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Algal Degradation of a Known Endocrine Disrupting Insecticide, α-Endosulfan, and Its Metabolite, Endosulfan Sulfate, in Liquid Medium and Soil

N. SETHUNATHAN,† M. MEGHARAJ,*,† Z. L. CHEN,† B. D. WILLIAMS,‡ GARETH LEWIS,‡ AND R. NAIDU†

Centre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes Campus, Mawson Lakes, SA 5095, Australia, and Department of Soil and Water, The University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia

The role of algae in the persistence, transformation, and bioremediation of two endocrine disrupting chemicals, α-endosulfan (a cyclodiene insecticide) and its oxidation product endosulfan sulfate, in soil (incubated under light or in darkness) and a liquid medium was examined. Incubation of soil under light dramatically decreased the persistence of α-endosulfan and enhanced its transformation to endosulfan sulfate, over that of dark-incubated soil samples, under both nonflooded and flooded conditions. This enhanced degradation of soil-applied α-endosulfan was associated with profuse growth of indigenous phototrophic organisms such as algae in soil incubated under light. Inoculation of soil with green algae, *Chlorococcum* sp. or *Scenedesmus* sp., further enhanced the degradation of α-endosulfan. The role of algae in α-endosulfan degradation was convincingly demonstrated when these algae degraded α-endosulfan to endosulfan sulfate, the major metabolite, and endosulfan ether, a minor metabolite, in a defined liquid medium. When a high density of the algal inoculum was used, both metabolites appeared to undergo further degradation as evident from their accumulation only in small amounts and the appearance of an endosulfan-derived aldehyde. Interestingly, β-endosulfan was detected during degradation of α-endosulfan by high density algal cultures. These algae were also capable of degrading endosulfan sulfate but to a lesser extent than α-endosulfan. Evidence suggested that both α-endosulfan and endosulfan sulfate were immediately sorbed by the algae from the medium, which then effected their degradation. Biosorption, coupled with their biotransformation ability, especially at a high inoculum density, makes algae effective candidates for remediation of α-endosulfan-polluted environments.

KEYWORDS: Endosulfan; endosulfan sulfate; algae; endocrine disruptor; biodegradation; soil

INTRODUCTION

Although several persistent organochlorine pesticides have been banned from agricultural and public health use during the last few decades, the cyclodiene insecticide endosulfan (1,4,5,6,7,7-hexachloro-5-norbornene-2, 3-dimethanol cyclic sulfite) continues to be used globally. Endosulfan is primarily used as a broad spectrum insecticide in agriculture, especially in cotton, a pesticide intensive crop, and rice. A standing cotton crop may receive repeated sprays of very high levels of endosulfan since this crop is prone to attack from several pests, *Helicoverpa* sp. in particular (*1*). Both endosulfan (*2*−*5*) and endosulfan sulfate (*3*) are known strong endocrine disrupting chemicals (EDCs) and therefore have the potential to impact human health and wildlife. In soil and water environments, endosulfan can undergo hydrolysis, especially under alkaline conditions, and/or oxidation to the more persistent and toxic endosulfan sulfate. Unlike hydrolysis, oxidation of endosulfan to its sulfate analogue is not a detoxification process. Off-site transport of both endosulfan and endosulfan sulfate to water resources, such as groundwater and rivers long after application of endosulfan to cotton (*6*, *7*), rice (*8*, *9*), and other crops, is a major problem of great concern, because endosulfan and endosulfan sulfate are extremely toxic to fish (*10*, *11*) and other aquatic invertebrates of economic importance.

Many researchers (*6*, *12*−*14*) have studied the soil persistence of endosulfan. The reported half-life values of α-endosulfan and endosulfan sulfate in Australian soils under cotton fields are 43 and 100 days, respectively (*15*). Fate and persistence of endosulfan, as with most pesticides, are governed by edaphic and environmental factors. For instance, the moisture regime is one of the major factors affecting the persistence and pathway

* To whom correspondence should be addressed. Tel: +61 8 8302 5044. E-mail: Megharaj.Mallavarapu@unisa.edu.au.
† University of South Australia, formerly at CSIRO Land and Water, Adelaide, Australia.
‡ The University of Adelaide.

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of endosulfan degradation in soils (*16*−*18*). Awasthi et al. (*17*) reported the formation of endosulfan diol as a major metabolite of α-endosulfan in flooded soils, but according to our earlier study (*18*), endosulfan sulfate was formed in both nonflooded and flooded soils, but to a lesser extent, paralleling the concomitant with slow degradation of α-endosulfan in the flooded soils.

A wide range of microorganisms, mostly bacteria and, to a lesser extent, fungi, has been implicated in the degradation of endosulfan (*19*−*21*) and endosulfan sulfate (*22*) in soils. Microbial involvement in the degradation of endosulfan (*20*, *21*) and endosulfan sulfate (*22*) has been demonstrated mostly in mixed bacterial cultures using growth media rich in nutrients. A white rot fungus, *Phanerochaete chrysosporium*, metabolized endosulfan under nitrogen deficient, nitrogen rich, and carbon deficient conditions via both oxidative and hydrolytic pathways, with endosulfan sulfate, endosulfan diol, endosulfan hydroxyether, and an unknown compound (tentatively identified as endosulfan dialdehyde) as the metabolites (*23*). Algae are ubiquitous in soils and are present even under semiarid and arid conditions. They grow profusely on moistening of the soils after rain or irrigation in tropical and subtropical environments. However, their role in the degradation of endosulfan and other pesticides has not been intensively studied. Recently, Shivaramaiah (*24*) reported the degradation of endosulfan by a blue−green alga, *Anabaena* sp., in pure culture and river water, but no endosulfan sulfate was detected. In our earlier laboratory study on soil persistence of α-endosulfan (*18*), soil samples were incubated under dark conditions that did not favor active growth and proliferation of algae. We have therefore examined the persistence of α-endosulfan and endosulfan sulfate in nonflooded and flooded soil samples incubated under light and dark conditions and the metabolism of these compounds in pure cultures of two algae (*Chlorococcum* sp. and *Scenedesmus* sp.) in a liquid algal medium and in soils inoculated with these algae.

MATERIALS AND METHODS

Pesticides and Metabolites. Standards of α-endosulfan (99.8%), β-endosulfan (98.7%), endosulfan sulfate (97.6%), endosulfan ether (99.9%), and endosulfan lactone (99.9%) were obtained from Riedel-deHaen AG (Seelze, Germany) while endosulfan diol was synthesized in the laboratory (*25*). Working standard solutions of these compounds were prepared by appropriate dilution of stock solutions using *n*-hexane.

Algae. A soil alga, *Chlorococcum* sp. (*26*), and an aquatic alga, *Scenedesmus* sp., used in this study were from the algal collection maintained in our laboratory.

Soil Incubation Studies. The soil used in this study was exactly the same as that used in the previous study (*18*). Air-dried, sieved (<2 mm) soil (organic carbon, 1.6%; total N, 0.13%; clay, 14.1%; silt, 30.1%; and sand, 55.8%; pH 6.6) from the experimental farm of the Waite Agricultural Research Station (Urrbrae) was placed in 10 g portions in 50 mL culture tubes. Soil samples were spiked with 20 μL of 2000 μg α-endosulfan mL^{-1} in acetone. After 1 h, to allow evaporation of acetone, one set of soil samples was maintained at 70% water holding capacity to provide nonflooded conditions while another set of soil samples was flooded with 12.5 mL of distilled water. Soil samples were incubated under 2000 Lux using a cool white fluorescent lamp (NEC, Japan) and under dark conditions at 21−23 °C in the laboratory. At desired intervals, endosulfan residues were extracted in acetone−hexane and analyzed by gas chromatography (GC). All treatments were conducted in duplicate.

In another experiment, nonflooded and flooded soil samples, spiked with α-endosulfan (as in the previous experiment) or 20 μL of 5000 μg endosulfan sulfate mL^{-1} acetone, were inoculated (after 1 h for evaporation of acetone) with 1 mL suspensions of *Chlorococcum* sp. and *Scenedesmus* sp. in Bold's medium (*27*) and incubated under light as described above. A set of soils were sterilized by autoclaving (121 °C, 1 h, three times on alternate days) prior to treatment. At desired intervals, endosulfan residues were extracted in acetone−hexane and analyzed by GC.

Estimation of Algal Population. The population density (by most probable number estimate) and biodiversity of indigenous algae in soil samples, untreated or treated with α-endosulfan or endosulfan sulfate at 40 μg g^{-1} soil, were monitored under both nonflooded and flooded conditions as described by Megharaj et al. (*28*).

Endosulfan Degradation in Algal Cultures. The degradation of α-endosulfan and endosulfan sulfate by pure cultures of *Chlorococcum* sp. and *Scenedesmus* sp. was examined as follows. Sterilized Bold's basal medium (5 mL portions), contained in glass test tubes, was spiked with 5 μL of 5000 μg of α-endosulfan or endosulfan sulfate mL^{-1} acetone and subsequently inoculated with 1 mL of a suspension of an actively growing culture of *Chlorococcum* sp. or *Scenedesmus* sp. in Bold's medium. Uninoculated medium served as a control. Both uninoculated and inoculated media were incubated under light (2000 Lux). At regular intervals, α-endosulfan and endosulfan sulfate and their metabolites, if any, in the algal medium were extracted in acetone−hexane (1:1 v/v) and the residues in the hexane layer were analyzed by GC.

Residue Extraction and Analysis. Before extraction of endosulfan residues from the soil, 11 mL of water was added to the nonflooded soil samples to provide the same soil−water ratio as in the flooded soil. Soil samples were vortexed with 20 mL of 1:1 acetone−hexane for 2 min. After 500 mg each of sodium sulfate and florisil was added to the soil samples, the contents in each tube were vortexed again for 1 min and centrifuged and the hexane layer in duplicate tubes was analyzed for α-endosulfan and endosulfan sulfate by GC.

In pure culture studies, duplicate samples of liquid growth medium (uninoculated and inoculated with algae) were vortexed with 10 mL of 1:1 acetone−hexane and 500 mg each of sodium sulfate and florisil for 2 min and the hexane layer was analyzed for endosulfan residues by GC.

GC Analysis. GC was performed with Perkin-Elmer Auto System gas chromatograph equipped with an autosampler, an on-column, split/splitless capillary inlet system, electron capture detector (ECD), and data processor (Turbochrom Workstation). Injection was made in the split mode at 260 °C. A DB-5 column (30 mm × 0.20 mm i.d., J&W Scientific, Folsom, CA) was held at an initial column temperature of 120 °C for 1 min, then at 30 °C min^{-1} to 180 °C, then at 10 °C min^{-1} to 250 °C, and finally held at that temperature for 3 min. The injector and detector were held at 260 and 300 °C, respectively. The flow rate was 6 mL min^{-1} for carrier gas (helium) and 30 mL min^{-1} for makeup gas (nitrogen). The volume for the sample injection was 1 μL. Under these conditions, the retention time (min) was 8.23 for α-endosulfan, 10.1 for endosulfan sulfate, and 8.57 for endosulfan diol. Recovery of α-endosulfan and endosulfan sulfate, using the extraction and analytical procedures described, was around 90% from soil samples and about 100% from the liquid medium.

GC-MS Analysis. GC-MS was performed using a Hewlett-Packard (Palo Alto, CA) model 6890 instrument equipped with a mass selective detector and a spilt/splitless injector operated in the splitless mode, with 1 μL injected on-column using a HP autosampler. HP Chemstation software was used for instrument control, data acquisition, and treatment. The column used was a 30 m × 0.25 mm i.d. × 0.25 μm film thickness BP5 S.G.E. (Melbourne, Australia). Helium (purity 99.99%) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The column was held at 50 °C for 3 min, then ramped at 20 °C min^{-1} to 210 °C, then at 5 °C min^{-1} to 260 °C, then at 30 °C min^{-1} to 300 °C, and held at 300 °C for 13 min. The transfer line to the mass selective detector was maintained at 280 °C. The ionization was carried out at a potential of 70 eV and an E.M. voltage of 2000 V. Mass scanning was carried out at scan rate of 0.5 s/scan over a mass range of 35−350 amu. The solvent delay was 5 min.

RESULTS

Effect of Light vs Dark conditions on Soil Persistence of α-Endosulfan. As reported earlier (*18*), the degradation of α-endosulfan in soil samples incubated in the dark was more

Table 1. Persistence of α-Endosulfan (μg) in Nonflooded and Flooded Soil Samples Incubated under Light vs Dark Conditions

treatment	light/dark	0 day	50 days
nonflooded	dark	33.5[a] ± 1.2 (0)	25.2 ± 0.8 (6.4)
nonflooded	light	31.6 ± 1.0 (0)	13.6 ± 2.8 (8.9)
flooded	dark	33.1 ± 1.8 (0)	31.5 ± 1.7 (0.5)
flooded	light	36.1 ± 1.8 (0)	18.8 ± 1.3 (6.8)

[a] α-Endosulfan (μg per 10 g soil) recovered; mean of duplicate estimations ± standard deviation. Values in parentheses: endosulfan sulfate (μg per 10 g soil) formed from α-endosulfan.

Table 2. Species Composition of Algae and Cyanobacteria (*28*) in Soil Samples Untreated and Treated with α-Endosulfan or Endosulfan Sulfate[a]

	untreated		α-endosulfan		endosulfan sulfate	
organism	NF	F	NF	F	NF	F
			alga			
Chlamydomonas sp.	+	+++	+++	+++	++	+++
Chlorococcum sp.	+	+	+	+	++	–
			cyanobacteria			
Gleocapsa sp.	++	++	–	–	–	–
Nostoc sp.	+	+	–	–	–	–
Phormidium sp.	++	+	+		+	+

[a] Frequency of occurrence of species: –, absent; +, low; ++, moderate; and +++, abundant. NF, nonflooded; F, flooded. Population density of microalgae including cyanobacteria in all of the treatments and controls are as follows: >153.7 × 10^3 g^{-1} soil (upper limit, >282.2, and lower limit, >83.7 × 10^3 g^{-1} soil).

pronounced in nonflooded soil than in flooded soil, with concomitant accumulation of endosulfan sulfate as the major metabolite, in greater amounts under the former moisture regime (**Table 1**). Soil incubation under light enhanced the degradation of α-endosulfan by 2-fold in nonflooded soil samples and by 5−6-fold in flooded soil samples over that in corresponding dark-incubated soil samples. Overall, degradation of α-endosulfan was faster under nonflooded conditions than under flooded conditions, irrespective of the presence or absence of light. Enhanced degradation of α-endosulfan under light also led to increased accumulation of endosulfan sulfate.

Untreated and α-endosulfan-treated soil samples, when incubated under light, supported visible growth of indigenous algae, especially in the surface layer of the nonflooded soil and surface layer and standing water column in flooded soil. There was no visible growth of algae in dark-incubated soil samples. The population density of algae in both untreated and α-endosulfan-treated soil samples incubated under light, based on most probable number estimates, was identical (>153.7 × 10^3 g^{-1} soil), irrespective of the moisture regime at 50 days. However, addition of α-endosulfan or endosulfan sulfate adversely affected the biodiversity of the cyanobacteria in the soil (**Table 2**). Although the density of green algae, *Chlorococcum* sp. and *Chlamydomonas* sp., was not affected by α-endosulfan or endosulfan sulfate, cyanobacteria, *Gleocapsa* sp. and *Nostoc* sp., were totally eliminated by α-endosulfan or endosulfan sulfate under both water regimes.

α-Endosulfan Persistence in Soils Inoculated with Algae. The persistence of α-endosulfan in nonflooded and flooded soil samples, inoculated with the soil alga, *Chlorococcum* sp., and an aquatic alga, *Scenedesmus* sp., and then incubated under light, was monitored. In uninoculated soil samples, the concentration of α-endosulfan decreased to about 30% of the original level under nonflooded conditions and to about 50% under flooded conditions at 50 days, with concomitant accumulation of endosulfan sulfate as the major metabolite (**Table 3**). Both algae effected a further, but marginal, increase in the transformation of α-endosulfan and the accumulation of endosulfan sulfate over that in uninoculated soil (evidently caused by indigenous soil algae or other microorganisms) under both water regimes. Substantial accumulation of endosulfan sulfate in the soil even at 50 days suggested that this metabolite may be refractory to degradation by algae (introduced and/or indigenous) and other microorganisms in the soil. In separate experiments, about 75% of the originally added endosulfan was recovered at 50 days when the soil samples were sterilized by autoclaving (121 °C for 1 h, three times on alternate days) and incubated under light after addition of endosulfan. Evidently, degradation of endosulfan in light-incubated nonsterile soil samples was caused by microorganisms, especially the dominant algae. Also, photolysis of endosulfan appears to be negligible under the conditions used in the study. The reduced recovery after long periods of time is not uncommon especially for persistent organochlorine compounds (*29*).

Algal Degradation of α-Endosulfan and Endosulfan Sulfate in Liquid Medium. The degradation of α-endosulfan by *Chlorococcum* sp. and *Scenedesmus* sp. in a liquid medium was examined. The presence of algae in the medium hastened the degradation of α-endosulfan (**Table 4**). Thus, either *Chlorococcum* sp. or *Scenedesmus* sp. effected the degradation of 65−75% of added α-endosulfan at 20 days and almost complete degradation (95−99%) at 30 days, as compared to 35% degradation in uninoculated controls during a 30 day incubation. During this degradation of α-endosulfan, endosulfan sulfate was formed as the major metabolite, along with endosulfan ether as a minor metabolite, but not in stoichiometric amounts. Endosulfan sulfate appeared to undergo further degradation since its concentration decreased between 20 and 30 days after inoculation of the medium with either alga. In uninoculated control, endosulfan sulfate or endosulfan ether was not detected.

In an uninoculated control, there was no decrease in the concentration of endosulfan sulfate during the 30 day incubation. However, its concentration decreased by 41% in the presence of *Chlorococcum* sp. and by 17% with *Scenedesmus* sp. during the same period (**Table 4**). These two algae appeared to be more effective in degrading α-endosulfan than its oxidation product. No metabolite was detected during algal degradation of endosulfan sulfate.

Degradation by High Density Inoculum. In another study, a high density of algal inoculum (*Scenedesmus* sp. 1550 × 10^6 cells mL^{-1}; *Chlorococcum* sp. 600 × 10^6) was used for accelerating the degradation of a relatively high concentration of α-endosulfan (20 μg mL^{-1}) or endosulfan sulfate (30 μg mL^{-1}). At this inoculum density, α-endosulfan decreased to <50% in 10 days and less than 10% in 20 days when inoculated with *Chlorococcum* sp. (**Table 5**). Similarly, *Scenedesmus* sp. effected almost complete degradation of α-endosulfan within 20 days. Despite the rapid disappearance of α-endosulfan with a high density of algal cells, endosulfan sulfate and endosulfan diol were detected only in small amounts, presumably due to their further metabolism by the algae. Both algae also degraded endosulfan sulfate, added at a concentration (30 μg mL^{-1}) higher than that for α-endosulfan, but more slowly than α-endosulfan, and not completely during the 20 day incubation. When high density algal cells (*Scenedesmus* sp. 1550 × 10^6 cells mL^{-1}; *Chlorococcum* sp. 600 × 10^6 cells mL^{-1}) were exposed to α-endosulfan for 1 h and centrifuged, all of the added insecticide was recovered from the cells of both algae, with only traces in the supernatant. Evidently, within 1 h of exposure of algal cells

Table 3. Effect of Algae on Degradation of α-Endosulfan (μg Recovered) in Nonflooded and Flooded Soil

	nonflooded			flooded		
		inoculated			inoculated	
days	uninoculated	*Chlorococcum* sp.	*Scenedesmus* sp.	uninoculated	*Chlorococcum* sp.	*Scenedesmus* sp.
0	36.8 ± 0.6[a]	38.1 ± 3.0	31.3 ± 3.6	31.4 ± 1.2	38.3 ± 4.2	36.5 ± 1.8
20	27.6 ± 1.8 (6.01)	27.9 ± 1.2 (3.28)	16.4 ± 1.2 (7.09)	23.0 ± 1.8 (0.5)	21.0 ± 2.4 (0.86)	25.5 ± 2.7 (1.11)
50	12.3 ± 1.2 (14.6)	8.1 ± 0 (21.4)	8.7 ± 0.6 (17.9)	14.5 ± 1.2 (7.0)	25.5 ± 0.6 (1.11)	12.9 ± 0.6 (17.1)

[a] α-Endosulfan (μg recovered per 10 g soil); mean of duplicate estimations ± standard deviation. Values in parentheses: endosulfan sulfate (μg) formed from α-endosulfan.

Table 4. Effect of Green Algae on Degradation of α-Endosulfan and Endosulfan Sulfate in Liquid Medium

	α-endosulfan			endosulfan sulfate		
		inoculated			inoculated	
days	uninoculated	*Chlorococcum* sp.	*Scenedesmus* sp.	uninoculated	*Chlorococcum* sp.	*Scenedesmus* sp.
0	21.2 ± 1.2[a]	21.1 ± 1.8[a]	21.3 ± 0.4	42.2 ± 2.4[d]	42.7 ± 3.0	42.2 ± 1.2
20	17.8 ± 1.8	7.6 ± 0.6 (3.11[b])	5.4 ± 1.5 (11.05[b])	42.0 ± 1.2	44.4 ± 2.4	39.3 ± 1.8
30	13.2 ± 1.2	0.9 ± 0 (2.76[b]; 0.01[c])	0.2 ± 0(6.28[b]; 2.36[c])	42.4 ± 3.0	25.0 ± 1.2	35.1 ± 0.6

[a] α-Endosulfan (μg recovered per 5 mL medium); mean of duplicate estimations ± standard deviation. [b] Endosulfan sulfate (μg per 5 mL medium) formed from α-endosulfan. [c] Endosulfan ether (μg per 5 mL medium) formed from α-endosulfan. [d] Endosulfan sulfate (μg per 5 mL medium) recovered after spiking.

Table 5. Degradation of α-Endosulfan and Endosulfan Sulfate by a High Density of Green Algae in Liquid Medium

	α-endosulfan[a]		endosulfan sulfate[a]	
incubation (days)	*Chlorococcum* sp.	*Scenedesmus* sp.	*Chlorococcum* sp.	*Scenedesmus* sp.
0	18.37	18.78	30.0	29.67
10	8.67	1.20	26.67	23.0
20	1.72 (0.15[b]; 0.14[c])	0.22 (0.28[a]; 0.81[b])	16.80	15.5

[a] Micrograms mL^{-1} recovered. [b] Micrograms endosulfan sulfate mL^{-1} formed from α-endosulfan. [c] Micrograms endosulfan ether mL^{-1} formed from α-endosulfan.

to α-endosulfan, the cells accumulated the insecticide and presumably algal degradation of α-endosulfan was occurring after biosorption/bioaccumulation.

The products of α-endosulfan metabolism by high density algal inoculum were subjected to GC-MS analysis for further characterization. Under the GC-MS operating conditions described, elution order for authentic standards was as follows: endosulfan ether < endosulfan lactone < α-endosulfan < β-endosulfan < endosulfan diol < endosulfan sulfate.

Either three or five metabolites were detected by GC-MS following algal exposure to α-endosulfan, depending on the algal species used. Degradation of α-endosulfan by *Scenedesmus* sp. produced five peaks in the region of interest. A peak at 15.87 min was identified as the starting material α-endosulfan by comparison of its retention time with authentic material and the mass spectrum that had a base peak at *m*/*z* 195 and other significant ions at *m*/*z* 241, *m*/*z* 265, *m*/*z* 277, and *m*/*z* 339. Computer library matching of the mass spectrum with authentic material showed the match to be >95%.

There was some conversion of the α-isomer to the β-isomer as evidenced by a peak at 16.86 min, the mass spectrum of which corresponded to that of authentic β-endosulfan. A peak at 18.20 min was identified as endosulfan ether on the basis of its mass spectrum. The mass spectrum had a base peak at *m*/*z* of 70 $[C_4H_6O]^+$ derived from the pentacyclic ether moiety and significant ions at *m*/*z* 207, 239, and 259. The observed fragmentation pattern was distinctly different from that of authentic endosulfan diol, which had a similar retention time, but whose base peak was at *m*/*z* 57 $[CH_3COCH_2]^+$ with other major ions at *m*/*z* 70, *m*/*z* 85, *m*/*z* 170, *m*/*z* 237, and *m*/*z* 272.

A peak at 17.49 min was assigned to an aldehyde metabolite, probably arising from oxidation in situ of the diol formed by hydrolysis of endosulfan sulfate. The mass spectral evidence favors a monoaldehyde with one loss of [CO] being observed, although in the absence of a molecular ion, it is difficult to characterize it as either endosulfan dialdehyde or monohydroxyaldehyde. Sutherland et al. (*21*) have reported the formation of a monoaldehyde during the degradation of α-endosulfan although it is not clear from their work whether other structural variations could be conclusively eliminated.

The peak at 20.17 min was easily identified as endosulfan sulfate, by comparison of its retention time and mass spectrum with the authentic material. The fragmentation pattern showed a parent ion at *m*/*z* 422, a base peak at *m*/*z* 272 $[M - Cl - C_4H_6SO_4]^+$, and major ions at *m*/*z* 387 [M − Cl] and *m*/*z* 229.

Chlorococcum sp. produced only three major peaks in addition to the starting material: residual α-endosulfan (15.86 min), β-endosulfan (16.86 min), endosulfan ether (18.20 min), and endosulfan sulfate (20.16 min). *Chlorococcum* sp. caused a significant conversion of the α-isomer to the β-isomer. *Chlorococcum* sp. appeared to be less efficient than *Scenedesmus* sp. in degrading α-endosulfan.

DISCUSSION

Past studies on the biodegradation and transformations of a myriad of pesticides in soil environments focused on the role of mostly bacteria and to some extent fungi. The experimental conditions used in these studies were probably highly selective for bacteria and fungi. For instance, extensive worldwide research on the soil persistence of pesticides has been based mostly on laboratory incubation data generated under dark or low light conditions. These conditions would restrict the growth of phototrophic organisms such as green algae and cyanobacteria in the soil. However, under field conditions, algae and cyanobacteria constitute an important portion of the diverse microbial community in the soil under adequate moisture and abundant sunlight, especially in the tropics and subtropics. According to our present study (**Table 1**), soil samples, incubated under light, accelerated the degradation of α-endosulfan to endosulfan sulfate over that in dark-incubated soil samples. Light also promoted a profuse growth of algae and cyanobacteria especially on the

surface soil, both under nonflooded and flooded conditions (**Table 3**). These phototrophic organisms, by generating oxic conditions through oxygen release during photosynthesis, may be directly responsible for the enhanced oxidation of α-endosulfan in soil samples incubated under light. Indigenous or introduced algae and cyanobacteria, by virtue of their photosynthetic (algae and cyanobacteria) and nitrogen-fixing (cyanobacteria) capabilities, can promote the proliferation of other members of the microbial community, such as bacteria and fungi, that may, in turn, facilitate the transformation of α-endosulfan, independently or together with phototrophs (*30*). The implication of such biodiversity on pesticide transformations in soils is little understood.

The results from this study indicte that algae (introduced and/or indigenous) can be directly involved in the degradation of α-endosulfan and probably endosulfan sulfate. This is because (i) introduced algae, *Chlorococcum* sp. or *Scenedesmus* sp., hastened the transformation of α-endosulfan and its metabolite, endosulfan sulfate, in liquid culture and (ii) exceptionally rapid degradation of endosulfan occurred also in soil (nonflooded and flooded) incubated under light that promoted the proliferation of indigenous and introduced algae. Photolysis of endosulfan was negligible under the experimental conditions used. α-Endosulfan is known to undergo rapid chemical hydrolysis with increased pH of the medium or soil above 7.0 (*10*). However, the pH of the soil or medium inoculated with the algae was around pH 7.0 and endosulfan ether accumulated in inoculated medium, and not in soil, but only in small amounts. Moreover, endosulfan sulfate, and not the hydrolysis product endosulfan diol, was the major product of α-endosulfan metabolism by algae. Evidently, algal transformations of α-endosulfan and endosulfan sulfate are not chemically catalyzed. It is worth noting here that α-endosulfan and the more persistent endosulfan sulfate can undergo very fast metabolism in cotton leaves (*14*). It is not clear whether a common mechanism is responsible for the rapid metabolism of α-endosulfan and endosulfan sulfate in phototrophic systems in algae and cotton leaves.

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