Bull Environ Contam Toxicol (2011) 87:324–329
DOI 10.1007/s00128-011-0347-3

Effects of Carbaryl and 1-Naphthol on Soil Population of Cyanobacteria and Microalgae and Select Cultures of Diazotrophic Cyanobacteria

Mallavarapu Megharaj · Kadiyala Venkateswarlu · Ravi Naidu

Received: 19 January 2011 / Accepted: 20 June 2011 / Published online: 26 June 2011

Abstract Carbaryl application to soil collected from a rice fallow field was relatively less toxic to viable estimates of cyanobacteria and microalgae under nonflooded conditions than under flooded conditions. Application of 1-naphthol, the hydrolysis product of carbaryl, to soil under both the regimes increased the population of both cyanobacteria and microalgae. Soil application of carbaryl and 1-naphthol in combination, up to 1.0 kg ha^{-1}, was nontoxic to the viable population. The toxicity exerted by carbaryl and 1-naphthol towards growth, measured in terms of chlorophyll *a*, and nitrogenase activity was more pronounced in *Anabaena* spp. than in *Nostoc* spp.

Keywords Cyanobacteria · Microalgae · Carbaryl · 1-Naphthol · Nontarget effects

Carbaryl (1-naphthyl *N*-methylcarabamate), an agricultural and garden insecticide, is used worldwide on more than 120 different food and fibre crops, trees, and ornamentals (Ware 2000), mostly as a substitute for some persistent organochlorine insecticides (Ribera et al. 2001). Very low mammalian oral and dermal toxicity together with short half-life in the environment and exceptionally broad spectrum of insect control made carbaryl the most popular carbamate effective against 160 harmful insect pests (Ware 2001). Sevin®, the most common commercial formulation of carbaryl, is used extensively against many major agricultural pests at rates ranging from 0.25 to 2.7 kg active ingredient per hectare (Rajagopal et al. 1984). In view of its extensive use (4.5–6.8 million kg/year) in the United States alone (Cox 1993) as a broad spectrum contact and ingestion insecticide with some systemic properties, carbaryl is the second most widely detected insecticide in surface waters (Martin et al. 2003). 1-Naphthol is the major product of carbaryl degradation following hydrolysis in soils (Venkateswarlu et al. 1980; Rajagopal et al. 1983; Gunasekara et al. 2008) and in pure cultures of microorganisms (Rajagopal et al. 1984). The wide use of carbaryl has increased the concern about possible pollution of water supplies and soils (Marco et al. 1993).

Cyanobacteria and microalgae occur virtually in every terrestrial habitat on earth (Rindi 2007). The ecological position of cyanobacteria and microalgae at the base of food webs together with their essential roles in nutrient cycling and oxygen production is critical to many ecosystems (Sabater and Carrasco 2001). Diazotrophic cyanobacteria that can fix atmospheric nitrogen have important implications for other biota (An and Kampbell 2003). Microalgae have been considered indicators of the bioactivity of environmental pollutants as they are comparatively sensitive to many chemicals and vary in their response to a variety of toxicants (Real et al. 2003). Alterations of the species composition of soil communities of cyanobacteria and microalgae as a result of toxic stress may affect the structure and functioning of the whole ecosystem (Campanella et al. 2000; Wong 2000; Verdisson

M. Megharaj · R. Naidu
Centre for Environmental Risk Assessment and Remediation, University of South Australia, Adelaide, SA 5095, Australia

M. Megharaj · R. Naidu
Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, University of South Australia, Adelaide, SA 5095, Australia

Present Address:
K. Venkateswarlu (✉)
Department of Microbiology, Sri Krishnadevaraya University, Anantapur 515055, India
e-mail: v_kadiyala@hotmail.com

et al. 2001). Although much information on the toxicological aspects of insecticides on microalgae has been compiled, very limited studies were conducted to understand the nontarget effects of insecticides on cyanobacteria (Abou-Waly et al. 1991; Venkateswarlu 1993; Sabater and Carrasco 2001). It has been realized that toxicological studies must involve a wide array of species with different sensitivities while assessing the effects of environmental pollutants on cyanobacterial and microalgal communities (Sanchez and Tarazona 2002). A few studies reported the nontarget effects of carbaryl and/or 1-naphthol on soil isolates of cyanobacteria and microalgae; however, virtually no information is available on the impact of carbaryl and its hydrolysis product, 1-naphthol, towards native populations of cyanobcateria and microalgae in soil (Ramakrishnan et al. 2010, 2011). The present study therefore examined the effects of carbaryl and 1-naphthol alone and in combination toward soil populations of cyanobacteria and microalgae, and compared the differential sensitivity of select soil isolates of diazotrophic cyanobacteria to these two chemical pollutants.

Materials and Methods

The soil used in this study was collected from a rice fallow field in Guntur district, India up to a depth of 6 cm, air-dried and passed through a 2-mm mesh sieve. Measured characteristics of the soil include organic matter, 1.5%; total N, 0.29%; cation exchange capacity, 18.2 meq 100 g^{-1} soil; and pH, 7.3 (1:1.25 soil–water slurry). Technical grade carbaryl (99% pure) and analytical grade 1-naphthol were obtained from Rhone-Poulenc Ltd. (Dagenham, England) and Sigma Chemical Co. (St. Louis, Missouri, USA), respectively.

To study the effects of carbaryl and 1-naphthol, alone and in combination, on the native populations of cyanobacteria and microalgae, soil samples contained in 15-mm × 150-mm test tubes were treated with aqueous solutions of these chemicals, at different levels of 5–50 mg kg^{-1} soil (equivalent to 0.5–5.0 kg ha^{-1}). Untreated soil samples served as controls. A set of tubes was maintained at 60% water-holding capacity to provide nonflooded conditions while another set was flooded with distilled water to provide 1:1.25 soil to water ratio. The tubes were incubated at 37°C. After 20 days of incubation, duplicate soil samples, maintained at both the water regimes, were withdrawn for estimation of total population (both cyanobacteria and microalgae) by enrichment culture technique using Bold's basal medium (BBM) followed by the 5-tube (dilution) most-probable number (MPN) method as described earlier (Muralikrishna and Venkateswarlu 1984). Fiducial limits for the MPN values of viable cells at the 95% confidence level were calculated according to Fisher and Yates (1963). Most of the predominant microalgae and cyanobacteria were identified at least to genus, and the frequency of their occurrence was also recorded.

Axenic cultures of *Anabaena cylindrica*, *Anabaena* sp., *Nostoc punctiforme* and *Nostoc* sp., all isolated from the experimental soil, were developed by repeated culturing on BBM-agar medium, and maintained under continuous illumination at a temperature of 26 ± 2°C (Megharaj et al. 1993a, 2000). Toxicity of carbaryl and 1-naphthol towards the growth of four species of cyanobacteria isolated from the soil incubation studies was determined according to Megharaj et al. (1993b). Stock solutions of technical grade carbaryl and 1-naphthol were prepared in acetone. Aliquots from stock solutions of carbaryl and 1-naphthol were dispensed, alone or in combination, into sterilized 100-mL Erlenmeyer flasks to provide the desired concentrations as described earlier (Megharaj et al. 1990). After complete evaporation of the carrier solvent, 25-mL portions of N-free Allen's medium were added to the flasks and equilibrated for 1 day. The medium in untreated controls and treatments were inoculated with 1.0-mL culture of homogeneous suspensions from 1-week-old cyanobacterial test cultures. At the end of 10-days incubation period, growth of the cyanobacteria was determined in terms of chlorophyll *a* (Megharaj et al. 1993a). Activity of dinitrogenase was measured following acetylene reduction assay (Stewart et al. 1971) as described elsewhere (Megharaj et al. 1993b).

The data for chlorophyll *a* and dinitrogenase activity were subjected to analysis of variance, and the means were compared by Duncan's new multiple range test at $p \leq 0.05$ (Megharaj et al. 1991).

Results and Discussion

The results presented in Table 1 indicate the viable count estimates, in terms of MPN, of cyanobacteria and microalgae in nonflooded and flooded soil under the influence of carbaryl and 1-naphthol alone or in combination after 20 days of exposure. Carbaryl at 0.5 kg ha^{-1} under nonflooded conditions significantly increased the soil populations of cyanobacteria and microalgae; 1.0 kg ha^{-1} concentration was innocuous while the two other higher concentrations used exerted pronounced toxicity. In flooded soil, application of carbaryl at the lowest concentration of 0.5 kg ha^{-1} was not toxic, but all other concentrations were significantly toxic to the native populations. This observation could be expected because carbaryl disappears quickly with an approximate half-life of 4 days in aerobic soil, and carbaryl degrades more slowly in anaerobic soil with an estimated half-life of 72 days (Xu 1998).

Table 1 Effect of carbaryl and 1-naphthol, alone and in combination, on population (MPN $\times$ 10^3 g^{-1} soil) of microalgae and cyanobacteria in soil under nonflooded or flooded conditions

Treatment (mg kg^{-1})	Nonflooded	Flooded
Untreated	25.3 (46.4, 13.8)[a]	29.6 (54.4, 16.1)
Carbaryl		
5	34.9 (64.1, 19.0)	21.6 (39.6, 11.7)
10	21.6 (39.6, 11.7)	15.9 (29.2, 8.7)
20	13.6 (25.1, 7.4)	13.6 (25.1, 7.4)
50	8.7 (15.9, 4.7)	10.2 (18.6, 5.5)
1-Naphthol		
5	34.9 (64.1, 19.0)	44.4 (76.1, 22.6)
10	49.5 (90.9, 26.9)	59.7 (109.6, 32.5)
20	49.5 (90.9, 26.9)	59.7 (109.6, 32.5)
50	21.6 (39.6, 11.7)	49.5 (90.9, 26.9)
Carbaryl + 1-naphthol		
2.5 + 2.5	25.3 (46.4, 13.8)	25.3 (46.4, 13.8)
5 + 5	25.3 (46.4, 13.8)	21.6 (39.6, 11.7)
10 + 10	29.6 (54.4, 16.1)	18.5 (33.9, 10.1)
20 + 20	11.7 (21.6, 6.4)	13.6 (25.1, 7.4)
50 + 50	4.2 (6.9, <4.1)	4.6 (8.4, <4.1)

[a] Numbers in parentheses are the upper and lower fiducial limits (at 95% confidence levels), respectively, of each MPN value (means of duplicates)

1-Naphthol, on the other hand, greatly enhanced cyanobacteria and microalgae under flooded soil conditions at all the concentrations tested. Furthermore, treatment of soil with 1-naphthol at levels from 0.5 to 2.0 kg ha^{-1} under nonflooded conditions was stimulatory for the total viable populations, and even the highest concentration of 5.0 kg ha^{-1} was nontoxic. Interestingly, the application of carbaryl and 1-naphthol in combination each at 0.25–1.0 kg ha^{-1} had no toxic effect on total populations of cyanobacteria and microalgae in nonflooded soils; however, the two higher concentrations exerted significant toxicity after 20 days. The combination of both chemicals at the lower levels of 0.25 and 0.5 kg ha^{-1} did not cause any measurable numerical change in the viable population of cyanobacteria and microalgae in flooded soil, while the MPN estimates were significantly reduced at higher concentrations.

The above results clearly indicate that the native soil population of cyanobacteria and microalgae respond differentially to the treatments of carbaryl and its hydrolysis product, 1-naphthol. Thus, the soil application of carbaryl under both water regimes was relatively toxic to the indigenous cyanobacteria and microalgae, while 1-naphthol significantly stimulated these phototrophs under nonflooded or flooded soil conditions. Moreover, simultaneous presence of 1-naphthol with carbaryl either stimulated the soil population or alleviated the toxicity of the parent compound. In contrast, 1-naphthol was shown to be more toxic to molluscs and to some marine fish than carbaryl (Murty 1986).

Of the 14 predominant microbial species that appeared in the MPN tubes, five were microalgae and nine were cyanobacteria (Table 2). In general, filamentous cyanobacteria were abundant from soil samples that received 1-naphthol alone or in combination with carbaryl. The cyanobacteria which were consistent in their occurrence include *Anabaena* sp., *Lyngbya* sp., *Nostoc punctiforme*

Table 2 Occurrence of cyanobacteria and microalgae at the end of 20 days as influenced by carbaryl and 1-naphthol in flooded soil conditions

Cyanobacterium/microalga	Untreated soil	Carbaryl (mg kg^{-1})		1-Naphthol (mg kg^{-1})		Carbaryl + 1-Naphthol (mg kg^{-1})	
		20	50	20	50	20	50
Chlamydomonas sp.	++	+	−	+	−	+	−
Chlorella sp.	+	+	+	−	+	−	−
Chlorococcum sp.	+	+++	−	+	+	+	+
Gloeocystis sp.	++	++	−	+	+	++	+
Scenedesmus sp.	+	+	−	+	−	+	−
Anabaena cylindrica	+	+	−	++	+	+	−
Anabaena sp.	+	+	−	++	+	+	+
Lyngbya sp.	+	+	+	++	+	+	+
Nostoc muscorum	+	+	−	+	+	++	−
N. punctiforme	+	+	+	++	+	+	+++
Nostoc sp.	+	−	−	++	+	+	−
Nostoc sp.	+	+	+	+	−	+	+
Phormidium sp.	−	−	−	++	+	+	+++
Synechocystis sp.	+	−	−	+	−	+	−

− Absent, + common, ++ abundant, +++ most abundant

Table 3 Effect of carbaryl and 1-naphthol on chlorophyll *a* ($\mu g\ mL^{-1}$ culture) in cyanobacteria grown under autotrophic conditions

Treatment ($\mu g\ mL^{-1}$ medium)	*Anabaena cylindrica*	*Anabaena* sp.	*Nostoc punctiforme*	*Nostoc* sp.
Untreated	2.19[a]	2.43[a]	2.31[b]	2.68[a]
Carbaryl				
5	0.96[b] (43.8)[1]	1.57[b] (64.6)	2.94[a] (127.2)	1.93[b] (72.1)
10	0.58[c] (26.7)	0.87[c] (35.8)	1.43[c] (61.7)	1.21[c] (45.2)
20	0.44[c] (20.2)	0.45[d] (18.4)	0.24[d] (10.2)	0.32[e] (12.1)
50	0[d]	0[e]	0[e]	0[f]
1-Naphthol				
5	0.99[b] (45.5)	1.53[b] (62.9)	2.90[a] (125.7)	2.36[b] (88.2)
10	0.49[c] (22.7)	0.50[d] (20.6)	1.45[c] (62.6)	1.42[c] (52.9)
20	0.35[c] (16.1)	0.41[d] (16.7)	0.24[d] (51.1)	0.91[d] (33.8)
50	0_{d}	0[e]	0[e]	0[f]

[1] Numbers in parentheses are percentages relative to untreated controls

Means (n = 4) in each column followed by the same superscript letter are not significantly different from each other ($p \leq 0.05$) according to Duncan's multiple range test

and *Phormidium* sp. A similar trend in the occurrence of cyanobacteria and microalgae was noticed under nonflooded soil conditions (results not shown).

The autotrophic growth response of the selected cultures of diazotrophic cyanobacteria (*Anabaena* sp., *A. cylindrica*, *Nostoc* sp. and *N. punctiforme*) under the influence of carbaryl or 1-naphthol treatment was examined by measuring chlorophyll *a* content. Excepting *N. punctiforme*, all the other isolates were significantly inhibited by both carbaryl and 1-naphthol even with the treatment of 5 $\mu g\ mL^{-1}$ (Table 3). Such a differential toxicity toward cyanobacteria (*N. linckia* and *N. muscorum*) was observed earlier with carbofuran, another methylcarbamate (Megharaj et al. 1993a). In particular, the next higher concentration of 10 $\mu g\ mL^{-1}$ of 1-naphthol also effected an increase in chlorophyll *a* in *N. punctiforme*. The highest concentration of 50 $\mu g\ mL^{-1}$ used in the present study was detrimental to all the cultures. These results indicate that carbaryl even below the field application doses is toxic to the growth of cyanobacteria. Obulakondaiah et al. (1993) observed alteration in growth and metabolic activities of a cyanobacterium, *Anabaena torulosa,* under the influence of carbaryl, at field application rates, alone or in combination with its hydrolysis product, 1-naphthol. In an earlier study, photosynthesis and in vivo nitrate reductase activity, and ultrastructure of another diazotrophic cyanobacterium, *Nostoc linckia*, isolated from soil, were also affected by carbaryl or its hydrolysis product, 1-naphthol, at concentrations ranging from 2 to 20 $\mu g\ mL^{-1}$ (Megharaj et al. 1993b). Yet in another study, carbaryl and 1-naphthol at concentrations from 5 to 50 $\mu g\ mL^{-1}$ interacted either additively by increasing the overall toxicity towards *Chlorella vulgaris*, *Synechococcus elongatus* and *N. linckia* or synergistically by enhancing the toxicity of both the compounds (Megharaj et al. 1990). Hammouda (1999), on the other hand, demonstrated the utilization of carbofuran at low concentrations by a nitrogen-fixing cyanobacterium, *Anabaena doliolum*. It was also observed that growth and chlorophyll *a* were stimulated at a concentration range of 1–10 $\mu g\ mL^{-1}$ carbofuran, but higher concentrations and subsequent accumulation of hydrolysis products were significantly toxic to the cyanobacterium.

Nitrogenase activity, measured in terms of ethylene formed from acetylene, in the test cultures of cyanobacteria also responded in a manner similar to that for chlorophyll *a* in the carbaryl treatment (Table 4). Concentrations ranging from 5 to 20 $\mu g\ mL^{-1}$ of carbaryl significantly affected nitrogen-fixing ability in all four cyanobacteria. This observation conforms to our earlier report of significant toxicity of carbaryl to nitrogenase activity in *N. linckia* (Megharaj et al. 1993b). However, in the present study, nitrogenase activity was greatly affected in *Anabaena* spp. by 1-naphthol at the lowest concentration of 5 $\mu g\ mL^{-1}$ while activity of the enzyme was significantly stimulated in the two species of *Nostoc*. The inhibition of nitrogenase activity at 10 and 20 $\mu g\ mL^{-1}$ naphthol was also greater with *Anabaena* spp. than with *Nostoc* spp. The present observation of less sensitivity with *Nostoc* compared to *Anabaena* toward carbaryl or 1-naphthol thus supports our earlier report that *Nostoc linckia* secreted mucous around the filaments as a protective layer when treated with nitrophenols (Megharaj et al. 1991).

Our data indicate that carbaryl even at field application rate may pose a risk to diazotrophic cyanobacteria, and consequently to the nitrogen economy of soils. It is currently understood from the extensive studies conducted so far on the impacts of many pollutants on cyanobacteria and microalgae that an evaluation with a wide taxonomic range

Table 4 Effect of carbaryl and 1-naphthol on nitrogenase activity (nmol ethylene mg chlorophyll a^{-1} produced from acetylene after 24 h of incubation) in cyanobacteria of cyanobacteria grown under autotrophic conditions

Treatment (μg mL^{-1} medium)	*Anabaena cylindrica*	*Anabaena* sp.	*Nostoc punctiforme*	*Nostoc* sp.
Untreated	24.5^{a}	19.8^{a}	24.1^{b}	22.7^{b}
Carbaryl				
5	6.2^{c} (25.4)[1]	6.4^{b} (9.9)	6.7^{d} (27.8)	16.9^{c} (74.3)
10	2.2^{d} (8.7)	1.4^{c} (6.9)	6.5^{d} (27.0)	6.7^{d} (29.5)
20	0.9^{d} (3.7)	0.3^{d} (1.5)	3.6^{e} (14.9)	4.6^{d} (20.3)
50	0^{e}	0^{e}	0^{f}	0^{e}
1-Naphthol				
5	9.7^{b} (39.4)	6.4^{b} (32.5)	32.1^{a} (133.3)	25.9^{a} (114.3)
10	5.4^{c} (22.1)	1.9^{c} (8.0)	15.8^{c} (65.7)	14.3^{c} (62.9)
20	0.8^{d} (3.1)	0.5^{d} (2.5)	7.5^{d} (30.9)	4.1^{d} (18.0)
50	0^{e}	0^{e}	0^{f}	0^{e}

[1] Numbers in parentheses are percentages relative to untreated controls

Means (n = 4) in each column followed by the same superscript letter are not significantly different from each other ($p \leq 0.05$) according to Duncan's multiple range test

in different ecosystems is necessary to arrive at a generalization on the nontarget effects of a pollutant (Ramakrishnan et al. 2010). In view of the observed differential sensitivity exhibited by the two genera of cyanobacteria toward the test chemicals, the present study further emphasizes the need to include large number of nontarget microorganisms to assess the toxic influences of environmental pollutants.

Acknowledgments KV thankfully acknowledges the Government of Australia (Department of Education, Employment and Workplace Relations) for the Endeavour Executive Award.

References

Abou-Waly H, Abou-Setta MM, Nigg HN, Mallory LL (1991) Growth response of freshwater algae, *Anabaena flos-aquae* and *Selenastrum capricornutum* to atrazine and hexazinone herbicides. Bull Environ Contam Toxicol 46:223–229

An Y-J, Kampbell DH (2003) Monitoring chlorophyll-*a* as a measure of algae in lake texoma marins. Bull Environ Contam Toxicol 70:606–611

Campanella L, Cubadda F, Sammartino MP, Saoncella A (2000) An algal biosensor for the monitoring of water toxicity in estuarine environments. Water Res 25:69–76

Cox C (1993) The problems with Sevin (carbaryl). J Pestic Reform 13:31–36

Fisher RA, Yates F (1963) Statistical tables for biological, agricultural and medical research, 6th edn. Oliver & Boyd, Edinburgh, p 146

Gunasekara AS, Rubin AL, Goh KS, Spurlock FC, Tjeerdema RS (2008) Environmental fate and toxicology of carbaryl. Rev Environ Contam Toxicol 196:95–121

Hammouda O (1999) Response of the paddy field cyanobacterium *Anabaena doliolum* to carbofuran. Ecotoxicol Environ Saf 44:215–219

Marco M-P, Gee SJ, Cheng HM, Liang ZY, Hammock BD (1993) Development of an enzyme-linked immunosorbent assay for carbaryl. J Agric Food Chem 41:423–430

Martin JD, Crawford CG, Larson SJ (2003) Pesticides in streams: preliminary results from cycle I of the National Water Quality Assessment Program (NAWQA), 1992–2001

Megharaj M, Prabhakara Rao A, Rao AS, Venkateswarlu K (1990) Interaction effects of carbaryl and its hydrolysis product, 1-naphthol, towards three isolates of microalgae from a rice soil. Agric Ecosyst Environ 31:293–300

Megharaj M, Pearson HW, Venkateswarlu K (1991) Toxicity of phenol and nitrophenols towards growth and metabolic activities of *Nostoc linckia*, isolated from soil. Arch Environ Contam Toxicol 21:578–584

Megharaj M, Pearson HW, Venkateswarlu K (1993a) Toxicity of carbofuran to soil isolates of *Chlorella vulgaris*, *Nostoc linckia* and *N. muscorum*. Appl Microbiol Biotechnol 39:644–648

Megharaj M, Pearson HW, Venkateswarlu K (1993b) Physiological and morphological alterations induced by combinations of carbaryl and 1-naphthol in *Nostoc linckia* isolated from soil. Curr Microbiol 27:41–45

Megharaj M, Singleton I, McClure NC, Naidu R (2000) Influence of petroleum hydrocarbon contamination on microalgae and microbial activities in a long-term contaminated soil. Arch Environ Contam Toxicol 38:439–445

Muralikrishna PVG, Venkateswarlu K (1984) Effect of insecticides on soil algal population. Bull Environ Contam Toxicol 33:241–245

Murty AS (1986) Toxicity of pesticides to fish. CRC Press, Boca Raton, pp 27–33

Obulakondaiah M, Sreenivasulu C, Venkateswarlu K (1993) Nontarget effects of carbaryl and its hydrolysis product, 1-naphthol, towards *Anabaena torulosa*. Biochem Mol Biol Int 29:703–710

Rajagopal BS, Chendrayan K, Reddy BR, Sethunathan N (1983) Persistence of carbaryl in flooded soils and its degradation by soil enrichment cultures. Plant Soil 73:35–45

Rajagopal BS, Brahmaprakash GP, Reddy BR, Singh UD, Sethunathan N (1984) Effect and persistence of selected carbamate pesticides in soils. Residue Rev 93:87–203

Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R, Sethunathan N (2010) The impacts of environmental pollutants on microalgae and cyanobacteria. Crit Rev Environ Sci Technol 40:699–821

Ramakrishnan B, Megharaj M, Venkateswarlu K, Sethunathan N, Naidu R (2011) Mixtures of environmental pollutants: effects on microorganisms and their activities. Rev Environ Contam Toxicol 211:63–120

Real M, Muñoz I, Guasch H, Navarro E, Sabater S (2003) The effect of copper exposure on a simple aquatic food chain. Aquat Toxicol 63:283–291

Ribera D, Narbonne JF, Arnaud C, Denis MS (2001) Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil: effects of carbaryl. Soil Biol Biochem 33:1123–1130

Rindi F (2007) Diversity, distribution and ecology of green algae and cyanobacteria in urban habitats. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, New York, p 619

Sabater C, Carrasco JM (2001) Effects of pyridaphenthion on growth of five freshwater species of phytoplankton. A laboratory study. Chemosphere 44:1775–1781

Sanchez P, Tarazona JV (2002) Development of a multispecies system for testing reproductive effects on aquatic invertebrates. Experience with *Daphnia magna*, *Chironmus prasinus* and *Lymnaea peregra*. Aquat Toxicol 60:249–256

Stewart WDP, Mague T, Fitzerald GP, Burris RH (1971) Nitrogenase activity in Wisconsin lakes of different degrees of eutrophication. New Phytol 70:497–509

Venkateswarlu K (1993) Pesticide interactions with cyanobacteria in soil and culture. In: Bollag J-M, Stotzky G (eds) Soil biochemistry, vol 8. Marcel Dekker Inc., New York, pp 137–179

Venkateswarlu K, Chendrayan K, Sethunathan N (1980) Persistence and biodegradation of carbaryl in soils. J Environ Sci Health B15:421–429

Verdisson S, Couderchet M, Vernet G (2001) Effects of procymidone, fludioxonil and pyrimethanil on two non-target aquatic plants. Chemosphere 44:467–474

Ware GW (2000) The pesticide book, 5th edn. Thomson Publications, Fresno, p 193

Ware GW (2001) An introduction to insecticides, 3rd ed. University of Minnesota, Minneapolis, USA. http://ipmworld.umn.edu. Accessed 15 Dec 2010

Wong PK (2000) Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll-a synthesis of *Scenedesmus quadricauda* Berb614. Chemosphere 41:177–182

Xu S (1998) Environmental fate of carbaryl. Environmental monitoring and pest management. Department of Pesticide Regulation, Sacramento, p 18