular extracts of *Scytonema hofmanni* (UTEX 1581), specifically based on inhibition of cyanobacteria, and chemically characterized as a chlorinated aromatic γ -lactone (see Fig. 3). Extracts of *S. hofmanni* were shown to inhibit (presumably due to cyanobacterin) a wide range of microorganisms, particularly including a diversity of cyanobacteria and

green algae, as well as rhodophytes, and was suggested, as such, to play a role in allelopathy of the species (Mason et al., 1982). Subsequently, species of the cyanobacterial genus, *Nostoc*, were found (Gromov et al., 1991; Vepritskii et al., 1991) to produce antialgal metabolites, termed cyanobacterin LU-1 and LU-2, which were, likewise, shown to inhibit a variety of cyanobacteria and other microalgae. Although the structures of LU-1 and LU-2 (not completely characterized) were generally considered to be unrelated to those isolated from *S. hofmanni*, it was found that both cyanobacterins from *S. hofmanni* and *Nostoc* (i.e. LU-1 and LU-2) seemed to act via inhibition of photosystem II (PSII), a target of several commercial herbicides (discussed further below).

Based on this, several screening studies (Flores & Wolk, 1986; Shlegel et al., 1998; Volk, 2005; Gantar et al., 2008) followed, using algicidal assays to identify metabolites with potential allelopathic roles. The presumably high importance of such compounds is underscored by the fact that up to 40% of cyanobacterial isolates evaluated in these screening studies were found to produce algicidal metabolites (Volk et al., 2005). Of particular note, it was found by Flores and Wolk (1986) and Schlegel et al. (1998), who screened sixty-five and approximately 200 strains of cyanobacteria, respectively, that algicidal metabolites were primarily restricted to heterocystous, nitrogen-fixing filamentous taxa (of Sections IV and V of the standard classification scheme of Rippka et al., 1979). In fact, this was somewhat confirmed by subsequent screening by Volk et al. (2005) who, likewise, found that the majority of algicide producers belong to these, or closely akin filamentous groups (e.g. *Oscillatoria*, *Arthrospira*, *Phormidium* from Section III). Moreover, it was found in this latter study that members of these same groups were, in fact, the more susceptible (versus unicellular cyanobacterial or green algal representative) to the apparent algicidal metabolites, suggesting a role in the allelopathy toward most related (and perhaps ecologically similar) photoautotrophic competitors. This is further supported by recent screening studies (Gantar et al., 2008) that used co-cultivation of sympatric isolates cyanobacteria and chlorophytes (specifically from the Florida Everglades), and found apparent allelopathic activity among all ecologically co-occurring cyanobacterial, and 4 of 6 green algal, strains examined the study.

Within Section IV and V cyanobacteria, two groups - the genus, *Nostoc*, and several members of the family Stigonemataceae - are particularly notable producers of apparent allelochemicals. Perhaps the most frequently identified producer of algicidal metabolites is the cyanobacterial genus, *Nostoc*. In addition to being consistently represented, among algicide producers in screening studies (Flores & Wolk, 1986; Shlegel et al., 1998; Volk, 2005), several apparently allelopathic metabolites (see Fig. 3) have been characterized from the genus, including alkaloids (e.g. nostocarboline), phenolics (e.g. 4'-dihydroxybiphenyl) and peptides (e.g. nostocyclamide). Although perhaps not as chemically diverse, members of the family of branched filamentous cyanobacteria, Stigonemataceae, particularly including the genera *Hapalosiphon* and *Fischerella*, have been, likewise, widely cited as producers of allelopathic compounds (e.g. Doan et al., 2000 and 2001; Leflaive and Ten-Hage, 2006; Gantar et al., 2008; Graneli et al., 2008; Leão et al., 2009).

In addition to an apparent phylogenetic conservation, there is some evidence to support chemical conservation within the algicidal chemistry of microalgae. In particular, a number of metabolites belonging to the indole class of alkaloids have been found to possess anti-algal activity, and consequently associated with allelopathic interactions. Perhaps the most frequently cited allelochemicals from cyanobacteria are the hapalindoles and related alkaloids (e.g. 12-*epi*-hapalindole E isonitrile, Fig. 3), including ambiguines, welwitindolinones and fischerindoles, that have been isolated from both marine and

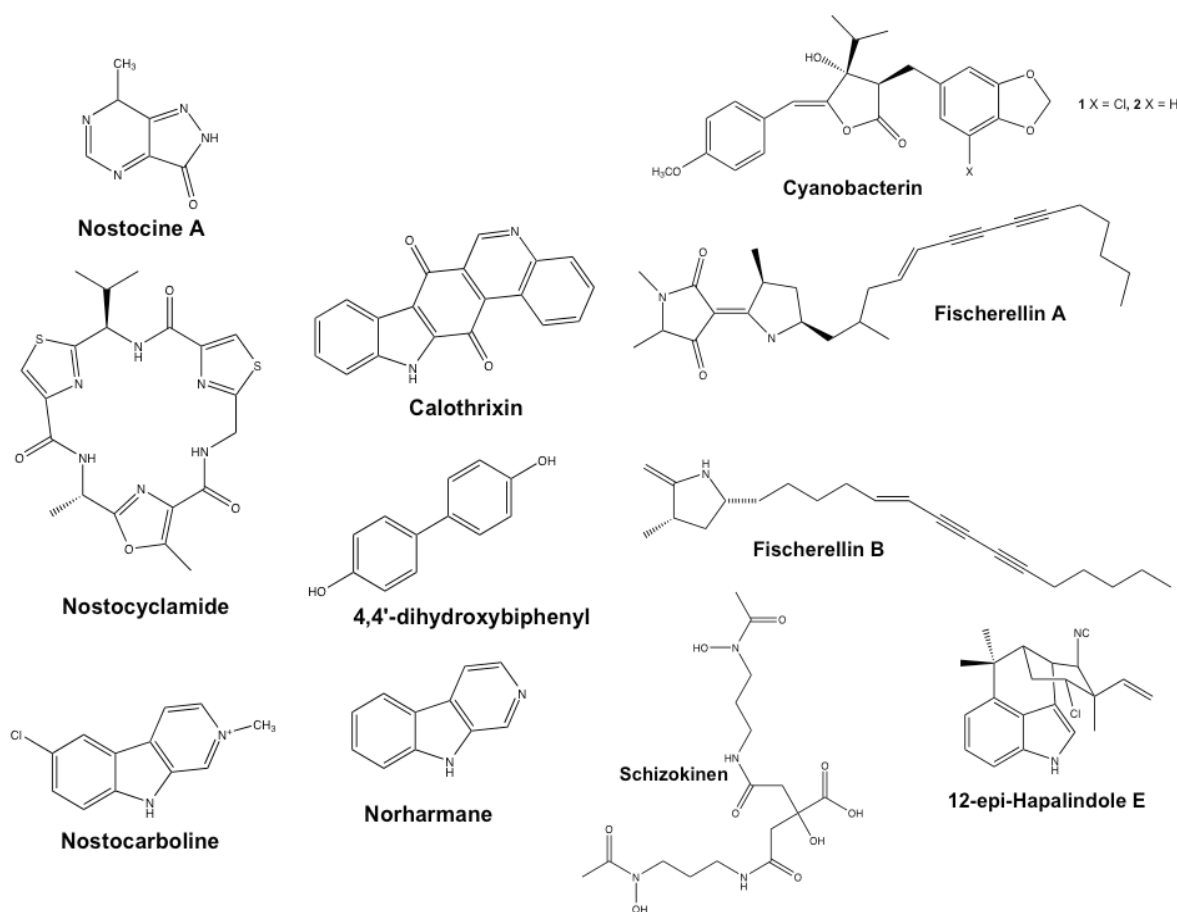


Fig. 3. Algicidal metabolites from microalgae with proposed role in allelopathy.

freshwater representatives of the family Stigonemataceae, and particularly the genera *Hapalosiphon* and *Fischerella*. Indeed, the first member of the class, hapalindole A, was initially identified, in part, based on algicidal activity (Moore et al., 1984). Several subsequent studies pointed to the apparent role of hapalindoles, as algicidal metabolites, in allelopathy (Doan et al., 2000; Etchegaray et al., 2004; Gantar et al., 2008). Doan et al. (2000), for example, purified 12-epi-hapalindole E isonitrile (Fig. 3) as an algicidal metabolite of an isolate of *Fischerella*, previously identified from screening studies (Schlegel et al., 1999), and specifically demonstrated inhibition of RNA synthesis. Although only a few hapalindole alkaloids have been specifically characterized with respect to algicidal activity, and possible allelopathy, it is likely that other members of the class, and related indole alkaloids in the taxonomic group (e.g. welwitindolinones, fischerindoles), may have similar roles and biological activities, particularly due to structural similarity, and generally widespread antimicrobial and antimitotic activity among these compounds. In fact, Etchegaray et al. (2004), in their identification of the 12-epi-hapalindole isothiocyanate, identified several other uncharacterized, algicidal metabolites from a strain of *Fischerella*, including those with chemical similarity (e.g. molecular weight) to the hapalindoles.

Moreover, other taxonomically distinct groups have, likewise, been found to produce compounds (Fig. 3 and 6) with a chemically similar indole core, including calothrixins isolated from *Calothrix*, and β -carbolines, and the chlorinated nostocarboline and norharmane (and related harmane alkaloids), isolated from species of *Nostoc* and *Nodularia*,

respectively (Doan et al., 2000; Becher et al., 2005; Blom et al., 2006; Volk, 2005). Interestingly an indole metabolite, harmaline (or 1-methyl norharmaline) - chemically related to one of these apparent algal allelochemicals (i.e. norharmaline) - was also identified separately (Kodani et al., 2002) from cultures of a non-algal bacterium, *Pseudomonas*, isolated from a eutrophic freshwater system, specifically based on apparent algicidal activity (i.e. against several cyanobacterial species). Furthermore, in some case (e.g. calothrixin), there is also shared biological activity (e.g. inhibition of RNA synthesis) with other cyanobacterial indole alkaloids (e.g. hapalindoles, see above). The possibility of an indole "pharmacophore" is particularly intriguing, in the context of possible herbicides, since the same heterocycle is found among naturally occurring auxins, including the major - and most potent - congener, indole-3-acetic acid (IAA), and synthetic auxins (e.g. 2,4-dichlorophenoxyacetic acid, or "2,4-D") which mimic these plant growth regulators (see section 4.2 *Herbicidal Target-Based Approach*, below), and consequently represent an important and widely used class of commercial herbicides.

In fact, algicidal metabolites from cyanobacteria and other microalgae encompass a fairly diverse array of secondary metabolites. In addition to the previously discussed indole alkaloids, algicidal metabolites so far identified (see Fig. 3) include various other alkaloids (e.g. the aminoacylpolyketide fischerellins; nostocine A), phenolics (e.g. 4,4'-dihydroxybiphenyl), hydroxamate chelators (e.g. schizokinen) and fatty acids (e.g. 2,5-dimethyldodecanoic acid, polyunsaturated fatty acids), as well as peptides (e.g. nostocyclamide). The latter is perhaps of particular interest. This group of metabolites is, as discussed above, a particularly characteristic class of secondary metabolites from cyanobacteria (and, to a lesser extent, other microalgae). Moreover, this class of compounds presents a number of compelling attributes toward the development of commercially viable candidate compounds (e.g. modification via semi-synthesis, heterologous expression; see *Commercial Potential of Herbicides from Microalgae*, below). Moreover, the biosynthetic genes (NRPSs), associated with this class of metabolites, appear to be especially widespread in the Section IV/V cyanobacteria that (as noted above) are most frequent producers of algicidal metabolites. Specifically, it was found (Christiansen et al., 2001) using sequence-specific primers that nearly all (97% and 100%, respectively) of the cyanobacteria in these two sections were positive for NRPS genes (compared to only 52%, 80% and 64%, respectively, for Sections I, II and III).

In a very recent study, Leão et al. (2010) used bioassay-guided fractionation, specifically based on inhibition of the green alga, *Chlorella vulgaris*, to isolate a series of cyclic peptides, the portoamides (Fig. 4) as apparent allelopathic agents from the blue-green alga, *Oscillatoria*. In addition to *C. vulgaris*, as well as other chlorophyte species, these metabolites differentially inhibited cyanobacteria, including *Cylindrospermopsis raciborskii*, but not several other cyanobacterial species (e.g. *Microcystis*, *Aphanizomenon* and *Anabaena*), nor other microalgae (e.g. the diatom, *Cyclotella meneghiniana*) tested. Interestingly, allelopathic activity was found to specifically peak during the early stages of exponential growth of the cyanobacterial culture, suggesting an ecological role (i.e. to "open" a niche for colonization), and consequently providing, in this case, an opportunity to purify a sufficient quantity of the otherwise low concentration metabolite for chemical characterization (Leão et al., 2010). Even more interesting, the investigators documented an apparent synergism of these metabolites. Specific mixtures of the most abundant congeners, portoamides A and B, in the ratios of 2:1 and 1:2.6 inhibited *C. vulgaris* at 30 ppm, but not in other ratios (e.g. 4.4:1) tested (Leão et al., 2010). This finding is particularly stunning given the rather minimal difference

between the structure of the two (differing only in the presence of a methoxy group), and is further underscored by the apparent lack of activity associated with other chemically related congeners (e.g. metabolites specifically lacking an esterified *N*-acetyl-*N*-methyl tyrosine found in portoamides A and B).

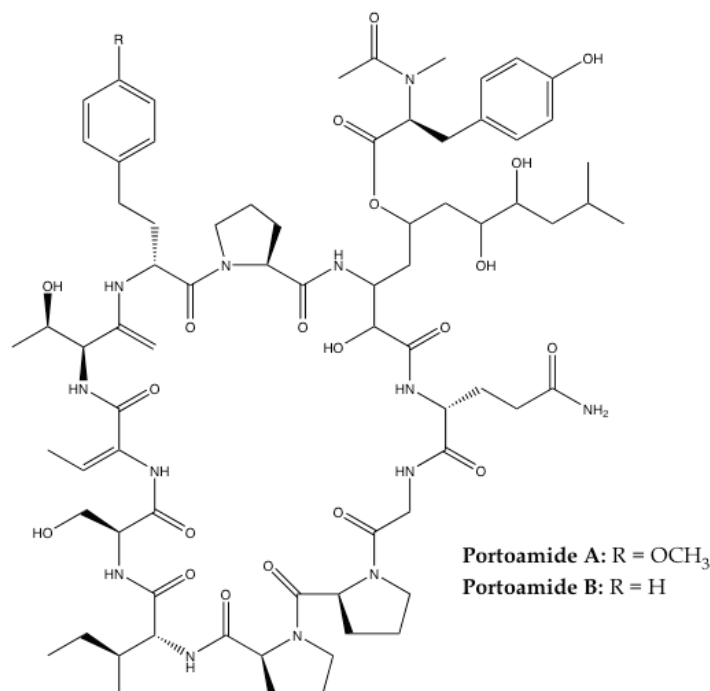


Fig. 4. Portoamides A and B isolated from a species of *Oscillatoria* as synergistic allelopathic metabolites (Leão et al., 2010).

In addition to metabolites identified based on their algicidal activity, it has been suggested that various 'HAB toxins,' largely recognized based on their effects on human health, may also play an ecological role in allelopathy. In fact, it has been suggested (e.g. Graneli et al., 2008) that nutritional imbalance (and consequent limitation) associated with eutrophication in aquatic systems may drive (via competitions for these resources) production of allelochemicals, and consequently production of these toxins. Elegant work by Kearns and Hunter (2000, 2001), for example, investigated allelopathic interactions between the cyanobacterial species, *Anabaena flos-aquae*, and green alga, *Chlamydomonas reinhardtii*, and found that well characterized toxins, microcystin-LR and anatoxin-a, from *A. flos-aquae* inhibited the green alga specifically via apparent paralysis, and subsequent settling. Interestingly, production of the latter of the two toxins was found to be stimulated by extracellular products of *C. reinhardtii*, while microcystin production was seemingly inhibited by the same (Kearns and Hunter, 2000). Subsequent studies (e.g. Pflugmacher, 2002) have, likewise, shown an allelopathic role of microcystins. Interestingly, both microcystin and anatoxin-a have been also shown (Mitrovic et al., 2004; Jang et al., 2007) to inhibit growth of plants, specifically using an aquatic plant model, "duckweed" (*Lemna japonica*).

Moreover, allelopathic interactions are not limited to the freshwater cyanobacteria, and a possible role of various metabolites from marine microalgae, including dinoflagellates, diatoms, haptophytes and raphidophytes, as well as from microalgal chlorophytes, have been, likewise, documented (see, for example, a good review by Graneli et al., 2008). In

some cases, allelopathy has been associated with known HAB-associated toxins. For dinoflagellate, haptophytes and diatom HAB species, in particular, studies have suggested a possible role of various polyethers – characteristic metabolites of these groups – including brevetoxins (Kubanek and Hicks, 2005), okadaic acid (Sugg and Van Dolah, 1999), prymnesin (Graneli and Johansson, 2003), gymnomidine (cf. Legrand et al., 2003) and cooliatxin (cf. Legrand et al., 2003). However, in several cases, it has been suggested that these metabolites, although algicidal, likely are not solely responsible for allelopathic activity, and that other extracellular metabolites (which remain to be characterized) may play a larger role (e.g. Sugg and Van Dolah, 1999; Kubanek and Hicks, 2005). Indeed, although apparent allelopathy has been demonstrated for a wide range of HAB species, specifically using culture-based experiments and/or extracellular preparations (e.g. culture filtrates), the allelochemicals responsible largely remain, in most cases, to be characterized.

Spanning eukaryotic and prokaryotic microalgae, one particularly interesting class of compounds that has been suggested to play a role in allelopathy are fatty acids – and specifically a polyunsaturated fatty acids (PUFAs) – either in free acid form, or as part of acylated sugars (i.e. glycolipids). A thorough review of these metabolites, and their possible role in allelopathy, has been previously presented by Ikawa (2004). Indeed, a growing number of studies have generally pointed to the biological activity of fatty acids. However, it is becoming generally clear that desaturation – as well as other modifications (e.g. oxidations, aldehydes) – may be particularly important to the observed toxicity or other bioactivity, and accordingly their possible role in allelopathy. Table 1 illustrates this trend, specifically based on several prior studies, in which algicidal activity has been consistently associated with relative desaturation of fatty acids isolated from microalgae. As an example of the importance of PUFAs in allelopathy, Arzul et al. (1993, 1995) investigated the apparent inhibition of the marine diatom, *Chaetoceros gracile*, by blooms of the dinoflagellate, *Gyrodinium*, and identified a high concentration of octadecapentaenoic acid (OPA, C18:5n3) and docosahexaenoic acid (DHA, C22:6n3) in the fatty acid profiles of *Gymnodinium*. These studies identified further found that these metabolites specifically inhibited the growth of *C. gracile*. Similarly, Uchida et al. (1988) identified relatively large amounts of DHA, along with eicosapentaenoic acid (C20:5n3), and small amounts of C18:2 and C18:3 fatty acids, from *Peridinium bipes*, and characterized their inhibition of cyanobacteria.

Chemically related to fatty acids are a family of mono- and diacylglycerides in which acyl groups are esterified to the 2' and/or 3' position of glycerol (typically with either a mono- or disaccharide, and particularly galactose, at the 1' position). As for free fatty acids, a variety of biological activities (e.g. inhibition of DNA polymerase, antimicrobial activity) have been described for these compounds (e.g. Kurihara et al., 1996; Ohta et al., 1998; Hanashima et al., 2000; Eitsuka et al., 2004; Mizushima et al., 2005; Cantillo-Ciau et al., 2010). Of particular note are the sulfated galactosyl (i.e. sulfoquinovosyl) substituted mono- and diacylglycerides which are found alongside non-sulfated glycolipids in the thylakoid membranes of prokaryotic and eukaryotic photoautotrophs (including higher plants). In fact, it has been proposed that, in addition to other possible mechanisms (e.g. acting as secondary messengers for biochemical pathways, phospholipases), the importance of both free fatty acids and acylglycerides in cellular membranes – and the consequent disruption and destabilization of these membranes by non-endogenous variants – may be responsible for the widespread biological activity of these compounds (Ikawa, 2004). Given the obvious and unique importance of subcellular plastids in photosynthetic organisms, including algae

and plants alike, further exploration of these compounds as selective inhibitors of photoautotrophs, and specifically as a novel target for herbicidal agents, is warranted.

	Cyanobacteria	Green Algae (Chlorophyta)			
	<i>Phormidium tenuis</i>	<i>Chlamydomonas s.</i>	<i>Haematococcus s.</i>	<i>Pandorina</i>	<i>Ankistrodesmus</i>
Myristic Acid (14:0)	>100	>100	>100	>100	>100
Palmitic Acid (C16:0)	>100	>100	>100	>100	>100
Palmitoleic Acid (C16:1)	2.5	12.5	50	50	50
Stearic Acid (C18:0)	n.d.	>100	>100	>100	>100
Oleic Acid (C18:1 cis-9)	1	50	100	100	100
Cis-Vaccenic Acid (C18:1 cis-11)	5	n.d.	n.d.	n.d.	n.d.
Linoleic Acid (C18:2)	0.5	50	12.5	25	25
Linolenic Acid (C18:3)	0.5	50	<12.5	12.5	50

Table 1. Algicidal activity of saturated and unsaturated fatty acids against cyanobacteria and green algae (Chlorophyta). Data are taken from McCracken et al. (1980) and Yamada et al. (1993) for chlorophyte and cyanobacteria data, respectively. Given are the minimum concentration (mg/L) that inhibits algal culture growth >50%. "n.d." indicates evaluation not done. Apparent increase in algicidal activity, associated with desaturation, highlighted in **bold** text.

Finally, allelopathy is not, by any means, limited to microalgae, and similar interspecific, chemically mediated interactions have been, likewise, suggested in marine and freshwater macroalgae. For example, several studies (Friedlander et al., 1996; Jin & Dong, 2003; Nan et al., 2004; Jin et al., 2005; Wang et al., 2007; Nan et al., 2008) have documented the apparent allelopathy of the macroalgal chlorophyte, *Ulva*, toward various microalgae and other macroalgae. Friedlander et al. (1996) identified apparently lipophilic metabolites from culture media of *Ulva lactuca* that inhibited the growth of the red alga, *Gracilaria conferta*, and suggested that these exometabolites may play a role in the observed growth inhibition of the latter species when grown together, under otherwise controlled conditions, in culture. Based on these studies, and citing a documented reciprocal relationship between growth of micro- and macroalgae, Nan et al. (2004) evaluated allelopathy of *U. pertusa* against eight species of microalgae, including dinoflagellates, haptophytes, diatoms and microalgal chlorophytes. These studies found that all eight species were inhibited in co-cultivation with the macroalga. However, only one of the species (i.e. the haptophyte, *Chroomonas placoidea*) was inhibited by culture filtrates. This was taken, by the authors, citing several related examples, to be due to instability of released metabolites (Nan et al., 2004). This observation

was confirmed by several subsequent studies (Jin et al., 2005; Wang et al., 2007) which additionally showed that continuous release of allelochemicals by *Ulva*, either by semi-continuous addition of filtrate, or presence of fresh algal material or dried powdered biomass, was sufficient to inhibit growth of microalgae. Such effects, moreover, are not limited to *Ulva*, and similar studies (e.g. Kim et al., 2004; Wang et al., 2006; Wang et al., 2007; An et al., 2008; Wang et al., 2009) have, likewise, shown inhibition of microalgae by other green algae (e.g. *Enteromorpha*), as well as coralline red algae (e.g. *Lithophyllum*, *Corallina*, *Porphyra*, *Gracilaria*) and brown algae (e.g. *Sargassum*, *Undaria*, *Laminaria*).

By-and-large, algicidal metabolites from macroalgae remain to be well characterized, however, several studies suggest a role of PUFAs, and associated metabolites (e.g. acylglycerides), as discussed above for microalgae. Work by Alamsjah et al. (2005, 2007) screened a collection of thirty-seven macroalgae, including representatives of the Chlorophyta, Rhodophyta and Phaeophyta, and specifically found extracts of the genus, *Ulva*, to be the most potently algicidal against the HAB organism, *Heterosigma akashiwo*. The investigators subsequently identified (Alamsjah et al., 2005, 2007) a series of PUFAs - particularly including unsaturated C16 and C18 acids - from several species of *Ulva* (*U. fasciata*, *U. pertusa*, *U. arasaki*, *U. conglobata*) with algicidal activity toward a range of microalgal representatives. Based on the relatively higher production of these by the most algicidal species (*U. fasciata* and *U. pertusa*) it was argued that they likely play a role in observed allelopathy. Fatty acids, including PUFAs, have similarly been identified as apparent allelopathic agents of other macroalgae, including brown algae (e.g. inhibition of various microalgae by *Cladosiphon*, Kakisawa et al., 1988), red algae (e.g. *Neodilsea*, *Chondrus* and *Ptilota*; Macias et al., 2007) and Charophytes (e.g. inhibition of the cyanobacterium, *Microcystis aeruginosa*, by *Chara vulgaris*, Zhang et al., 2009).

PUFAs, moreover, are not the only class of bioactive metabolites from macroalgae, and various bioactive metabolites, including alkaloids (e.g. Gross et al., 2006; Arunkumar et al., 2010; Güven et al., 2010), phenolics (e.g. Hassan and Ghareib, 2009) and terpenoids (e.g. Fenical & Paul, 1984; Paul & Fenical, 1984; Lane et al., 2007; Arunkumar et al., 2010), have been characterized. Although the potential of these compounds as herbicides remains to well-studied, based on the apparent importance of allelopathy among photoautotrophs in aquatic habitats, the chemical diversity of the macroalgae, like the microalgae, represents a wealth of secondary metabolites to be explored to this end.

4. Microalgal metabolites as herbicides

In contrast to algicidal metabolites - specifically in relation to allelopathy - relatively scant studies have investigated biological activity of microalgal toxins with respect to higher plants (i.e. herbicides). The current body of knowledge with regards to potential herbicidal metabolites from microalgae currently remains limited. However, given the importance of allelopathy among aquatic photoautotrophs, and presumptive homologies of the biochemistry and physiology between both algae and higher plants, as photosynthetic organisms, the chemical diversity of bioactive secondary metabolites represents a likely rich source of such compounds. As such three approaches are proposed as a means to explore the, otherwise well recognized, chemical repertoire of these taxa with respect to potential herbicidal agents. These include: (1) a *in vivo* bioassay approach, based on identifying phytotoxicity in representative plant species; (2) a target-based approach, specifically incorporating assays based on recognized herbicide targets; and (3) a pharmacophore-based

approach, specifically surveying algal metabolites to identify those with recognized structural features associated with herbicidal compounds.

4.1 *In vivo* bioassay approach

Perhaps the primary means of identifying potentially herbicidal metabolites from microalgae has been a variety of *in vivo* biological assays, relevant to herbicidal activity, which are readily available (and adaptable to most laboratory environments). Such assays, typically utilize a variety of endpoints relevant to herbicidal activity, including inhibition of seed germination and seedling growth, and inhibition of photosynthesis and/or “bleaching” of photosynthetic pigments, as well as various biochemical markers (e.g. oxidative stress).

In particular, the aquatic monocotyledonous angiosperm, “duckweed”, including *Lemna minor* and *L. gibba*, and related species, have been widely utilized as a model system for algae-derived herbicides due, in part to its rapid growth (and presumptively its role of these metabolites in aquatic allelopathy). *Lemna* has, in fact, been adopted by the U.S. Environmental Protection Agency (EPA) as a general model for aquatic phytotoxicity (EPA 712-C-96-156). As an early example of this, Entzeroth et al. (1985) used *Lemna* to identify herbicidal compounds from ethanol extracts of the cyanobacterial species, *Lyngbya aesturii*. Using the species for bioassay-guided fractionation, herbicidal activity was found to be associated with an unusual fatty acid, 2,5-dimethyldodecanoic acid, which inhibited growth at concentrations as low as 200 ng/mL. (Entzeroth et al., 1985). Although not an algal metabolite in the strict sense, *Lemna* was also used to evaluate the phytotoxic activity of usnic acid, a metabolite of lichens (composite organisms comprised of a fungal and algal symbiont).

More recently, several studies (Weiss et al., 2000; Leblanc et al., 2005; Mitrovic et al., 2004 and 2005; Jang et al., 2007; Saqrane et al., 2007; Yi et al., 2009) have utilized *Lemna* to investigate the apparent effects of the widespread cyanobacterial toxin, microcystin, on plant growth. Such studies have indicated that microcystins can inhibit growth and photosynthesis, as well as oxidative stress, in *Lemna* (e.g. Weiss et al., 2000; Mitrovic et al., 2005). Interestingly, not all studies have equally shown inhibitory effects of microcystins on *Lemna*, likely due to the lack of a standardized genetic background (and associated variability). Leblanc et al. (2005), for example, showed that – contrary to various other studies in the same species (e.g. Saqrane et al., 2007) – microcystin-LR had no apparent effect on *L. gibba* growth or photosynthesis in the species.

Aside from studies in *Lemna*, as well as a number of other aquatic plant species (e.g. *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Phragmites australis*; Pflugmacher et al., 2001; Pflugmacher, 2002; Yi et al., 2009) studies have used a range of other plant models, including agriculturally important ones, to investigate phytotoxicity of algal metabolites. For example, Sanevas et al. (2006) evaluated aqueous methanolic extracts from a species of the cyanobacterial genus, *Hapalosiphon*, in a range of monocotyledonous and dicotyledonous agricultural plant species, including radish, cabbage, carrot, lettuce, wheat, onion, rice and maize, and specifically demonstrated a dose-dependent inhibition of roots and, to a lesser extent, shoot elongation, apparently due to inhibition of cell division. Hassan and Ghareib (2009) more recently utilized tomato (*Lycopersicon esculentum*) and lettuce (*Lactuca sativa*) to demonstrate inhibition of seed germination and seedling growth by apparent phenolic compounds extracted from the green alga, *Ulva lactuca*. Similarly, extracts of a strain of *Nostoc*, isolated from an agricultural pond, was found to specifically inhibit root and shoot growth of seedling, but not affect seed germination, in rice (*Oryza sativa*). Phytotoxicity of the lichen-derived usnic acid has been evaluated and found (Lasceve and Gaugain, 1990;

Ozturk et al., 1999) to inhibit growth in several monocot (e.g. maize, onion) and dicot (e.g. sunflower) species. Gleason and Case (1986) showed that cyanobacterin from *Scytonema hofmanni* equally inhibited crop plant species, including maize (*Zea mays*) and peas (*Pisum sativum*), as well as wild plant species, including dock (*Rumex crispus*), wild buckwheat (*Polygonum convolvulus*) and wild oats (*Avena fatua*). Moreover, several studies (McElhiney et al., 2001; Pflugmacher et al., 2007; Saqrane et al., 2008) investigated the possible impacts of microcystin on crop plants by evaluating phytotoxicity in a range of species including potato (*Solanum tuberosum*), mustard (*Synapis alba*), bean (*Phaseolus vulgaris*), maize (*Z. mays*), lentils (*Lens esculentum*), peas (*P. sativum*) wheat (*Triticum durum*) and spinach (*Spinacia oleracea*). In particular, microcystin-LR inhibited both epicotyl and root length, in addition to seed germination in maize (Saqrane et al., 2008).

Here we propose - and briefly present preliminary data on - the novel use of the model angiosperm, *Arabidopsis thaliana*, as the basis of a biological assay for investigation of herbicides from microalgae (and potentially other sources). *A. thaliana* ("thale cress"), a member of the mustard family (Brassicaceae), has been well studied - dating back to the work of George Rédei in the 1950s. However it took nearly four decades before it would be widely accepted as a model organism in plant biology. Specific advantages of the model include small size and ease of cultivation (e.g. ability to grow on agar plates), a small (ca. 120 Mb) and now completely sequenced genome, and prolific seed production and rapid life cycle (ca. 6 weeks from seed germination to mature seed), as well as a large collection of described genetic mutants and various features that make it amenable to transgenic manipulations. An early, seminal review of the species as a model organism was presented by Meinke et al. (1998), following its acceptance by the Security Council of Model Genetic Organisms in 1998 (Fink, 1998). *The Arabidopsis Information Resource* (TAIR; www.arabidopsis.org) currently maintains a database, specifically relevant to the genetic and molecular biology of *Arabidopsis*.

Owing, in particular, to various practical advantages (e.g. small size, laboratory cultivation, rapid germination and growth), *A. thaliana* represents an ideal candidate for investigation of herbicides generally, and specifically as a means to rapidly assess herbicidal compounds including, as relevant to the current topic, those from microalgae. Moreover, as a model organism, and particularly one with well described genetic background, as well as general amenability to various molecular biological methodologies, herbicidal activity can, in principle, be readily investigated via, for example, comparison to available genetic mutants, genetic manipulation, etc toward the goal of target identification. As such, we have developed a phytotoxicity assay based on *A. thaliana*, and specifically applied this to the screening of metabolites from a collection of freshwater cyanobacteria.

As illustrated in Fig. 5, extracts of cyanobacterial culture biomass were assayed in agar plates seeded with *A. thaliana*, and grown in a standard light- and temperature-controlled environmental chamber. Biomass (ca. 100 mg) from eleven strains of freshwater cyanobacteria, specifically isolated from the Florida Everglades (see Berry et al., 2007; Gantar et al., 2008), was sequentially extracted in non-polar (chloroform) and polar (30% ethanol) solvents. Prior to seeding, small "treatment wells" were made into the agar medium (i.e. Murashige-Skoog medium with 0.8% agar) in the approximate center of each "sector" of a standard square Petri dish, specifically using an autoclave-sterilized Pasteur pipet, and low vacuum (see Fig. 5). Each well was filled with aliquots (20 µL) of prepared extracts. Subsequently, seeds of the wild-type *Columbia* ecotype of *A. thaliana* (Col-0), obtained from commercial sources (e.g. Lehle Seeds, Round Rock, TX, U.S.A.), were seeded

into assay plates, as per standard techniques. Briefly, an appropriate amount of seeds (ca. 50 per milligram, or approximately 8 mg per assay plate) were weighed into microcentrifuge tubes, and sterilized (for 5 minutes) with 30% bleach, followed by repeated (3-4 times) rinsing with nanopure water, and subsequent centrifugation. Rinsed, sterilized seeds were re-suspended into 0.1% agar, and seeded into appropriate sectors of the test plate (ca. 10-15 seeds). Seeded plates were kept for approximately two weeks (to germinate and grow) in an environmental growth chamber (Therm Scientific Precision Model 818) at 28° C with a 14:10 light/dark cycle.

As seen in Fig. 5, the assay specifically demonstrated apparent inhibition of *A. thaliana* seedling growth by a polar (30% ethanol) extract of the isolate, *Microcystis* 95-13. Although germination was not apparently inhibited, the growth of hypocotyls was clearly reduced in seedlings exposed to the extract (Fig. 5). None of the other polar or non-polar extracts evaluated showed any apparent inhibition of seed germination or seedling growth in the two-week test period. Likewise, no effect of solvent (neither 30% ethanol or chloroform) was detected relative to untreated (i.e. “no solvent”) controls. As referenced above, *Microcystis* is, as a genus, recognized to produce the potentially toxic microcystins that have been associated with human and other animal intoxication events, as well as phytotoxicity. These results support the latter, but the recognized toxicity of the microcystins – if they are, in fact, responsible for phytotoxicity against *A. thaliana* – would, as further discussed below, likely preclude their application as herbicides. We are currently investigating the phytotoxic metabolites identified here, but these results suggest the assay presented here may represent an effective means to rapidly screen – and subsequently characterize targets of – microalgal metabolites with respect to herbicidal activity.

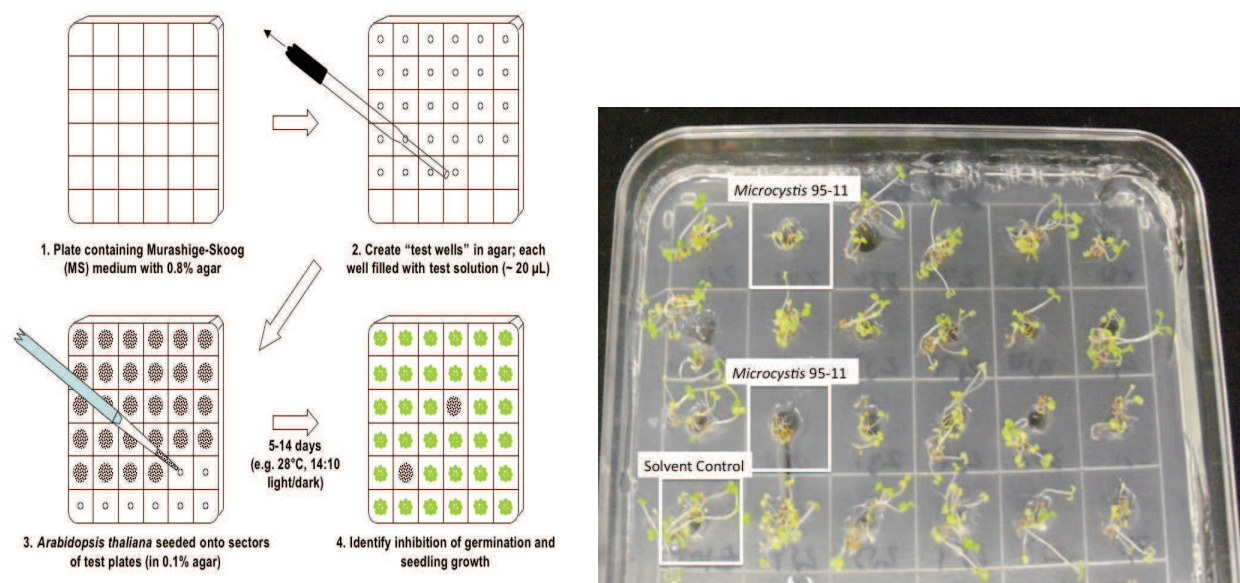


Fig. 5. *A. thaliana* phytotoxicity assay of cyanobacterial culture extracts showing inhibition of seedling growth by the isolate *Microcystis* 95-11. Shown are the polar (30% extracts).

4.2 Herbicidal target-based approach

With respect to the target-based approach, commercial herbicide generally fall into several categories based on their biochemical, molecular or cellular “mechanisms of actions” (MOA), including: (1) inhibition of primary metabolism, particularly including biosynthesis

of lipids and amino acids, and specifically “branched” and aromatic amino acids, e.g. inhibitors of acetolactate synthase (ALS) and enolpyruvulshikimate 3-phosphate synthase (ESPS), respectively; (2) inhibition of oxygenic photosynthesis, including photosystems I and II (PSI/II), as well as biosynthesis of associated pigments, e.g. protoporphyrinogen oxidase (PPO), a key enzyme in the synthesis of chlorophyll; (3) synthetic “mimics” of plant growth regulators, e.g. auxins, cytokinins; and (4) inhibitors of microtubules or other components of cell-division.

By far, the most well investigated target of phytotoxic metabolites from microalgae has been inhibition of oxygenic photosynthesis, and associated molecular/biochemical targets. A good review of photosynthesis inhibitors from microalgae has been previously presented by Smith & Doan (1999). Recent studies by Gantar et al. (2008), for example, used pulse amplitude-modulated (PAM) fluorescence to show that lipophilic extracts of the cyanobacterial strain, *Fischerella* 52-1 (isolated from the Florida Everglades), specifically inhibited photosystem II (PSII), in addition to apparent degeneration of thylakoids. This finding is particularly notable as the strain was found to produce hapalindoles (Gantar et al., 2008; Walton et al., in press) previously associated with allelopathy (see *Algal Allelopathy* above). Within the same genus, the fischerellins A and B (Fig. 3) were shown (Srivastava et al., 1998) to potently inhibit PSII, specifically acting at multiple sites of PSII, distinct from that of the photosynthesis-inhibiting herbicide, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Likewise, cyanobacterin, originally isolated from the cyanobacterial species, *Scytonema hofmanni*, inhibited PSII at a site distinct from - and with a potency nearly five times - that of DCMU, and specifically at the oxidizing site of quinone-B electron acceptor (Gleason & Paulson, 1984; Gleason & Case, 1986; Gleason et al., 1986). On the other hand, the peptide, nostocyclamide (Fig. 3), from the genus, *Nostoc*, seems to inhibit PSII by uncoupling electron transport (Jüttner et al., 1997 cf. Smith & Doan, 1999).

A second important class of herbicides are those that mimic plant growth regulators (“hormones”) including auxins, cytokinins and gibberellins. Both macroalgae and microalgae - and particularly cyanobacteria and chlorophytes - have been found to produce extracellular compounds that act as all three of these, including metabolites chemically identical (e.g. the auxin, indole-3-acetic acid) to the plant hormones (see review by Tsavkelova et al., 2006). The realization that microalgae may produce metabolites with plant growth regulator activity extends back to Zulpa de Caire et al. (1979) who demonstrated that culture medium of the cyanobacterium, *Nostoc*, exhibited auxin-like activity. Zaccaro et al. (1996) subsequently showed that extracellular products from *Scytonema hofmanni* stimulated growth of *Lilium alexandrae* in a manner similar to the synthetic auxin, 1-naphthaleneacetic acid (NAA), and implied that metabolites might represent a non-toxic alternative to this agent. Sergeeva et al. (2002) screened thirty-four strains of cyanobacteria, and not only identified auxin-like activity in 21 of the 34 strains, but confirmed (using GC-MS, and analytical standards) the presence of the main auxin, indole-3-acetic acid (IAA), in two species of *Nostoc*. Interestingly, a higher amount of auxin-like activity was found for symbiotic (83%), versus free-living (38%), species of cyanobacteria. Similarly, isopentanyl adenine cytokinins, including zeatin, and specifically *cis* isomers, as well as aromatic cytokinins, including benzyladenine (chemically similar to the synthetic cytokinin,) have been previously isolated from several species of macroalgae (Stirk et al., 1999; Stirk et al., 2002; Stirk et al., 2003). More recently, Hussain et al. (2010) identified both IAA and zeatin-like cytokinins in a variety of cyanobacterial species. Cyanobacteria have been similarly

shown to produce gibberellin-like metabolites (Gupta and Agarwal, 1973; Rodriguez et al., 2006).

Although plant growth regulators are, obviously, involved in the positive growth of higher plants (e.g. cell division, elongation), directed application of agonists can lead to uncontrolled growth, and consequently act as herbicides. Perhaps the best example of this strategy is the widely used synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D). In fact, several studies (Jäger et al., 2005; Manickavelu et al., 2006) have shown that cyanobacterial metabolites can replace 2,4-D in plant cell culture. In accordance with the dose-dependent effects of plant growth regulators, Hassan and Ghareib (2009) evaluated phenolics extracted from *Ulva lactuca*, and found moderate concentration (30 ppm) stimulated germination of lettuce and tomato seeds. However, at higher concentrations (300 ppm), the same extracts reduced both seed germination and seedling growth.

A third target of several herbicides is cell division. Acting in some ways like synthetic plant growth regulators (e.g. effects on elongation, cell swelling), such compounds differ in affecting cell division via direct mechanisms (e.g. inhibition of tubulin/microtubule assembly) rather than indirect routes (e.g. regulation of gene expression). Perhaps the best described are the pre-emergence dinitroaniline herbicides (DNHs). DNHs, such as the widely used trifluralin, inhibit mitosis (as well as other effects, e.g. cell swelling, induction of multiple nuclei) by interaction with tubulin subunits of microtubules, in a fashion similar to the well described "spindle poison," colchine (Upadhyaya & Noodén, 1977, 1980).

Indeed, microalgae produce a wide diversity of cell division inhibitors, although these metabolites have been largely investigated with respect to their potential as antibiotics or anticancer drugs. Perhaps the best investigated microalgal metabolite in this regard, are the cryptophycins (Trimurtulu et al., 1994), comprised of more than twenty-five congeners specifically isolated from the cyanobacterial genus, *Nostoc*, that have been shown (Smith et al., 1994) to act via depolymerization of microtubules, and consequently investigated as both antifungal and antitumor compounds (including, in the latter case, Phase I clinical trials). Likewise, other cyanobacterial metabolites, including curacin A (from the marine cyanobacterium, *Lyngbya majuscula*) and the dolastatins (from the marine cyanobacterium, *Symploca*) are inhibitors of microtubule assembly and tubulin polymerization (Verdier-Pinard et al., 1998; Luesch et al., 2001), and have also been investigated with respect to potential anticancer drug development. Indeed, many such compounds – and particularly an array of non-ribosomal peptides – have been isolated and characterized (to some extent) from cyanobacteria and other microalgae, and a number of good reviews on these are available (Moore, 1996; Gerwick et al., 2001; Tan, 2007). Moreover, aside from cyanobacteria, other algae have been, likewise, found to produce inhibitors of mitosis. For example, various species of brown algae have been found to produce terpenoids, including the mediterraneols and bifurcarenone (Sun et al., 1980; Francisco et al., 1986) that inhibit cell-division.

Despite this focus on cell division inhibitors from microalgae with respect to drug development, limited studies have also shown apparent cell division in plant tissues. Extracts from the cyanobacterium, *Hapalosiphon*, for example, were shown (Sanevas et al., 2006) to inhibit root and shoot growth in a wide range of crop plants (see above), and subsequently found to inhibit mitosis, as evidenced by a dose-dependent reduction in the mitotic index (in the onion root model). In a study (Baskin and Wilson, 1997) using the *A. thaliana* model (see above), the effect of the protein phosphatase inhibitors, okadaic acid and microcystin-LR – metabolites from dinoflagellate and cyanobacterial species, respectively –

were evaluated with respect to cortical microtubules of roots. Interestingly, although both toxins are recognized inhibitors of type 1/2A serine/threonine protein phosphatases, they exerted markedly different effects. Okadaic acid affects both cell elongation and radial expansion (at higher concentrations), as well as microtubule disorganization, whereas microcystin-LR only minimally inhibited elongation. Although inherent toxicity of both metabolites may obviously limit the potential of these compounds themselves, as commercial herbicides, the differential activity of these metabolites suggests an otherwise uncharacterized difference in their effects (via protein phosphatase inhibition) on cell division, and supports a need to further investigate their specific targets, particularly in relation to phytotoxic effects such as those observed.

A final important target of commercial herbicides is the inhibition of biosynthetic pathways of primary metabolites, including lipids and amino acids. Among important examples of these are the amino acid biosynthesis inhibitors (see review by Kishore and Shah, 1988), including specific inhibitors of acetolactate synthase (ALS, e.g. sulfonylureas) and enolpyruvylshikimate 3-phosphate synthase (EPSPS, e.g. glyphosate), key steps in the synthesis of branched chain, and aromatic, amino acids, respectively. In particular, the ALS pathway is one that exists only in plants (and not animals) making it a particularly good target for herbicides. Similarly, inhibitors of the enzyme acetyl coenzyme A carboxylase (ACCase) act via inhibition of a key step in lipid biosynthesis, and show selective activity, specifically between monocots and dicots.

To date, there are no known (to the author's knowledge) metabolites from microalgae that have been found to target either amino acid or lipid biosynthesis. That said, the unique diversity of both lipid and amino acids in the secondary metabolites of microalgae makes them seemingly rife with opportunities to explore possible effects of these as, for example, potential inhibitors of their respective primary metabolic pathways. In particular, the cyanobacteria are recognized to produce and utilize a wide range of "unusual amino acids" including D-isomers, β -hydroxy, N-methylated or otherwise modified version of essential amino acids, as well as completely unique representatives (see, for example, review by Gerwick et al., 2001). Also along these lines, it is worth noting that several studies (e.g. Powell et al., 1991; Forlani et al., 2008) have shown an apparently widespread tolerance of cyanobacteria to the EPSPS inhibitor, glyphosate, specifically, it seems, via insensitivity of this enzyme in these microalgae. Interestingly, although such a possible role of these small molecules with respect to amino acid metabolism remains to be investigated, at least two studies have shown inhibition, by algal (or related) metabolites, of enzymes involved in catabolism of amino acids. Specifically, the lichen-derived toxin, usnic acid, was found to inhibit hydroxyphenylpyruvate dioxygenase (HPPD), similar to several commercial herbicides (e.g. triketones), leading to blockage of plastoquinone synthesis, and consequent "bleaching" of plant cells (Duke et al., 2002). Likewise, norharmane (isolated from *Nostoc* and several other cyanobacteria; Volk, 2008) has been shown to inhibit the equivalent enzyme - indoleamine 2,3-dioxygenase - involved in the catabolism of the amino acid, tryptophan. In general, the paucity of studies represents an open area for research.

4.3 Pharmacophore-based approach

In addition to their classification based on target or MOA, commercially important herbicides also span a wide range of chemical classes. Based on established structure-activity relationships of either herbicides - or, alternatively, endogenous/exogenous

regulators – the identification of structurally related metabolites (i.e. shared “pharmacophores”) serves as a means to identify potential herbicidal candidates. To be sure, there have been, to date, very limited use of this approach as a systematic means to screen microalgal metabolites for herbicidal compounds. However, at least one study arguably has. Specifically, a recent study by Volk (2008) utilized a combination of high-performance thin-layer chromatography, and high-performance liquid chromatography (HPLC), to screen thirty-three species of microalgae for norharmane, based on prior (Volk, 2006) identification of this metabolite as an algicidal metabolite of *Nostoc*. Interestingly, this study identified an additional seven strains of cyanobacteria that produced norharmane or related compounds (Volk, 2008). Similarly, the pharmacophore-based approach has been used direct synthetic studies of microalgal metabolites. As an example, Blom et al. (2006) used norharmane (as a synthetic starting material), and the structurally related metabolite, nostocarboline (Fig. 3), to prepare several synthetic analogs of the latter, and demonstrate structure-activity relationships, as well as (in, at least, one case) enhanced algicidal activity.

Moreover, several cases of structural similarity between microalgal metabolites and established herbicides exist. For example, as discussed above (see *Algal Allelopathy*) - and as shown in Fig. 6 - several of the purportedly allelopathic metabolites from microalgae contain an indole core, structurally similar to that of the naturally occurring auxins (e.g. IAA, IBA, Cl-IAA). Although bioactive indole alkaloids are widespread in the natural world, including marine and freshwater habitats (see, for example, review by Gul and Hamann, 2005), and the algicidal activity of these microalgal metabolites, as currently understood, do not parallel the effects of auxins, this supports the importance of indoles as a target for pharmacophore-based approaches.

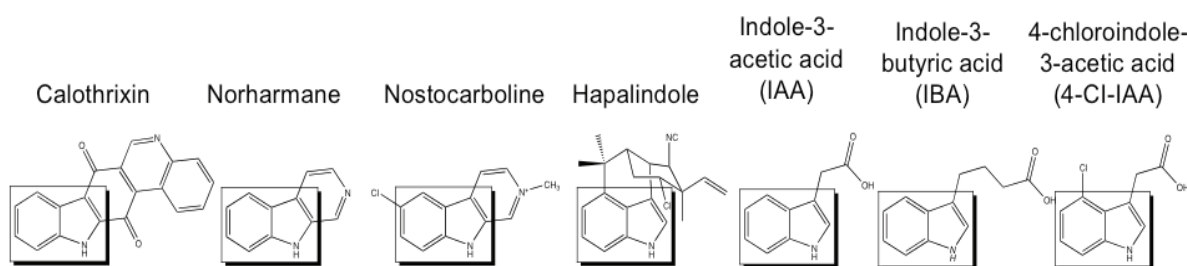


Fig. 6. Indole-containing allelochemicals from cyanobacteria, and structural comparison to plant auxins. From left to right are calothrixin A, norharmane, nostocarboline and 12-eip-hapalindole E isonitrile, and the naturally-occurring auxins, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 4-chloroindole-3-acetic acid.

Likewise, various other chemical classes, represented by commercially important herbicides, are found in marine and freshwater microalgae. For example, oxazole rings – characteristic of a number of herbicides (e.g.) - are commonly found among bioactive non-ribosomal peptides from cyanobacteria (e.g. muscoride from *Nostoc muscorum*, Nagatsu et al., 1995; microcyclamides from *Microcystis* sp., Raveh et al., 2010; raocyclamides from *Oscillatoria raoi*, Admi et al., 1996). Indeed, the recognized allelopathic metabolite, nostocyclamide, contains this heterocyclic ring system (Fig. 3). Likewise, various marine algae are known to produce chlorinated hydrocarbons similar to the halogenated aliphatic class of herbicides (e.g. trihaloacetic acids, such as dalapon), and which have been associated with possible allelopathy (see, for example, review by Kladi et al. (2004)).

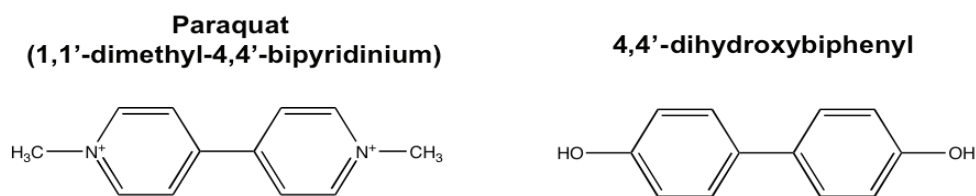


Fig. 7. General structural resemblance between the algicidal exometabolite, 4,4'-dihydroxybiphenyl, from cyanobacteria, and the herbicide, paraquat.

In other cases, the structure of microalgal metabolites, rather than containing identical structural features, closely resemble, in a more general way, recognized herbicides. For example, the algicidal phenolic compound, 4,4'-dihydroxybiphenyl, isolated (Volk, 2006) as an “exometabolite” of cyanobacterial cultures, bears general structural resemblance to the commercially important herbicide, paraquat (Fig. 7). Similarly, bioactive metabolites structurally resembling (Fig. 8) halogenated diphenyl ether herbicides (e.g. oxyfluorfen, acifluorfen, diclofop), have been isolated from algae. In particular, bioactive polybrominated diphenyl ethers, including hydroxylated and methoxylated variants, have been isolated from rhodophytes, chlorophytes and cyanobacteria (Malmvärn et al., 2008). One such example is shown in Fig. 8.

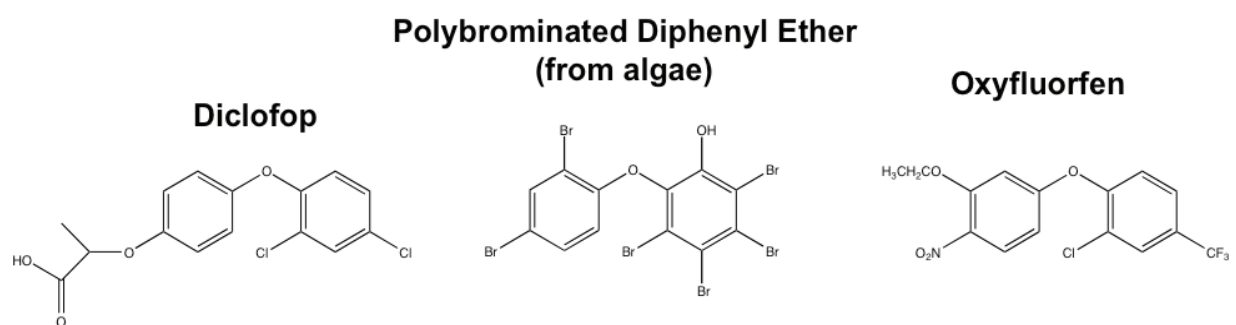


Fig. 8. Comparison of polybrominated diphenyl ethers from microalgae, and the diphenyl ether herbicide, oxyfluorfen).

5. Commercial potential of herbicides from microalgae

As demonstrated in this chapter, microalgae represent a rich repository of bioactive metabolites, including numerous compounds with biological activity either indirectly (e.g. algicidal) or directly (e.g. photosynthesis inhibiting, antimetabolic) related to herbicidal potential. Moreover, a number of features make these metabolites particularly compelling as a source of potential herbicides.

As discussed, the cyanobacteria, in particular, are recognized producers of an array of non-ribosomal peptides, while the secondary metabolism of eukaryotic microalgae is particularly characterized by a diversity of metabolites derived from the polyketide synthesis pathway. As modular enzymes, in both cases, these megasynthases (e.g. NRPSs, PKSs) are highly amenable to “*in vivo* combinatorial” approaches (through directed manipulation of gene, and consequently enzyme, modules), as well as heterologous expression. The former, in principle, enables a means for rapid biosynthetic modification, and essentially “design,” of molecules for improved activity and uptake potential, as well as reduced toxicity, and other

characteristics germane to effective and safe herbicides. As one example, Christiansen et al. (2003) demonstrated, using targeted mutation of one module (*mcyJ*, an *O*-methyltransferase) of the NRPS/PKS gene responsible for microcystin biosynthesis chemical modification specifically resulting in a novel variant with modified activity. Heterologous expression, on the other hand, enables the potential for large-scale production of these metabolites in suitable hosts (e.g. *E. coli*) which can be readily cultured with high yields on a large-scale (e.g. biofermentation). Such an approach has, in fact, been demonstrated for a very limited number of algal or other microbial metabolites. In one example, the biosynthetic NRPS gene cluster for the algal metabolite, patellamide A, naturally produced by an endosymbiotic algae (from didemnid ascidians), was heterologously expressed in *E. coli* (Schmidt et al., 2005), and proposed (Long et al., 2005) as a means of sustainable production of the bioactive metabolite.

Other compelling feature of these algal metabolites as herbicides include a presumptive “biodegradability” of many of these natural products, as well as water solubility of many (e.g. peptides) which might minimize concerns of bioaccumulation, and associated environmental concerns. Although these are not universally true of algal metabolites, both water solubility and lability have been described in at least some of the allelopathic metabolites from algae (e.g. apparent algicides from the macroalga, *Ulva*, as discussed above).

In addition to potential application as herbicides, particularly with regards to, for example, crop pests (i.e. weeds), microalgal metabolites hold an equally important potential as algicides and related “antifouling” agents. As pointed-out by Duke et al. (2002) aquaculture represents “one of the fastest growing areas of agriculture in the world,” and although not traditionally grouped with herbicides, the diversity algicides represent a “niche market.” Presence of algae in aquaculture facilities is associated with a number of concerns related to both health (e.g. toxic contaminants) and quality (e.g. “off-flavors” associated with the cyanobacterial metabolite, 2-methylisoborneol, MIB) that are consequently responsible for substantial economic losses. Currently there are rather limited approved agents for control of noxious algae in aquaculture, and as such, discovery of natural products, as potentially “environmentally safe” algicides, from microalgae (or other sources) holds tremendous – albeit largely untapped – promise.

Although a number of microalgal metabolites have been shown to have either directly, or potentially, herbicidal or algicidal activity, there is an obvious need for further study. In many cases, compounds, studied with respect to herbicidal or algicidal activity, have recognized potential for human or animal toxicity which would presumably limit their direct use as herbicides. This is perhaps particularly true for a number of so-called “HAB toxins” which despite demonstrated allelopathic, and even herbicidal, activity have well documented human toxicity, as well as impacts on wildlife and domestic animals. That said, although such toxicity would likely preclude any direct commercial application of these compounds, their continued investigation would provide a means of identify possible novel targets for development of (less toxic) herbicides based on their MOAs. Furthermore, aside from continued identification of metabolites with herbicidal activity, evaluation of taxa specific (e.g. monocots versus dicots, crop plant versus weed” species) differences in activity, as well as further elucidation of MOAs, will be required for potential of these compounds to be realized in any commercially relevant sense.

Finally, despite the obvious potential of the microalgal secondary metabolites with respect to discovery and development of herbicides, there remains a relatively limited commercial

exploration of this resource. In fact, to the author's knowledge, in only one case - specifically based on the work of Gleason et al. (1986, 1990) with respect to the algicidal and phototoxic activity of cyanobacterin - has a patent (United States Patent 4626271) been submitted for explicit use of the compound (specifically in conjunction with a surfactant) as a green plant herbicide. It is, of course, the hope that continued exploration of this chemical diversity will lead to future realization of its tremendous potential.

6. References

- Admi, V.; Afek, U. & Carmeli, S. (1996) Raocyclamides A and B, novel cyclic hexapeptides isolated from the cyanobacterium *Oscillatoria raoi*. *Journal of Natural Products* 59, 396-399.
- Alamsjah, M. A.; Hirao, S.; Ishibashi, F. & Fujita, Y. (2005) Isolation and structure determination of algicidal compounds from *Ulva fasciata*. *Bioscience, Biotechnology and Biochemistry* 69, 2186-2192.
- Alamsjah, M. A.; Hirao, S.; Ishibashi, F.; Oda, T. & Fujita, Y. (2007) Algicidal activity of polyunsaturated fatty acids derived from *Ulva fasciata* and *U. pertusa* (Ulvaceae, Chlorophyta) on phytoplankton. *Journal of Applied Phycology* 20, 713-720.
- An, Z.; Wang, Z.; Li, F.; Tian, Z. & Hu, H. (2008) Allelopathic inhibition on red tide microalgae *Skeletonema costatum* by five microalgal extracts. *Frontiers of Environmental Science & Engineering in China* 2, 297-305.
- Apstein, C. (1896) *Das Süßwasserplankton*. Kiel, Leipzig, Germany.
- Arunkumar, K.; Sivakumar, S. R. & Rengasamy, R. (2010) Review on bioactive Potential in seaweeds (marine macroalgae): a special emphasis on bioactivity of seaweeds against plant pathogens. *Asian Journal of Plant Sciences* 9, 227-240.
- Arzul, G.; Erard-Le Denn, E.; Videau, C.; Jegou, A. M. & Gentien, P. (1993) Diatom growth repressing factors during an offshore bloom of *Gyrodinium cf. aureolum*. In: *Toxic Phytoplankton Blooms in the Sea*, T. J. Smayda & Y. Shimizu (Eds.), pp. 719-724, Elsevier, Amsterdam.
- Arzul, G.; Gentien, P.; Bodenec, G.; Toularastel, F.; Youenou, A. & Crassous, M. P. (1995) Comparison of toxic effects of *Gyrodinium cf. nagasakiense* polyunsaturated fatty acids. In: *Harmful Marine Algal Blooms*, P. Lassus, G. Arzul, E. Erard, P. Gentien & C. Marcaillou (Eds.), Lavoisier, Paris.
- Baskin, T. I. & Wilson, J. E. (1997) Inhibitors of protein kinase and phosphatase alter root morphology and disorganize cortical microtubules. *Plant Physiology* 113, 493-502.
- Becher, P. G.; Beuchat, J.; Gademann, K. & Jüttner, F. (2005) Nostocarboline: isolation and synthesis of a new cholinesterase inhibitor from *Nostoc* 78-12A. *Journal of Natural Products* 68, 1793-1795.
- Berry, J. P.; Gantar, M.; Perez, M. H.; Berry, G. & Noriega, F. G. (2008) Cyanobacterial toxins as allelochemicals with potential applications as algacides, herbicides and insecticides. *Marine Drugs* 15, 117-146.
- Blom, J. F.; Brütsch, T.; Bethuel, B. D.; Locher, H. H.; Hubschwerien, C. & Gademann, K. (2006) Potent algicides based on the cyanobacterial alkaloid nostocarboline. *Organic Letters* 16, 737-740.
- Cardozo, K. H. M.; Guaratini, T.; Barros, M. P.; Falcão, V. R.; Tonon, A. P.; Lopes, N. P.; Campos, S.; Torres, M. A.; Souza, A. O.; Colepicolo, P. & Pinto, E. (2007)

- Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology, Part C* 146, 60-78.
- Cantillo-Ciau, Z.; Moo-Puc, R.; Quijano, L. & Freile-Pelegri, Y. (2010) The tropical brown alga *Lobophora variegata*: a source of antiprotozoal compounds. *Marine Drugs* 16, 1291-1304.
- Christiansen, G. E.; Dittmann, L. V., Ordorika, R.; Rippka, M.; Herdman, T.; Börner, T. (2001) Nonribosomal peptide synthetase genes occur in most cyanobacterial genera as evidenced by their distribution in axenic strains of the PCC. *Archives of Microbiology* 176, 452-458.
- Christiansen, G.; Fastner, J.; Erhard, M.; Börner, T. & Dittman, E. (2003) Microcystin biosynthesis in *Planktothrix*: genes, evolution and manipulation. *Journal of Bacteriology* 185, 564-572.
- Codd, G. A.; Bell, S. G.; Kaya, K.; Ward, C. J.; Beattie, K. A. & Metcalf, J. S. (1999) Cyanobacterial toxins, exposure routes and human health. *European Journal of Phycology* 34, 405-415.
- Copping, L. G. & Duke, S. O. (2007) Natural products that have been used commercially as crop protection agents. *Pest Management Science* 63, 524-554.
- Dayan, F. E.; Cantrell, C. L. & Duke, S. O. (2009) Natural products in crop protection. *Biorganic & Medicinal Chemistry* 17, 4022-4034.
- Dittmann, E.; Neilan, B. & Börner, T. (2001) Molecular biology of peptide and polyketide biosynthesis in cyanobacteria. *Applied Microbiology and Biotechnology* 57, 467-473.
- Doan, T. N.; Rickards, R. W.; Rothschild, J. M. & Smith, G. D. (2000) Allelopathic actions of the alkaloid 12-*epi*-hapalindole E isonitrile and calothrixin A from cyanobacteria of the genera *Fischerella* and *Calothrix*. *Journal of Applied Phycology* 12, 409-416.
- Doan, N. T.; Stewart, P. R. & Smith, G. D. (2001) Inhibition of bacterial RNA polymerase by the cyanobacterial metabolite 12-*epi*-hapalindole E isonitrile and calothrixin. *FEMS Microbiology Letters* 196, 135-139.
- Duke, S. O.; Dayan, F. E.; Rimando, A. M.; Schrader, K. K.; Aliotta, G.; Oliva, A. & Romagni, J. (2002) Chemicals from nature for weed management. *Weed Science* 50, 138-151.
- Eitsuka, T.; Nakagawa, K.; Igarishi, M. & Miyazawa, T. (2004) Telomerase inhibition by sulfoquinovosyldiacylglycerol from edible purple laver (*Porphyra yezoensis*). *Cancer Letters* 212, 15-20.
- Entzeroth, M.; Mead, D. J.; Patterson, G. M. L. & Moore, R. E. (1985) A herbicidal fatty acid produced by *Lyngbya aestuarii*. *Phytochemistry* 24, 2875-2876.
- Erhard, D. & Gross, E. M. (2006) Allelopathic activity of *Elodea Canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. *Aquatic Botany* 85, 203-211.
- Etchegaray, A.; Rabello, E.; Dieckmann, R.; Moon, D. H.; Fiore, M. F.; von Döhren, H.; Tsai, S. M. & Neilan, B. A. (2004) Algicide production by the filamentous cyanobacterium *Fischerella* sp. CENA 19. *Journal of Applied Phycology* 16, 237-243.
- Fenical, W. & Paul, V. J. (1984) Antimicrobial and cytotoxic terpenoids from tropical green algae of the family Udoteaceae. *Hydrobiologia* 116-117, 135-140.
- Fink, G. R. (1998) Anatomy of a revolution. *Genetics* 149, 473-477.
- Flores, E. & Wolk, C. P. (1986) Production by filamentous, nitrogen fixing cyanobacteria of a bacteriocin and of other antibiotics that killed related strains. *Archives of Microbiology* 145, 215-219.

- Forlani, G.; Pavan, M.; Gramek, M.; Kafarski, P. & Lipok, J. (2008) Biochemical bases for a widespread tolerance of cyanobacteria to the phosphonate herbicide glyphosate. *Plant and Cell Physiology* 49, 443-456.
- Francisco, C.; Banaigs, B.; Valls, R.; Codomier, L. (1985) Mediterraneanol a, a novel rearranged diterpenoid-hydroquinone from the marine alga *Cytoseira mediterranea*. *Tetrahedron Letters* 26, 2629-2632.
- Friedlander, M.; Gonen, Y.; Kashman, Y. & Beer, S. (1996) *Gracilaria coferta* and its epiphytes: 3. Allelopathic inhibition of the red seaweed by *Ulva* cf. *lactuca*. *Journal of Applied Phycology* 8, 21-25.
- Gantar, M.; Berry, J. P.; Thomas, S.; Wang, M.; Perez, R.; Rein, K. S. & King, G. (2008) Allelopathic activity among cyanobacteria and microalgae isolated from Florida freshwater habitats. *FEMS Microbiology Ecology* 64, 55-64.
- Gerwick, W. H., Tan, L. T. & Sitachitta, N. (2001) Nitrogen-containing metabolites from marine cyanobacteria. *The Alkaloids: Chemistry and Biology* 57, 75-184.
- Gleason, F. K. & Paulson, J. L. (1984) Site of action of the natural algicide, cyanobacterin, in the blue-green alga, *Synechococcus* sp. *Archives of Microbiology* 138, 273-277.
- Gleason, F. K. & Case, D. E. (1986) Activity of the natural algicide, cyanobacterin, on angiosperms. *Plant Physiology* 80, 834-837.
- Gleason, F. K.; Case, D. E.; Sipprell, K. D. & Magnuson, T. S. (1986) Effect of the natural algicide, cyanobacterin, on a herbicide-resistant mutant of *Anacystis nidulans* R2. *Plant Science* 46, 5-10.
- Granéli, E. & Johansson, N. (2003) Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. *Harmful Algae* 2, 135-143.
- Granéli, E.; Weberg, M. & Salomon, P. S. (2008) Harmful algal blooms of allelopathic microalgal species: the role of eutrophication. *Harmful Algae* 8, 94-102.
- Gromov, B. V.; Veprikskii, A. A.; Titota, N. N.; Mamkayeva, K. A. & Alexandrova, O. V. (1991) Production of the antibiotic cyanobacterin LU-1 by *Nostoc linckia* CALU 892. *Journal of Applied Phycology* 3, 55-59.
- Gross, E. M. (2003) Allelopathy in aquatic autotrophs. *Critical Reviews in Plant Sciences* 22, 313-339.
- Gross, H.; Goeger, D.; Hills, P.; Mooberry, S. L.; Ballantine, D. L.; Murray, T. F.; Valeriote, F. A. & Gerwick, W. H. (2006) Lophocladines, bioactive alkaloids from the red alga *Lophocladia* sp. *Journal of Natural Products* 69, 640-644.
- Gul, W. & Hamann, M. T. (2005) Indole alkaloid natural products: an established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases. *Life Science* 78, 442-453.
- Gupta, A. B. & Agarwal, P. R. (1973) Extraction, isolation and bioassay of a gibberellin-like substance from *Phormidium foveolarum*. *Annals of Botany* 37, 737-741.
- Güven, K. C.; Percot, A. & Sezik, E. (2010) Alkaloids in marine algae. *Marine Drugs* 8, 269-284.
- Hanashima, S.; Mizushina, Y.; Ohta, K.; Yamazaki, T.; Sugawara, F. & Sakaguchi, K. (2000) Structure-activity relationship of a novel group of mammalian DNA polymerase inhibitors, synthetic sulfoquinovosylacylglycerols. *Japanese Journal of Cancer Research* 91, 1073-1083.

- Hassan, S. M. & Ghareib, H. R. (2009) Bioactivity of *Ulva lactuca* acetone extracts on germination and growth of lettuce and tomato plants. *African Journal of Biotechnology* 8, 3832-3838.
- Horrigan, L.; Lawrence, R. S. & Walker, P. (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. *Environmental Health Perspectives*, 110, 445-456.
- Hussain, A.; Krischke, M.; Roitsch, T.; Hasnain, S. (2010) Rapid determination of cytokinins and auxin in cyanobacteria. *Current Microbiology*, in press.
- Ikawa, M. (2004) Algal polyunsaturated fatty acids and effects on plankton ecology and other organisms. *UNH Center for Freshwater Biology Research* 6, 17-44.
- Irfanullah, H. M. & Moss, B. (2005) Allelopathy of filamentous green algae. *Hydrobiologia* 543, 169-179.
- Jäger, K.; Ördög, V.; Barnabás, B. (2005) Effect of cyanobacterial and microalgal biomass on anther culture response of wheat (*Triticum aestivum*). *Acta Agronomica Hungarica* 53, 99-107.
- Jang, M.-H.; Ha, K. & Takamura, N. (2007) Reciprocal allelopathic responses between toxic cyanobacteria (*Microcystis aeruginosa*) and duckweed (*Lemna japonica*). *Toxicon* 49, 727-733.
- Jin, Q. & Dong, S. L. (2003) Comparative studies on allelopathic effects of two different strains of *Ulva pertusa* on *Heterosigma akashiwo* and *Alexandrium tamarense*. *Journal of Experimental Marine Ecology and Biology* 293, 41-45.
- Jin, Q.; Dong, S. & Wang, C. (2005) Allelopathic growth inhibition of *Prorocentrum micans* (Dinophyta) by *Ulva pertusa* and *Ulva linza* (Chlorophyta) in laboratory cultures. *European Journal of Phycology* 40, 31-37.
- Jones, A. C.; Gu, L.; Sorrels, C. M.; Sherman, D. H. & Gerwick, W. H. (2009) New tricks from ancient algae: natural products biosynthesis in marine cyanobacteria. *Current Opinions in Chemical Biology* 13, 216-223.
- Kakisawa, H.; Asari, F.; Kusumi, T.; Toma, T.; Sakurai, T.; Oohusa, T.; Hara, Y. & Chiharai, M. (1988) An allelopathic fatty acid from the brown alga *Cladosiphon okamuranus*. *Phytochemistry* 27, 731-735.
- Kearns, K. D. & Hunter, M. D. (2000) Green algal extracellular products regulate antialgal toxin production in a cyanobacterium. *Environmental Microbiology* 2, 291-297.
- Kearns, K. D. & Hunter, M. D. (2001) Toxin-producing *Anabaena flos-aquae* induces settling of *Chlamydomonas reinhardtii*, a competing motile alga. *Microbiol Ecology* 42, 80-86.
- Keating, K. L. (1977) Allelopathic influences on blue-green sequence in a eutrophic lake. *Science* 196, 885-887.
- Keating, K. L. (1978) Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. *Science* 199, 971-973.
- Kim, M.-J.; Choi, J.-S.; Kang, S.-E.; Cho, J.-Y.; Jin, H.-J., Byung-Soo, C. & Hong, Y.-K. (2004) Multiple allelopathic activity of the crustose coralline alga *Lithophyllum yessoense* against settlement and germination of seaweed spores. *Journal of Applied Phycology* 16, 175-179.
- Kishore, G. M. & Shah, D. M. (1988) Amino acid biosynthesis inhibitors as herbicides. *Annual Reviews in Biochemistry* 57, 627-663.
- Kodani, S.; Imoto, A.; Mitsutani, A. & Murakami, M. (2002) Isolation and identification of the antialgal compound, harmaline (1-methyl-b-carboline), produced by the algicidal bacterium, *Pseudomonas* sp. K44-1. *Journal of Applied Phycology* 14, 109-114.

- Kubanek, J. & Hicks, M. K. (2005) Does the red tide dinoflagellate *Karenia brevis* use allelopathy to outcompete other phytoplankton? *Limnology and Oceanography* 50, 883-895.
- Kurihara, H.; Tada, S.; Takahashi, K. & Hatano, M. (1996) Digalactosyldiacylglycerol suppression of inhibition by sulfoquinovosyldiacylglycerol of alpha-glucosidase. *Bioscience, Biotechnology and Biochemistry* 60, 932-933.
- Lane, A. L.; Stout, E. P.; Hay, M. E.; Prusak, A. C.; Hardcastle, K.; Fairchild, C. R.; Franzblau, S. G.; Le Roch, K.; Prudhomme, J.; Aalbersberg, W. & Kubanek, J. (2007) Callophycoic acids and callophycols from the Fijian red alga *Callophycus serratus*. *Journal of Organic Chemistry* 72, 7343-7351.
- Lascève, E. & Gaugain, F. (1990) Effects of usnic acid on sunflower and maize plantlets. *Journal of Plant Physiology* 136, 723-727.
- Leão, P. N.; Vasconcelos, M. T. & Vasconcelos, V. M. (2009) Allelopathy in freshwater cyanobacteria. *Critical Reviews in Microbiology* 35, 271-282.
- Leão, P. N.; Pereira, A. R.; Liu, W.-T.; Ng, J.; Pevzner, P. A.; Dorrestein, P. C.; König, G. M.; Vasconcelos, V. M. & Gerwick, W. H. (2010) Synergistic allelochemicals from a freshwater cyanobacterium. *Proceedings of the National Academy of Sciences U. S. A.* 107, 11183-11188.
- Leblanc, S.; Pick, F. R. & Aranda-Rodriguez, R. (2005) Allelopathic effects of the toxic cyanobacterium *Microcystis aeruginosa* on duckweed, *Lemna gibba*. *Environmental Toxicology* 20, 67-73.
- Leflaive, J. & Ten-Hage, L. (2006) Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. *Freshwater Biology* 52, 199-214.
- Legrand, C.; Rengefors, K.; Fistarol, G. O. & Granéli, E. (2003) Allelopathy in phytoplankton - biochemical, ecological and evolutionary aspects. *Phycologia* 42, 406-419.
- Lindell, S. D.; Pattenden, L. C. & Shannon, J. (2009) Combinatorial chemistry in the agrosocieties. *Bioorganic & Medicinal Chemistry* 17, 4035-4046.
- Long, P. F.; Dunlap, W. C.; Battershill, C. N. & Jaspers, M. (2005) Shotgun cloning and heterologous expression of the patellamide gene cluster as a strategy to achieving sustained metabolite production. *Chembiochem* 6, 1760-1765.
- Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L. & Corbett, T. H. (2001) Symplostatin 3, a new dolastatin 10 analogue from the marine cyanobacterium *Symploca* sp. VP 452.
- Macías, F. A.; Galindo, J. L. G.; Garcia-Diaz, M. D. & Galindo, J. C. G. (2007) Allelopathic agents from aquatic ecosystems: potential biopesticides models. *Phytochemistry Reviews* 7, 155-178.
- Malmvärn, A.; Zebühr, Y.; Kautsky, L.; Bergman, A. & Asplund, L. (2008) Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-*p*-dioxins in red alga and cyanobacteria living in the Baltic Sea. *Chemosphere* 72, 910-916.
- Manickavelu, A.; Nadarajan, N.; Ganesh, S. K.; Ramalingam, R., Raguraman, S. & Gnanamalar, R. P. (2006) Organogenesis induction in rice callus by cyanobacterial extracellular product. *African Journal of Biotechnology* 5, 437-439.
- Mason, C. P.; Edwards, K. R.; Carlson, R. E., Pignatello, J.; Gleason, F. K. & Wood J. M. (1982) Isolation of a chlorine-containing antibiotic from the freshwater cyanobacterium, *Scytonema hofmanni*. *Science* 215, 400-402.

- McCracken, M. D.; Middaugh, R. E. & R. S. Middaugh (1980) A chemical characterization of an algal inhibitor obtained from *Chlamydomonas*. *Hydrobiologia* 70: 271-276.
- McElhiney, J.; Lawton, L. A. & Leifert, C. (2001) Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. *Toxicon* 39, 1411-1420.
- Meinke, D. W.; Cherry, J. M.; Dean, C.; Rounsley, S. D. & Koornneef, M. (1998) *Arabidopsis thaliana*: a model plant for genome analysis. *Science* 282, 662-682.
- Mitrovic, S. M.; Pflugmacher, S.; James, K. J. & Furey, A. (2004) Anatoxin-a elicits an increase in peroxidase and glutathione-S-transferase activity in aquatic plants. *Aquatic Toxicology* 10, 185-192.
- Mitrovic, S. M.; Allis, O.; Furey, A. & James, K. J. (2005) Bioaccumulation and harmful effects of microcystin-LR in the aquatic plants *Lemna minor* and *Wolffia arrhiza* and the filamentous alga *Chladophora fracta*. *Ecotoxicology and Environmental Safety* 61, 345-352.
- Mizushina, Y.; Kasai, N.; Iijima, H.; Sugawara, F.; Yoshida, H. & Sakaguchi, K. (2005) Sulfoquinovosylacylglycerol, a eukaryotic DNA polymerase inhibitor and anti-cancer agent. *Current Medicinal Chemistry and Anticancer Agents* 5, 613-625.
- Moffitt, M. C. & Neilan, B. A. (2004) Characterization of the nodularin synthetase gene cluster and proposed theory of evolution cyanobacterial hepatotoxins. *Applied and Environmental Microbiology* 70, 6353-6362.
- Molisch H. (1937) *Der Einflub einer Pflanze auf die andere Pflanze, Allelopathie*. Gustav Fischer, Jena, Germany.
- Moore, R. E.; Cheuk, C. & Patterson, G. M. L (1984) Hapalindoles: new alkaloids from the blue-green alga *Hapalosiphon fontinalis*. *Journal of the American Chemical Society* 106, 6456-6457.
- Moore, R. E. (1996) Cyclic peptides and depsipeptides from cyanobacteria: a review. *Journal of Industrial Microbiology* 16, 134-143.
- Nagatsu, A.; Kajitani, H. & Sakakibara, J. (1995) Muscoride A: a new oxazole peptide alkaloid from freshwater cyanobacterium *Nostoc muscorum*. *Tetrahedron Letters* 36, 4097-4100.
- Nan, C. R.; Zhang, H. Z. & Zhao, G. Q. (2004) Allelopathic interactions between the macroalga *Ulva pertusa* and eight microalgal species. *Journal of Sea Research* 52, 259-268.
- Nan, C.; Zhang, H.; Lin, S.; Zhao, G. & Liu, X. (2008) Allelopathic effects of *Ulva lactuca* on selected species of harmful bloom-forming microalgae in laboratory cultures. *Aquatic Botany* 89, 9-15.
- Ohta, K.; Mizushina, Y.; Hirata, N.; Takemura, M.; Sugawara, F.; Matsukage, A.; Yoshida, S. & Sakaguchi, K. (1998) Sulfoquinovosyldiacylglycerol, KM043, a new potent inhibitor of eukaryotic DNA polymerase and HIV-reverse transcriptase type 1 from a marine red alga, *Gigartina tenella*. *Chemical Pharmacology Bulletin* 46, 684-686.
- Öztürk, S.; Güvenc, S.; Arikan, N.; Yilmaz, Ö (1999) Effect of usnic acid on mitotic index in root tips of *Allium cepa*. *Lagascalia* 21, 47-52.
- Paul, V. J. & Fenical, W. (1984) Bioactive terpenoids from Caribbean marine algae of the genera *Penicillus* and *Udotea* (Chlorophyta). *Tetrahedron* 40, 2913-2918.
- Pflugmacher, S.; Wiegand, C.; Beattie, K. A.; Krause, E., Steinberg, C. E. W. & Codd, G. A. (2001) Uptake, effects, and metabolism of cyanobacterial toxins in the emergent reed plant *Phragmites Australia*. *Environmental Toxicology and Chemistry* 20, 846-852.

- Pflugmacher, S. (2002) Possible allelopathic effects of cyanotoxins, with reference to microcystin-LR, in aquatic ecosystems. *Environmental Toxicology* 17, 407-413.
- Pflugmacher, S.; Aulhorn, M. & Grimm, B. (2007) Influence of a cyanobacterial crude extract containing microcystin-LR on the physiology and antioxidative defence systems of different spinach variants. *New Phytologist* 175, 482-489.
- Potera, C. (2007) Florida Red Tide brews up drug lead for cystic fibrosis. *Science* 15, 1561-1562.
- Powles, S. B.; Preston, C.; Bryan, I. B. & Jutsum, A. R. (1996) Herbicide resistance: impact and management. *Advances in Agronomy* 58, 57-93.
- Powell, H. A.; Kerby, N. W. & Rowell, P. (2006) Natural tolerance of cyanobacteria to the herbicide glyphosate. *New Phytologist* 119, 421-426.
- Raveh, A.; Moshe, S.; Evron, Z.; Flescher, E. & Carmeli, S. (2010) Novel thiazole and oxazole containing cyclic hexapeptides from a waterbloom of the cyanobacterium *Microcystis* sp. *Tetrahedron* 66, 2705-2712.
- Rippka, R.; Deruelles, J.; Waterbury, J. B.; Herdman, M. & Stanier, R. Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111, 1-61.
- Rodriguez, A. A.; Stella, A. M.; Storni, M. M.; Zulpa, G. & Zaccaro, M. C. (2006) Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa*. *Saline Systems* 2, 7-11.
- Saqrane, S.; El Ghazali, I.; Ouahid, Y.; El Hassni, M.; El Hadrami, I.; Bourarab, L.; del Campo, F. F.; Oudra, B. & Vasconcelos, V. (2007) Phytotoxic effects of cyanobacteria extract on the aquatic plant *Lemna gibba*: microcystin accumulation, detoxification and oxidative stress induction. *Aquatic Toxicology* 83, 284-294.
- Saqrane, S.; El Ghazali, I.; Oudra, B.; Bouarab, L. & Vasconcelos, V. (2008) Effects of cyanobacteria producing microcystins on seed germination and seedling growth of several agricultural plants. *Journal of Environmental Science and Health B* 43, 443-451.
- Schmidt, E. W.; Nelson, J. T.; Rasko, D. A.; Sudek, S.; Elsen, J. A.; Haygood, M. G. & Ravel, J. (2005) Patellamide A and C biosynthesis by a microcin-like pathway in *Prochloron didemni*, the cyanobacterial symbiont of *Lissoclinum patella*. *Proceedings of the National Academy of Sciences U.S.A.* 102, 7315-7320.
- Sanevas, N.; Sunohara, Y. & Matsumoto, H. (2006) Crude extract of the cyanobacterium, *Hapalosiphon* sp., causes a cessation of root elongation and cell division in several species. *Weed Biology and Management* 6, 25-29.
- Sergeeva, E.; Liaimer, A. & Bergman, B. (2002) Evidence for production of phytohormone indol-3-acetic acid by cyanobacteria. *Plant* 215, 229-238.
- Schlegel, I.; Doan, N. T.; de Chazal, N. & Smith, G. D. (1999) Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *Journal of Applied Phycology* 10, 471-479.
- Smith, C. D.; Zhang, Z.; Mooberry, S. L.; Patterson, G. M. L. & Moore, R. E. (1994) Cryptophycin: a new microtubule agent active against drug-resistant cells. *Cancer Research* 54, 3779-3784.
- Smith, G. D. & Doan, N. T. (1999) Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. *Journal of Applied Phycology* 11, 337-344.
- Snyder, R. V.; Gibbs, P. D.; Palacios, A.; Aply, L.; Dickey, R.; Lopez, J. V. & Rein, K. S. (2003) Polyketide synthase genes from marine dinoflagellates. *Marine Biotechnology* 5, 1-12.

- Srivastava, A.; Jüttner, F. & Strasser, R. J. (1998) Action of the allelochemical, fischerellin A, on photosystem II. *Biochimica et Biophysica Acta* 1364, 326-336.
- Stirk, W.A.; Ördög, V. & Van Staden, J. (1999) Identification of the cytokinin isopentenyladenin in a strain of *Arthronema africanum*. *Journal of Phycology*, 35 89-92.
- Stirk, W. A.; Ördög, V.; Van Staden, J. and Jäger, K. (2002) Cytokinin- and auxin-like activity in cyanophyta and microalgae. *Journal of Applied Phycology* 14, 215-221.
- Stirk, W. A.; Novak, O.; Strnad, M. & van Staden, J. (2003) Cytokinins in macroalgae. *Plant Growth Regulation* 41, 13-24.
- Sugg, L. M. & Van Dolah, F. M. (1999) No evidence for allelopathic roles of okadaic acid among ciguatera-associated dinoflagellates. *Journal of Phycology* 35, 93-103.
- Sun, H. H.; Ferrara, N. M.; McConnell, O. J. & Fenical, W. (1980) Bifurcarenone, an inhibitor of mitotic cell division from the brown alga. *Tetrahedron Letters* 3123-3126.
- Tan, L. T. (2007) Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochemistry* 68, 954-979.
- Trimurtulu, G.; Ohtani, I.; Patterson, G. M. L.; Moore, R. E.; Corbett, T. H.; Valeriote, F. A. & Demchik, L. (1994) Total structures of cryptophycins, potent antitumor depsipeptides from the blue-green alga *Nostoc* sp. strain GSV 224. *Journal of the American Chemical Society* 116, 4729-4737.
- Tsavkelova, E. A.; Klimova, S. Y.; Cherdyntseva, T. A. & Netrusov, A. I. (2006) Microbial producers of plant growth stimulators and their practical use: a review. *Applied Biochemistry and Microbiology* 42, 117-126.
- Upadhyaya, M. K. & Noodén, L. D. (1977) Uptake and binding of [¹⁴C]oryzalin in corn root segments. *Plant Physiol.* 59, 5-46.
- Upadhyaya, M. K. & Noodén, L. D. (1980) Mode of dinitroaniline herbicide action. *Plant Physiology* 66, 1048-1052.
- Uchida, A.; Shimada, A.; Ishida, Y. (1988) Antibacterial and antialgal substance produce by the dinoflagellate *Peridinium bipes*. *Nippon Suisan Gakkaishi* 54, 1941-1945.
- Van Dolah, F. M. (2000) Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental Health Perspectives* 108, 133-141.
- Vepritskii, A. A.; Gromov, B. V.; Titota, N. N. & Mamkaeva, K. A. Production of the antibiotic-algicide cyanobacterin LU-2 by a filamentous cyanobacterium *Nostoc* sp. *Mikrobiologiya* 60, 21-25.
- Verdier-Pinard, P.; Lai, J.-Y.; Yoo, H.-D.; Yu, J.; Marquez, B.; Nagle, D. G.; Nambu, M.; White, J. D.; Falck, J. R.; Gerwick, W. H.; Day, B. W. & Hamel, E. (1998) Structure-activity analysis of the interaction of curacin A, the potent colchicines site antimitotic agent, with tubulin and effects of analogs on the growth of MCF-7 breast cancer cells. *Molecular Pharmacology* 53, 62-76.
- Volk, R. B. (2005) Screening of microalgal culture media for the presence of algicidal compounds and isolation and identification of two bioactive metabolites, excreted by the cyanobacteria *Nostoc insulare* and *Nodularia harveyana*, respectively. *Journal of Applied Phycology* 17, 339-347.
- Volk, R. B. (2008) Screening of microalgae for species excreting norharmane, a manifold biologically active indole alkaloid. *Microbiology Research* 163, 307-313.
- Wang, R.; Xiao, H.; Zhang, P.; Qu, L.; Cai, H. & Tang, X. (2007) Allelopathic effects of *Ulva pertusa*, *Corallina pilulifera* and *Sargassum thunbergii* on the growth of the dinoflagellates *Heterosigma akashiwo* and *Alexandrium tamarense*. *Journal of Applied Phycology* 19, 109-121.

- Wang, Y.; Bin, Z. & Xuexi, T. (2009) Effects of two species of macroalgae - *Ulva pertusa* and *Gracilaria lemaneiformis* - on growth of *Heterosigma akashiwo* (Raphidophyceae). *Journal of Applied Phycology* 21, 375-385.
- Weiss, J.; Libert, H.-P. & Braune, W. (2000) Influence of microcystin-RR on growth and photosynthetic capacity of the duckweed *Lemna minor*. *Journal of Applied Botany* 74, 100-105.
- Welker, M. & von Döhren, H. (2006) Cyanobacterial peptides - nature's own combinatorial biosynthesis. *FEMS Microbiology Reviews* 30, 530-563.
- Yamada, N.; Murakami, N.; Morimoto, T. & Sakakibara, J. (1993) Autogrowth inhibitory substances from the freshwater cyanobacterium *Phormidium tenue*. *Chemical Pharmaceutical Bulletin* 41, 1863-1865.
- Yi, D.; Yijun, Z.; Xue, B.; Zhihui, F. & Kai, C. (2009) Cytotoxic effects of cyanobacteria extract on *Lemna minor* and *Myriophyllum spicatum* phyto-tolerance and superoxide dismutase activity. *Environmental Toxicology* 24, 304-308.
- Zaccaro, M. C.; Kato, A.; Zulpa, G.; Storni, M. M.; Steyerthal, N.; Lobasso, K & Stella, A. M. (2006) Bioactivity of *Scytonema hofmanni* (Cyanobacteria) in *Lillium alexandrae* *in vitro* propagation. *Electronic Journal of Biotechnology* 9, 211-214.
- Zhang, T. T.; He, M.; Wu, A. P. & Nie, L. W. (2009) Allelopathic effects of submerged macrophyte *Chara vulgaris* on toxic *Microcystis aeruginosa*. *Allelopathy Journal* 23.
- Zulpa de Caire, G.; Zaccaro De Mulé, M. C.; Storni De Cano, M. (1979) Productos extracelulares de *Nostoc muscorum* obtenidos en medios con y sin nitrógenocombinado. I: Sus efectos sobre plantulas de arroz. *Phyton* 37, 1-13.

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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