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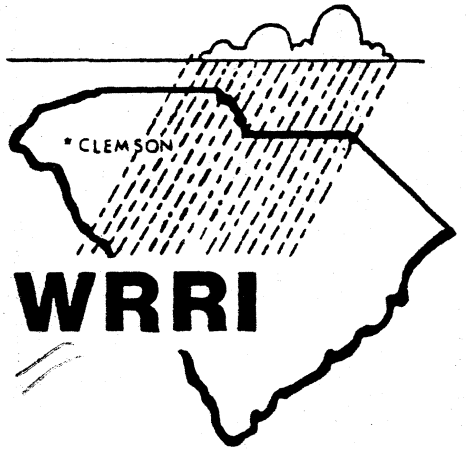

EFFECT OF HERBICIDE RESIDUES ON MICROBIAL PROCESSES IN CROPLAND POND OR IMPOUNDMENT SEDIMENT

by

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TABLE OF CONTENTS

ABSTRACT

An assay system is described for examination of potential effects of herbicides on non-target sediment microorganisms. The tests are designed to be carried out in two parts; a laboratory portion to initially screen for potential effects of a herbicide (1000 ppm), then a field trial for examining herbicides applied at recommended and/or tenfold field application levels (FAL). A key feature of the procedure is that the assays are miniaturized, thereby allowing sufficient replication to establish the statistical validity of the results while minimizing the size of the soil sample which must be collected. Sediments were obtained from a catfish aquaculture pond and an adjoining lake that are adjacent to croplands. Potted lake and pond sediment controls compared to undisturbed cores showed no difference in initial carbon mineralization, acetylene reduction, phosphatase and dehydrogenase activities, but did show a significant difference in methanogenesis. Diuron, after one week in lake sediment, stimulated glucose mineralization, dehydrogenase activity and acetylene reduction at all tested concentrations, and methanogenesis at FAL (2.35 ppm diuron); no effect was observed on phosphatase and cellulolytic activities. On the other hand, paraquat (1000 ppm) stimulated lake sediment glucose mineralization; inhibited phosphatase activity and methanogenesis (0.24 and 2.40 ppm paraquat); no effects were observed on acetylene reduction, dehydrogenase, or cellulolytic activities. In pond sediment, $CuSO_4$ (1000 ppm) inhibited phosphatase activity and methanogenesis (20 and 50 ppm $CuSO_4$); no effect was noted for acetylene reduction, dehydrogenase activity and glucose mineralization. Simazine (1000 ppm) stimulated pond sediment acetylene reduction; inhibited phosphatase activity and methanogenesis (20 and 50 ppm simazine); and had no effect on dehydrogenase activity and glucose mineralization. Chronic effects (4-8 wk incubation) and effects

at lower concentrations by these four herbicides, for the most part, were not observed. Our results support the concept that a battery of tests are required to profile and evaluate the effects of herbicides on sediment microbial communities.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In order to help the ailing farm income, the Clemson University Extension Service has proposed aquaculture as an alternative. However, a potential hazard in using cropland ponds or impoundments for aquaculture is their possible contamination from herbicides used in agriculture and aquatic weed control. Measurements of *in situ* herbicide effects on a variety of microbial processes will help to evaluate the effects of these chemicals in aquaculture.

It is expected that each herbicide can be characterized by its differential effect on selected microbial activities. In addition, any effect a herbicide has on microbial activity is likely to have a profound effect on water quality. This would be expected since microbial activities, including decomposition, are required to maintain a balanced ecological system. Laboratory technicians at the Clemson University catfish impoundments routinely measure water quality parameters such as pH, oxygen and inorganic nutrients. It is likely that during field-application of a herbicide, changes in water quality would be reflected by changes in microbial activities. These results would thus help aquacultural specialists in understanding and assessing the degree of hazard associated with drainage or use of herbicides in aquacultural impoundments.

Unfortunately, at this time techniques for assessing the impacts of chemicals upon microbial activities in sediments are not nearly as well developed as those for aquatic systems. Consequently, it is difficult to know the appropriate approach to provide the information needed for making informed decisions. It is likely, however, that many of the concepts developed for assessing the impacts of chemical in aquatic systems can be modified and applied to sediments. The purpose of the study was to adapt a number of

currently recognized procedures to the problem of assessing the impact of herbicides on microbial processes in sediments and to assess their effectiveness.

RELATED RESEARCH

Pesticide Effects on Microbial Activities

A review of the literature has shown that there have been few studies on the effect of pesticides on microbial processes in aquatic sediments. However, pesticide toxicity studies are well documented in soils. Such studies are valuable in gauging to what extent pesticides may affect specific microbial processes under various environmental conditions.

The effect of toxicants on the natural environment has been studied by measuring microbial mineralization and incorporation of radiolabeled substrates. Grossbard and Davies (12) have reported that simazine at 130 kg/ha inhibited carbon dioxide evolution, but had no effect at 2-10 kg/ha. Orndorff and Colwell (27) found that mineralization of radiolabeled amino acids by bacteria was inhibited by kepone. Anderson and Drew (1) reported that paraquat stimulated and inhibited mineralization of sucrose and phenol, respectively, in soil. They also observed that paraquat and its formula additives increased mineralization of radiolabeled plant material (1). In contrast, the herbicides dinoseb and linuron had no effect on decomposition of plant material (21). Barnhart and Vestal (3) used radiolabeled acetate incorporation into microbial lipids to measure biomass, and found that copper ions, sodium pentachlorophenate and phenol caused an inhibition.

Mineralization and incorporation of radiolabeled substrates by microorganisms are powerful tools for studying the effects of toxicants on microbial activity. The extreme sensitivity of detection of radiolabeled atoms and the opportunity to use *in situ* substrate concentrations, make radiotracer methods very useful for studying natural environments. With an appropriate substrate, this method can be used as a measure of either general or specific microbial activity.

Methanogens are important in anaerobic decomposition of organic matter in waterlogged soils, marshes, swamps, marine and freshwater sediment, and manure piles. Griffiths et al. (11) found that long term exposure to crude oil increased methane production in marine sediments, while Kiene and Capone (18) reported stimulation of the process in soils ammended with aldicarb, methomyl or carbyrl. Copper ions were reported to only initially inhibit methanogenesis (4).

A survey of the literature shows that many investigators use at least one reaction of the nitrogen cycle when examining soil microbial activity. Several investigators have measured N_2 fixation using the acetylene reduction method (15). A study of sandy loam soil showed that several pesticides (e. g. chlorpyrifos, carbofuran, chlordane) reduced ethylene production after two, but not after six days; while other pesticides (e.g. parathion, thionazin, trichlorburan) stimulated ethylene production (32). Malanchuk and Joyce (22) found that 2,4-D at a low level of application decreased acetylene reduction. Other investigators have amended soils with glucose to increase activity to a measurable level. In these studies, paraquat sometimes inhibited acetylene reduction (29), while glyphosate had no effect (6). Nayak and Rao (25,26), measuring N_2 fixation using the ^{15}N isotope method, found that benomyl and carbofuran stimulated nitrogenase activity. In aquatic sediment, diquat, paraquat and monuron inhibited acetylene reduction by blue-green algae (7).

Nitrogen is often a growth limiting nutrient for plants in both soil and sediment. Monitoring the effect of pesticides on nitrogen-fixation would be significant because of the important role of this reaction in soil fertility and its relative sensitivity to perturbances in the environment.

Enzyme assays are often used as a measure of microbial activity in soil and sediment. One enzyme assay that is useful, measures dehydrogenase

activity associated with the electron transport system (ETS). Microorganisms having different terminal electron acceptors often use similar dehydrogenases in preceding transport steps. Therefore, ETS activity is considered a relative measure of general microbial activity. Atlas et al. (2) reported that the herbicides folpet and captafol inhibited dehydrogenase activity in soils after 21 days. However, other herbicides such as diuron and linuron (21), and acid rain (19) failed to have an effect on dehydrogenase activity.

Phosphatases are required to mineralize organic phosphate. Because a large portion of a bacterial community may possess phosphatase activity, a phosphatase assay can be used to measure general microbial activity as well as potential phosphate mineralization. Studies have shown that phosphatase activity was not affected by folpet or captafol (2), but was influenced by acid rain (19) and agricultural practices (8).

Measurement of cellulolytic activity also is used as an index of decomposer activity. Grossbard and Marsh (13) measured cellulose decomposition by weight loss, and reported that linuron at 500 ppm inhibited cellulolytic activity, but had no effect at 50 ppm. Hoeninger (16), by measuring spectrophotometrically the loss of remazol brilliant blue dye from stained cellophane strips, showed that the activity of an aquatic cellulolytic decomposing community is affected by acidity levels. Smith and Mayfield (29) visually assessed cellulose decomposition of cotton duck strips, and reported that paraquat inhibited cellulolytic activity.

The previous studies have clearly shown that the effects of herbicides will vary greatly depending on the herbicide, the soil type and environmental conditions, and the specific microbial process observed. Such findings would make it imperative that site-specific conditions be maintained and that a variety of microbial activities be measured for assessing the impact of a

herbicide in an aquaculture impoundment. As far as I am aware, this work is one of the few studies that have addressed both these criteria for evaluating the effect of herbicides on non-target microbial populations.

RESEARCH OBJECTIVES.

The goal of this study was to adapt a number of currently recognized procedures to the problem of assessing the impact of herbicides on microbial processes in sediment and to assess their effectiveness. Within this overall goal the specific objectives were:

(1) To use intact miniature sediment core samples, thereby simulating natural conditions and allowing for sufficient replication to establish the statistical validity of the results.

(2) To characterize the effect of herbicides on nontarget microorganisms by profiling their differential effects on selected microbial activities.

(3) To assess the potential acute and chronic impact of herbicides upon water quality.

EXPERIMENTAL METHODS AND MATERIALS.

Sediment and Herbicides

Sediment was obtained from a man-made catfish pond and an adjoining lake site located at the Clemson University College of Agricultural Science Research Station, Clemson, SC. The sediment was lightly sieved to remove large rocks and organic debris. A sediment analysis (pH, mineral content and classification) was provided by the Clemson University Agricultural Service Laboratory.

Agricultural herbicide formulations were obtained from the Department of Fertilizer and Pesticide Control, Clemson University. Herbicides used in this study include paraquat, diuron, simazine and copper sulfate. Paraquat is used in this state to control weeds in crops including soybean and corn. Diuron, simazine and copper sulfate are used in aquatic weed control programs. Dr. T. E. Schwedler, Associate Professor of Fisheries at Clemson University, recommends using copper sulfate or simazine to control specific weeds in aquacultural impoundments containing catfish.

Laboratory Experiments

Wet sieved sediment (470 g) was added to cylindrical containers (276 ml). Diuron (80W), paraquat, $CuSO_4$ and simazine each were prepared at 4.7×10^{-3} g/ml H_2O and mixed thoroughly into pots of sediment to give a final herbicide concentration of 1000 ppm. Control containers received a corresponding amount of distilled water. Herbicide amended sediments and controls were incubated for 1-wk at 28°C before assaying for microbial activity. Distilled water was added as required to prevent samples from drying out.

Field Experiments

Wet sieved sediment (2.2 kg) was added to plant pots (2.4 liters). Diuron was added to sediment to achieve either a 1x field application level (FAL) (2.36 ppm) or a 10x FAL (23.6 ppm) (20). Paraquat was added to sediment to achieve either a 1x FAL (0.24 ppm) (20) or a 10x FAL (2.4 ppm). $CuSO_4$ and simazine were added at 20 ppm and 50 ppm, respectively, to achieve a 10x FAL (20). Replicate pots were placed into the same pool from which sediment was removed. Individual pots were removed at 1-, 4- and 8-wk intervals and the sediment temperature recorded. Sediment cores were obtained adjacent to the buried pots using a coring device (13.75 cm length x 10 cm diameter). Sediment pots and cores were transported to the laboratory and immediately processed for assaying microbial activities. Miniature subcores (3-ml) were removed vertically from sample pots using a 5-ml syringe with the end removed.

Glucose Mineralization

A modified method of Meyer-Reil (24) was used for the incubation of sediment subcores under *in situ* temperatures with the addition of a low concentration of ^{14}C-glucose. Briefly, undisturbed sediment cores were placed into individual environmental chambers consisting of a 1.7 cm diameter funnel attached at the bottom to a syringe (Figure 1). A subcore was placed snuggly inside each funnel on top of a paper filter disk. A small volume of ^{14}C-substrate was added on top of each sediment core without disturbing the sediment structure. A low vacuum was applied with the syringe in order to permit better penetration of the substrate into the sediment. The chamber funnel was then capped with a rubber septum fitted with a plastic rod and cup assembly containing a fluted strip of chromatography paper. After incubation, 5 ml of 5N H_2SO_4 was injected through the septum and flushed through the core using the syringe. Working through the septum, β-phenethylamine, a CO_2

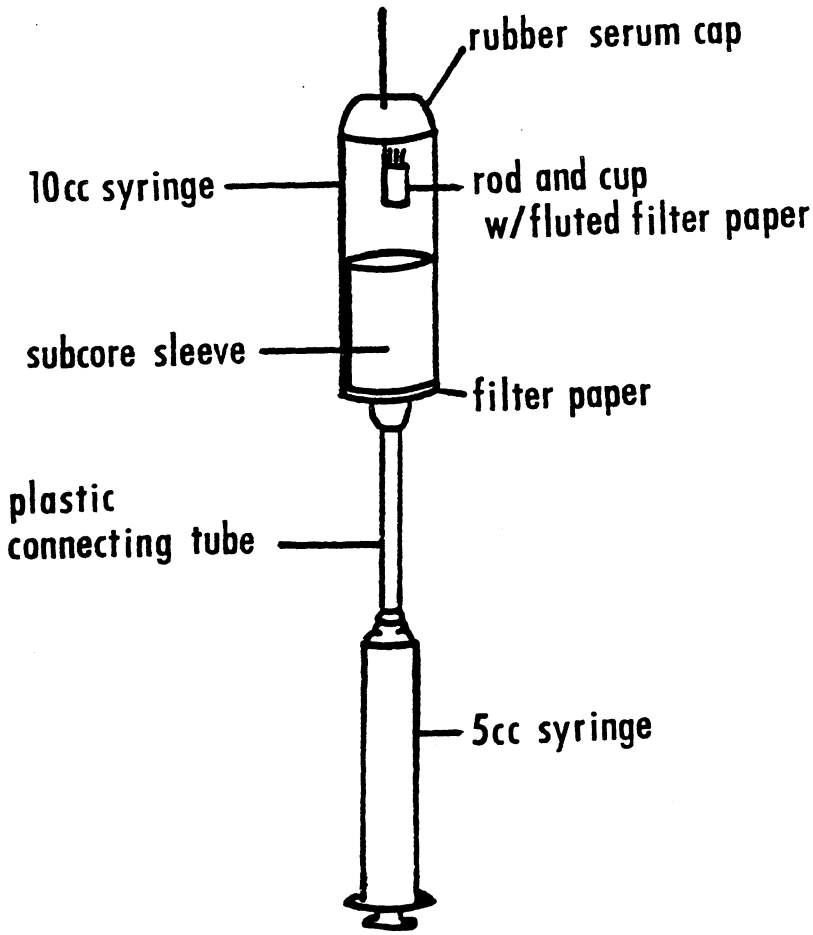

Figure 1. Environmental Chamber (Glucose mineralization).

absorbent, was then injected onto the fluted chromatography paper and $^{14}CO_2$ was collected for 30 min. The absorbent-saturated chromatography paper containing trapped $^{14}CO_2$ was then added to scintillation fluor and the radioactivity determined by liquid scintillation counting. Data are presented in mol of substrate respired per g dry sediment per h.

Methanogenesis

Sediment subcores were transferred into 1.7 cm diameter environmental chambers which had been purged with 80% N_2 - 10% CO_2 to ensure anaerobic conditions. A rubber septum was added to close the top of the chamber. The amount of methane released to the gas space was measured quantitatively with time. This was accomplished by inserting, through the septum, the needle of a 5 ml syringe previously filled with a gas atmosphere similar to that in the chamber. Any uptake of gas was measured and the process repeated until 2 to 5 ml of gas mixture remained in the syringe after the needle was inserted through the septum. Gas in the syringe was forced into the chamber and then the plunger released to allow gas to once again enter the syringe. This flushing process was repeated five times. Gas was then removed so that the tube was at atmospheric pressure. The volume of gas removed was noted and analyzed chromatographically for methane gas (17). The total amount of methane produced was calculated for each sampling time and corrected for methane removed in gas samples. Rates of methanogenesis were calculated as nmol of methane produced per g of dry sediment per h.

Nitrogen Fixation

Sediment subcores were transferred to 1.7 cm diameter environmental chambers which had been purged with nitrogen gas. A rubber septum was then added to close the top of the chamber. Nitrogen fixation was measured using the Acetylene Reduction technique (30). Working through the septum, acetylene

was added to the chamber to give a final concentration of 0.1 Atm. The amount of ethylene produced was measured quantitatively with time. Gas samples were removed through the septum with a syringe and needle while at the same time closing the plunger of the environmental chamber syringe to compensate for the gas being removed. Ethylene produced was measured by gas chromatography and the data presented in nmol of ethylene produced per g dry sediment per h.

Phosphatase Activity

Miniature sediment subcores were placed into individual environmental chambers. A 1-ml volume of 0.006 M p-nitrophenol phosphate in 0.05 M Tris buffer (pH 7.5) or 1-ml of the Tris buffer (control) (10) was slowly added on top of a sediment core and allowed to penetrate into the sediment by applying a low vacuum using the environmental chamber syringe. Cores were incubated at in situ temperature for an appropriate time before terminating the reaction by using the chamber syringe to flush and filter 1.5 ml of 5 N NaOH through the sediment core. The absorbance of the filtrate was read at 410 nm on a spectrophotometer. Absorbance values were converted to p-nitrophenol concentrations by comparing to a standard curve. Data are presented as nmol p-nitrophenol released per g dry weight sediment per h.

Dehydrogenase Activity

Miniature sediment subcores were placed into individual environmental chambers. A 1-ml aqueous solution of 3% triphenyltetrazolium chloride (21) was added on top of the core and allowed to permeate the sediment as previously described. Sediment cores were incubated for an appropriate time at in situ temperature. The reaction product formazan was extracted by flushing and filtering 10 ml of 95% ethanol through the sediment as previously described. Formazan absorbance was read at 485 nm on a spectrophotometer,

corrected for substrate and sediment controls, and converted to triphenylformazan concentrations using a standard curve. Data are presented as μl of H_2 transferred per g dry weight sediment per da.

Cellulose Degradation

Cellulose degradation was studied using a modified procedure of Swift (31). In laboratory experiments, a dyed cellulose strip was added to each of three 4 x 8 cm nylon bags and placed into slits cut into potted sediment. Samples were incubated in the laboratory at 28°C for 2-wk. In field experiments, cellulose strips in nylon bags were placed into pots to be buried in the lake, and subsequently retrieved after 1, 4 and 8 wk of incubation. Upon completion of incubation, cellulose strips were removed and gently washed free of sediment with water. The harvested strips and three unexposed strips were each placed into separate aluminum foil capped flasks containing 60 ml of 0.35% KOH and autoclaved for 20 min. Each extracted dye was diluted to 100 ml with distilled water in a volumetric flask and its absorbance (A) recorded at 595 nm on a Spectronic 20 colorimeter. The percentage of weight lost (W) for each treatment was calculated by the following equation:

$$W = \frac{\text{average A (unexposed)} - \text{average A (exposed)}}{\text{average A (unexposed)}} \times 100 \ .$$

Statistical Analysis

The General Linear Models Procedure of SAS (SAS Institute Inc., Cary, North Carolina) was used to analyze the data. All tests were performed at the 95% confidence level.

RESULTS

Sediment Analysis

Information on sediment pH, classification, and mineral content was supplied by the Clemson University Agricultural Service Laboratory (Table 1). Percentages of sand, silt and clay were determined using a soil hydrometer (34). Clay particles are usually defined as having a diameter of less than 0.002 mm; however, in this procedure, the separation of clay was made for particles of 0.005 mm diameter.

The lake sediment was characterized as course-loamy containing 90% sand and little organic material. In contrast, the pond sediment was classed as loam.

Amendment Procedure

A comparison of microbial activities between *in situ* control and undisturbed core sediment for both lake and pond samples showed that methane production was the only activity significantly affected by the potting procedure involved in sediment preparation (Tables 2 and 3). A control-core comparison was not made for cellulolytic activity because the test could not be initiated without disturbing the sediment.

Table 1. Sediment Characteristics

	pH	Mineral content (lb/A) P	K	Mg	Ca	% Sand	% Silt	% Clay	Class
Lake	5.3	4	51	49	240	90	2	8	course loamy
pond	6.0	8	63	55	310	48	42	10	loam

Table 2. Effect of Potting Lake Sediment on Microbial Activities.

Test[a]	1-wk Cont.	1-wk Core	4-wk Cont.	4-wk Core	8-wk Cont.	8-wk Core
Acetylene[b] Reduction	0.02	0	0.22	0	0.09	1.35
Methane[c] Production	38.11	12.68*	33.91	1.45*	16.00	23.20
Phosphatase[d] Activity	99.96	66.10	151.88	100.46	-	-
Dehydrogenase[e] Activity	0.70	0.61	1.71	1.18	1.80	0.98
Glucose[f] Mineralization	89.50	84.60	133.83	152.63	-	-

[a]Control sediment samples were sieved, placed in pots and buried in lake bottom sediment. Core sediment samples were obtained from sediment adjacent to buried potted control sediments.

[b]Each value listed is in units of nmol ethylene/gdwt sediment and is the average of ten replicates collected after 6 h incubation at 28°C.

[c]Each value listed is in units of nmol methane/gdwt sediment and is the average of ten replicates collected after 6 h incubation at 28°C.

[d]Each value listed is in units of nmol p-nitrophenol/gdwt sediment after 1 h and is the average of four replicates incubated at 28°C.

[e]Each value listed is in units of μl H_2 transferred/g sediment, and is the average of four replicates collected after 24 h incubation at 28°C.

[f]Each value listed is in units of x 10^{-3} nmol glucose mineralized/g sediment and is the average of three replicates collected after 6 h incubation at 28°C.

*Significant difference at 95% confidence level.

Table 3. Effect of Potting Pond Sediment on Microbial Activities.

Test[a]	1-wk Cont.	1-wk Core	4-wk Cont.	4-wk Core
Acetylene[b] Reduction	0.02	0.01	0.01	0.01
Methane[c] Production	2.44	1.37	3.06	1.33
Phosphatase[d] Activity	125.72	127.77	179.28	219.26
Dehydration[e] Activity	4.12	3.36	14.84	15.63
Glucose[f] Mineralization	175.24	194.24	81.30	107.35

[a]Control sediment samples were placed in pots and buried in pond bottom sediment. Core sediment samples were obtained from sediment adjacent to buried potted control sediments.

[b]Each value listed is in units of nmol ethylene/gdwt sediment and is the average of ten replicates collected after 24 h incubation at 28°C.

[c]Each value listed is in units of nmol methane/gdwt sediment and is the average of ten replicates collected after 6 h incubation at 28°C.

[d]Each value listed is in units of nmol p-nitrophenol/gdwt sediment after 1 h and is the average of four replicates incubated at 28°C.

[e]Each value listed is in units of 1 H_2 transferred/g sediment, and is the average of four replicates collected after 24 h incubation at 28°C.

[f]Each value listed is in units of x 10^{-3} nmol glucose mineralized/g sediment and is the average of three replicates collected after 6 h incubation at 28°C.

*Significant difference at 95% confidence level.

Glucose Mineralization

Lake sediment amended with 1000 ppm of diuron or paraquat and incubated in the laboratory showed significantly higher amounts of glucose mineralization than did the nonamended control sediment (Table 4). In field studies, lake sediment amended with 1x or 10x FAL of diuron showed significantly higher amounts of glucose mineralization in samples that were buried for 1-wk on the lake bottom (Table 4). Neither of the paraquat treatments showed a significant difference from the control (Table 4). After 4-wk on the lake bottom, the diuron 1x FAL sample was not recovered, but the 10x FAL sample still showed a significant increase in mineralization compared to the control.

Neither $CuSO_4$ nor simazine at the concentrations tested under laboratory or *in situ* conditions significantly affected glucose mineralization in pond sediment (Table 5).

Methane Production

Lake sediment samples treated with diuron or paraquat at a concentration of 1000 ppm and incubated in the laboratory did not significantly alter methane production compared to a control sediment sample (Table 6). In field studies, sediment treated with 1x FAL of diuron for 1- and 4-wk stimulated methane production, whereas a 10x FAL did not (Table 6). In contrast, paraquat at 1x and 10x FAL showed increasing inhibition of methane production over a 4-wk period. After 8-wk, sediment treated with paraquat at 10x FAL showed no significant difference in methanogenesis compared to the control.

Pond sediment samples treated with $CuSO_4$ or simazine at a concentration of 1000 ppm and incubated in the laboratory did not significantly alter methanogenesis (Table 7). However at 10x FAL, $CuSO_4$ or simazine inhibited methanogenesis for up to 4-wk.

Table 4. The Effect of Diuron and Paraquat on C^{14}-Glucose Mineralization in Lake Sediment.

Treatment	C^{14}-Glucose Mineralized (x 10^{-3} nmol/gdwt/h)[c] 1-wk	4-wk
Laboratory[a]		
Control	2.46	-
Diuron (1000 ppm)	17.77*	-
Paraquat (1000 ppm)	29.29*	-
In situ		
Control	14.52	11.57
Diuron (2.36 ppm)	34.07*	-
Diuron (23.60 ppm)	31.10*	36.20*
Paraquat (0.24 ppm)	6.88	15.77
Paraquat (2.40 ppm)	14.90	12.90

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for C^{14}-glucose mineralized (28°C).

[b]Sediment pots were exposed to a herbicide for either 1 or 4-wk in lake sediment before assaying for C^{14}-glucose mineralized (28°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 5. The Effect of $CuSO_4$ and Simazine on C^{14}-Glucose Mineralization in Pond Sediment.

Treatment	C^{14}-Glucose Mineralized (x 10^{-3} nmol/gdwt/h)[c]	
	1-wk	4-wk
Laboratory[a]		
Control	24.27	-
$CuSO_4$ (1000 ppm)	28.49	-
Simazine (1000 ppm)	24.17	-
In situ		
Control	36.11	17.19
$CuSO_4$ (20 ppm)	37.12	16.81
Simazine (50 ppm)	44.52	14.12

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for C^{14}-glucose mineralized (28°C).

[b]Sediment pots were exposed to a herbicide for either 1 or 4-wk in pond sediment before assaying for C^{14}-glucose mineralized (24°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 6. The Effect of Diuron and Paraquat on Methanogenesis in Lake Sediment.

Treatment	Methane ($\times 10^{-1}$ nmol/gdwt/h)[c]		
	1-wk	4-wk	8-wk
Laboratory[a]			
Control	8.35	-	-
Diuron (1000 ppm)	11.57	-	-
Paraquat (1000 ppm)	11.10	-	-
In situ[b]			
Control	20.23	55.78	18.78
Diuron (2.36 ppm)	64.02*	86.72*	-
Diuron (23.60 ppm)	26.83	31.80	-
Paraquat (0.24 ppm)	16.67	10.48*	-
Paraquat (2.40 ppm)	38.83	5.42*	14.67

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for methane (28°C).

[b]Sediment pots were exposed to a herbicide for either 1, 4 or 8-wk in lake sediment before assaying for methane (28°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 7. The Effect of $CuSO_4$ and Simazine on Methanogenesis in Pond Sediment.

Treatment	Methane ($\times 10^{-1}$ nmol/gdwt/h)[c] 1-wk	4-wk
Laboratory[a]		
Control	8.21	-
$CuSO_4$ (1000 ppm)	1.97	-
Simazine (1000 ppm)	3.46	-
In situ[b]		
Control	5.43	6.66
$CuSO_4$ (20 ppm)	2.73*	0.46*
Simazine (50 ppm)	1.72*	2.02*

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for methane (28°C).

[b]Sediment pots were exposed to a herbicide for either 1- or 4-wk in pond sediment before assaying for methane (24°C).

[c]Average of 10 replicates.

*Significant difference from control at 95% confidence level.

Nitrogen Fixation

Diuron at a concentration of 1000 ppm caused a substantial stimulation of acetylene reduction in lake sediment after 1-wk exposure in the laboratory (Table 8). In contrast, addition of paraquat at a concentration of 1000 ppm did not significantly change activity compared to a control. Lake sediment treated with diuron at 1x (2.36 ppm) and 10x (23.6 ppm) FAL showed a significant increase in acetylene reduction after a 1-wk incubation in lake sediment, but after a 4-wk incubation, these differences became less apparent (Table 8). In contrast, sediment samples that were exposed to 1x (0.24 ppm) and 10x (2.4 ppm) FAL of paraquat showed no significant difference in acetylene reduction from control samples.

$CuSO_4$ at the concentrations tested did not affect acetylene reduction in pond sediment (Table 9). In contrast, simazine stimulated acetylene reduction at a concentration of 1000 ppm, but not at 50 ppm.

Phosphatase Activity

Lake sediment amended with diuron at 1000 ppm and incubated in the laboratory showed no significant difference in the amount of p-nitrophenol released compared to a control sediment sample (Table 10). In contrast, paraquat amended sediment (1000 ppm) showed a significant decrease in p-nitrophenol produced compared to a control. For field studies, neither herbicide at 1x or 10x FAL showed a significant difference from their control (Table 10).

Pond sediment phosphatase activity was inhibited by $CuSO_4$ or simazine at 1000 ppm (Table 11). This inhibition was not observed at 20 ppm $CuSO_4$. However, simazine at a concentration of 50 ppm inhibited phosphatase activity after 1-wk, but not after 4-wk.

Table 8. The effect of Diuron and Paraquat on Nitrogen-Fixation (Acetylene Reduction) in Lake Sediment.

Treatment	Ethylene (x 10^{-2} nmol/gdwt/h)[c] 1-wk	4-wk	8-wk
Laboratory[a]			
Control	0.43	-	-
Diuron (1000 ppm)	1.70*	-	-
Paraquat (1000 ppm)	0.14	-	-
In situ[b]			
Control	0.05	0.46	0.19
Diuron (2.36 ppm)	15.96*	1.61	-
Diuron (23.60 ppm)	7.69*	5.24	-
Paraquat (0.24 ppm)	2.56	3.88	-
Paraquat (2.40 ppm)	0.34	5.35	1.90

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for ethylene (28°C).

[b]Sediment pots were exposed to a herbicide for either 1, 4 or 8-wk in lake sediment before assaying for ethylene (28°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 9. The Effect of $CuSO_4$ and Simazine on Nitrogen-Fixation (Acetylene Reduction) in Pond Sediment.

Treatment	Ethylene ($\times 10^{-2}$ nmol/gdwt/h)[c] 1-wk	4-wk
Laboratory[a]		
Control	0.07	-
$CuSO_4$ (1000 ppm)	0	-
Simazine (1000 ppm)	0.19*	-
In situ[b]		
Control	0.04	0.02
$CuSO_4$ (20 ppm)	0.03	0.02
Simazine (50 ppm)	0.04	0.02

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for ethylene (28°C).

[b]Sediment pots were exposed to a herbicide for either 1 or 4-wk in pond sediment before assaying for ethylene (24°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 10. The Effect of Diuron and Paraquat on Phosphatase Activity in Lake Sediment.

Treatment	p-Nitrophenol (nmol/gdwt/h)[c] 1-wk	4-wk
Laboratory[a]		
Control	237.93	-
Diuron (1000 ppm)	231.30	-
Paraquat (1000 ppm)	170.80*	-
In situ[b]		
Control	99.96	151.88
Diuron (2.36)	92.98	138.28
Diuron (23.60)	106.62	115.79
Paraquat (0.24 ppm)	70.80	154.86
Paraquat (2.40 ppm)	62.92	132.39

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for phosphatase activity (28°C).

[b]Sediment pots were exposed to a herbicide for either 1 or 4-wk in lake bottom sediment before assaying for phosphatase activity (28°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 11. The Effect of $CuSO_4$ and Simazine on Phosphatase Activity in Pond Sediment.

Treatment	p-Nitrophenol (nmol/gdwt/h)[c] 1-wk	4-wk
Laboratory[a]		
Control	95.80	-
$CuSO_4$ (1000 ppm)	44.52*	-
Simazine (1000 ppm)	46.35*	-
In situ[b]		
Control	125.78	179.20
$CuSO_4$ (20 ppm)	124.10	176.30
Simazine (50 ppm)	72.51*	177.84

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for phosphatase activity (28°C).

[b]Sediment pots were exposed to a herbicide for either 1 or 4-wk in pond sediment before assaying for phosphatase activity (24°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Dehydrogenase Activity

In the laboratory trial portion of the test, diuron at 1000 ppm concentration showed a significant stimulation of dehydrogenase activity in lake sediment (Table 12). Paraquat treated samples did not significantly differ in dehydrogenase activity from a control sample. Lake sediments treated with diuron 1x and 10x FAL under *in situ* conditions showed significantly higher activity than control samples after a 1-wk lake incubation. After 4-wk of lake incubation, neither treatment showed a significant difference in activity from control samples. At the same FALs, paraquat showed no stimulation or inhibition of activity when compared to the control sediment.

Neither $CuSO_4$ nor simazine at the concentrations tested in the laboratory or *in situ* significantly affected dehydrogenase activity in pond sediment (Figure 13).

Cellulose Degradation

There appears to be an average decrease in percent cellulose degradation for both laboratory and field studies when lake sediments were treated with either paraquat or diuron (Table 14). However, these differences are not significant at the 95% confidence level.

Table 12. The Effect of Diuron and Paraquat on Dehydrogenase Activity in Lake Sediment.

Treatment	H_2 transferred (ml/gdwt Sediment/da)[c]		
	1-wk	4-wk	8-wk
Laboratory[a]			
Control	1.71	-	-
Diuron (1000 ppm)	11.26*	-	-
Paraquat (1000 ppm)	5.49	-	-
In situ[b]			
Control	0.70	1.71	1.80
Diuron (2.36)	5.55*	3.01	-
Diuron (23.60)	8.16*	1.92	-
Paraquat (0.24 ppm)	0.46	2.13	-
Paraquat (2.40 ppm)	3.78	0.94	1.78

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for dehydrogenase activity (28°C).

[b]Sediment pots were exposed to a herbicide for either 1-, 4- or 8-wk in lake bottom sediment before assaying for dehydrogenase activity (28°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 13. The Effect of $CuSO_4$ and Simazine on Dehydrogenase Activity in Pond Sediment.

Treatment	H_2 transferred (ml/gdwt Sediment/da)[c]	
	1-wk	4-wk
Laboratory[a]		
Control	1.58	-
$CuSO_4$ (1000 ppm)	2.44	-
Simazine (1000 ppm)	3.48	-
In situ[b]		
Control	4.13	9.96
$CuSO_4$ (20 ppm)	2.19	5.80
Simazine (50 ppm)	5.92	8.46

[a]Sediment pots were exposed to a herbicide for 1-wk in the laboratory (28°C) before assaying for dehydrogenase activity (28°C).

[b]Sediment pots were exposed to a herbicide for either 1 or 4-wk in pond sediment before assaying for dehydrogenase activity (24°C).

[c]Average of ten replicates.

*Significant difference from control at 95% confidence level.

Table 14. The Effect of Diuron and Paraquat on Cellulolytic Activity in Lake Sediment.

Treatment	% Degradation[c] 1-wk	4-wk
Laboratory[a]		
Control	29.6	-
Diuron (1000 ppm)	13.0	-
Paraquat (1000 ppm)	24.1	-
In situ[b]		
Control	7.1	7.8
Diuron (2.36 ppm)	5.7	-
Diuron (23.60 ppm)	1.5	3.6
Paraquat (0.24 ppm)	5.0	7.1
Paraquat (2.40 ppm)	5.7	4.2

[a]Dyed cellulose strips were exposed to herbicide treated sediment for 2-wk in the laboratory (28°C) before determining degradation.

[b]Dyed cellulose strips were exposed to herbicide treated sediment for either 1- or 4-wk in lake bottom sediment (12°C) before measuring degradation.

[c]Average value of three replicates; values did not show a significant difference from control at 95% confidence level.

DISCUSSION

A number of currently recognized procedures were adapted to the problem of assessing the impact of herbicides on microbial processes in sediment. A key feature was to standardize a procedure for collecting and incubating soil samples for assaying a variety of microbial activities. This was accomplished by assaying miniature replicate sediment-subcores in environmental chambers. A sufficient number of replicate subcores were used to establish the statistical validity of the results while minimizing the size of the sediment sample which must be collected. Furthermore, because the procedure evaluates a variety of microbial processes, it will help assess the broad ecological impact of a herbicide on a microbial community.

Herbicides were slowly mixed into potted sediments to obtain homogeneous samples for optimal data reproducibility. However, potting and mixing sediment may adversely affect in situ activities of microorganisms due to possible changes in environmental conditions. Nevertheless, comparing potted lake and pond sediment with undisturbed sediment cores showed no difference in initial carbon mineralization, acetylene reduction, phosphatase, and dehydrogenase activities, but did show a significant effect on methanogenesis. Those activities not affected by potting and mixing may either reflect a minimal disruption of the sediment, a rapid equibration of the sediment back to in situ condition, the ability of the same microbial population to function equally well under another environmental condition, or that sediment microbial populations are sufficiently diverse that a different population would succeed as conditions changed. In contrast, methanogens are not physiologically diverse and do not readily tolerate exposure to oxygen. Therefore, it is not surprising that there was a significant difference in methanogenesis between potted and undisturbed samples, with differences diminishing with

time. It is apparent that using potted sediment may preclude using methanogenesis as a test assay. Nevertheless, this assay may prove useful in other sediments having higher amounts of reducing agents, which could buffer against redox changes. Likewise, measurement of cellulolytic activity may be more applicable for other sediments. In this study, the effects of herbicides were difficult to detect because of low cellulolytic activity.

Initially herbicides were examined in the laboratory at a higher level (1000 ppm) than would be expected to accumulate in sediment. This procedure was used as a screen to determine potential effects of the herbicides on non-target microbial populations. A review of the literature indicates that a variety of concentration dependent effects might be predicted. For example, if a high concentration of herbicide had no effect, one could expect no effect to a slight stimulation at in situ concentrations (2,6). If a stimulation is observed, it would diminish with decreasing herbicide concentration (9,11,12). If an inhibition is observed, lower concentrations would show less inhibition to even a slight stimulation (9,11,28). Our results strongly support these observations.

A battery of tests was carried out in order to assess the effect of the herbicides at field application and higher levels on non-target microorganisms. One could expect that each herbicide would have a characteristic spectrum of effects on a given set of microbial activities. However, this differential effect is not invariable, but depends on sediment type, residence time in the environment, season and herbicide formulation (wetting agents, etc.). This observation is supported by the sometimes contradictory reports on the effects of herbicides on microbial activities (13,23,29,33). Summarizing the data of this study, diuron after 1-wk in lake sediment stimulated glucose mineralization, dehydrogenase activity and acetylene reduction at all

tested concentrations and methanogenesis at FAL (2.35 ppm diuron); no effect was observed on phosphatase and cellulolytic activities. On the other hand, paraquat (1000 ppm) stimulated lake sediment glucose mineralization; inhibited phosphatase activity and methanogenesis (2.40 and 0.24 ppm paraquat); no effects were observed on acetylene reduction, dehydrogenase or cellulolytic activities. In pond sediment, $CuSO_4$ (1000 ppm) inhibited phosphatase activity and methanogenesis (20 and 50 ppm $CuSO_4$); no effect was noted for acetylene reduction, dehydrogenase activity and glucose mineralization. In contrast, simazine (1000 ppm) stimulated pond sediment acetylene reduction; inhibited phosphatase activity and methanogenesis (20 to 50 ppm simazine); no effect was observed on dehydrogenase activity and glucose mineralization. Chronic effects (4-8-wk incubation) and effects at lower concentrations by these four herbicides, for the most part, were not observed. These partial stimulations and inhibitions observed on different microbial activities probably would have little effect on total mineralization of organic matter in the sediment. Prolonged exposure could effect other environmental parameters, such as dissolved oxygen, Eh, and pH. However, chronic effects (4-8-wk incubation), for the most part were not observed; correlating with reports that these herbicides are degraded best at moderate temperatures, in soils having a low clay content, under aerobic and anaerobic conditions (14).

This study demonstrates a number of tests that would be applicable for examining different microbial activities under in situ sediment conditions. A battery of tests would be required to properly evaluate herbicide effect on organisms as physiologically diverse as those found in sediment microbial communities.

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