Bull. Environm. Contam. Toxicol. 24,306-312 (1980)

An Implanted Slide Technique for Examining the Effects of the Herbicide Diuron on Soil Algae

Annette E. Pipe and D. Roy Cullimore
Microbiology Unit, Department of Biology, University of Regina, Regina, Saskatchewan, Canada, S4S OA2

Methods available for the qualitative and quantitative observation of soil algae are few. They include direct observation of algae on soil particles (TCHAN 1952), chlorophyll extraction (PANTERA 1970, SHUBERT & STARKS 1979, SINGH 1961), and a number of more commonly used dilution techniques (ALI & SANDHU 1972, ANANTANI & MARATHE 1972, CULLIMORE & McCANN 1977, JURGENSEN & DAVEY 1968). None of these methods are entirely satisfactory. Direct observation reveals both an extremely low number and restricted range of algae when compared with other techniques (JOHNSON 1962); pigment extraction may be subject to interference from humic acids (FOGG et al. 1973); and dilution techniques fail to distinguish between algae actively growing in the soil and those present as resting stages (FOGG et al. 1973). Dilution techniques also restrict the genera recorded to those able to grow satisfactorily on the medium or media provided. There is, therefore, a serious need to investigate methods for monitoring soil algae and their activities.

The use of buried slides for the examination of soil microorganisms was first reported by ROSSI (1928) and subsequently by CHOLODNY (1930). The procedure has since become known as the "Rossi-Cholodny buried slide" or "contact slide" method (ALEXANDER 1961, BURGES & RAW 1967, HAWKER et al. 1960). It is one of the most widely used *in situ* methods for the examination of soil fungi (BROWN & HORNBY 1971, BURGES & RAW 1967, GOCHENAUR & WOODWELL 1974) and soil bacteria (ALEXANDER 1961), but, in contrast to the generally accepted use of glass slides and plates for the *in situ* observation of aquatic algae (SCHWOERBEL 1970), has not been used for soil algae. CHOLODNY (1930) did observe some growth of algae, mainly *Cyanophyceae*, on slides whose upper portions projected from the soil, but was more interested in the fungi and bacteria developing on completely buried slides.

This paper describes a modification of the Rossi-Cholodny buried slide technique for studying indigenous soil algae. The procedure involves the implantation of a microscope slide vertically in the soil, leaving the top 1.5 cm of the slide projecting above the soil surface. Light passes downwards from the upper exposed and illuminated portion of the slide, encouraging

0007-4861/80/0024-0306 $01.40

algal growth on the slide surface in contact with the soil. After incubation, the slide is removed from the soil and examined microscopically. A herbarium specimen can be made of the algal population colonizing the slide by covering the soil particles and microorganisms on its surface with a thin film of agar.

As a result of the increasing awareness of the potential effects of agricultural chemicals on the soil microorganisms and their biochemical activities, the need has arisen for efficient methods of monitoring these effects. The method described in this paper has been used to monitor changes in the soil algal population following treatment with the herbicide diuron.

MATERIAL AND METHODS

Microscope slides were marked with small scratches at 2 mm intervals along both edges of one face. Pairs of slides, marked faces together, were fastened together at one end with adhesive tape ("Scotch" magic transparent tape, width 12.5 mm). The slide pairs (taped ends uppermost) were pushed into moist soil, held in plastic-lined 200-mL paper cups, until only the top 1.5 cm projected from the soil surface. The soil used in this experiment was a heavy clay, with a pH (when mixed 1:1 with distilled water) of 7.7. The soil was enriched, prior to inserting the microscope slides, with Bold Basal medium (NICHOLS & BOLD 1965) (adjusted to a pH of 7.7) to give a final concentration of about 10% (v/w). The cups were covered with plastic sheeting and incubated for 30 days at 20±2°C under continuous illumination from General Electric "Gro and Sho" Plant Lights. Soil moisture levels were monitored weekly by weight and corrected, when necessary, with the appropriate volume of distilled water to maintain water holding capacity.

After incubation, vertical cuts were made in the sides of each cup, allowing it to be pulled away from the soil which then fell away from the slides. The slides were air-dried, after which any large aggregates of soil adhering to the slides were easily removed by tapping the slides sharply against a hard surface. Microscopic examination verified that this procedure did not cause significant losses of algae from the slides. The pairs of slides were then separated, and the surfaces exposed to the soil were covered with molten 2% water agar, applied as a fine spray. The agar hardened on contact with the slide. When the slide was fully coated, the agar was dehydrated in a dust-free flow of air from a sterile laminar flow cabinet, leaving a thin covering over the soil particles and microorganisms, and sealing them into position on the slide.

The slides were examined microscopically immediately, after the addition of a few drops of distilled water and application of a cover-slip, or were stored at 4°C wrapped in aluminium foil,

for examination at a later date. Slides could be stored thus for at least 2 years without observable damage to the algae. Upon examination of the slides, the algae present in each 2 mm horizontal etched strip were identified to genus. Cell numbers in each strip were recorded for each genus. Algae present in very large groups were difficult to count; in some cases it was necessary to count 100 cells in part of the aggregation and make an estimate of the total number in the group. Cell numbers of *Oscillatoria* were determined by dividing the total length of filaments observed by the average cell length (calculated from measurement of 100 cells). Repeated hydration and dehydration of the agar during successive observations of the algae did not impair their preservation, so long as the slides were not kept moist for longer than a few hours at a time.

For the herbicide studies, the required volume of a solution of diuron in acetone (20 μg/μL) was dispersed in distilled water and applied to the soil (about 150 g dry weight) in the cups with a pipet, to give final concentrations throughout the soil (moistened to water holding capacity) of 1, 5 and 10 ppm diuron (ppm/dry soil). These concentrations are within the range likely to occur in the field (SANBORN et al. 1977). The amount of acetone added to the soil was standardised (75 μL) in both treated and control soils. The amount of diuron present in successive layers (0-2 cm, 2-4 cm, 4-6.5 cm) of the soil treated with 1 ppm diuron was determined colorimetrically (KATZ 1966). The procedure was also conducted on untreated soil sections.

Dilution cultures were set up to compare the algal genera recovered on implanted slides with those developing in liquid medium. Ten 10 g samples of the same soil (untreated) as that used for the slide experiments were dispersed in 90 mL volumes of sterile Bold Basal medium (pH 7.7) contained in 250 mL Erlenmeyer flasks. The flasks were incubated under the same conditions of temperature and illumination as the cups of soil. After incubation for 60 days, the cultures were examined microscopically and the algal genera present were recorded.

RESULTS AND DISCUSSION

Algal genera observed on slides implanted in untreated soil. The algal genera observed most frequently on the surface of slides implanted in the untreated soil were *Chlorella*, *Hantzschia*, *Navicula*, *Oscillatoria* and *Stichococcus*. *Hantzschia* was present on all slides. Cultures of the soil in Bold Basal medium revealed *Anabaena*, *Lyngbya*, *Mesotaenium* and *Nostoc* in addition to those genera observed on the slides. Since these four genera were observed only sporadically in the dilution cultures (a maximum of two out of the ten replicates) they were probably not sufficiently numerous to colonize the slides, and

may not have been a significant fraction of the soil algal population. It is also possible that they were present as resting stages which would grow only under the enriched nutrient regime resulting from addition of the soil to the culture medium. The range of algae recorded on the implanted slides was, therefore, slightly lower than that observed in dilution cultures. However, the slide method is superior to the dilution technique in that it provides information concerning the arrangement of algae with respect to soil particles at various depths.

Chemical analysis of diuron in the soil. Soil analysis revealed that approximately 73% (110μg) of the 150μg herbicide applied to the soil in each cup was recovered by the analytical procedure used (Table 1).

TABLE 1

Concentration of diuron in successive depths of heavy clay soil treated with herbicide at the rate required to give a theoretical concentration of 1 ppm throughout the soil[a].

Soil Section (depth,cm)	Weight of soil (g)	Amount of diuron recovered (μg) Mean[b] ± S.D.	Concentration of diuron (ppm) Mean[b] ± S.D.
0-2	56	84.7 ± 13.0	1.5 ± 0.2
2-4	43	15.3 ± 8.6	0.3 ± 0.2
4-6.5	52	10.3 ± 17.9	0.2 ± 0.3

a. 150μg diuron in 150 g dry soil.
b. Three separate determinations.

Approximately 77% (85μg) of the diuron recovered was present in the top 2 cm (56 g) of soil. The concentration of diuron recovered from this section (the only one of significance to the algal counts presented in this work) was therefore approximately 1.5 ppm. If the total amount of undetected diuron (40μg) were present in the top 2 cm of soil, the maximum concentration would have been approximately 2.2 ppm. However, a proportion of the undetected diuron was probably present in the lower soil sections, and a very small amount might also have become adsorbed to the plastic lining of the cups. The maximum possible concentration of diuron present in the top 2 cm section of the soil was therefore probably no greater than 2.0 ppm.

GROVER (1975), working with the same soil as that used in the present study (Regina heavy clay) found that, at water holding capacity, more than 50% of the diuron applied became adsorbed to the soil particles. Thus, it can be assumed that

less than 50% of the diuron present in the top 2 cm of the soil in the cups (maximum 2.0 ppm) was in the soil water. Since adsorbed herbicide is not available for uptake by soil microorganisms (HANCE & McKONE 1976) the concentration of available diuron in the top 2 cm of soil would be no greater than 1 ppm. Similarly, it may be assumed that the actual concentrations of available diuron in the soils treated to contain 5 ppm and 10 ppm did not exceed the intended levels.

Effect of diuron on the colonization of implanted slides by soil algae. The effect of diuron on algal colonization of slides is shown in Table 2.

TABLE 2

Effect of diuron on the colonization of implanted slides by five genera of soil algae.

Algal genus	Cumulated numbers of cells observed in the top 10 mm of 10 slides[a] implanted in soil treated with:		
	0 ppm diuron	1 ppm diuron	5 ppm diuron
Chlorella	30.2×10^3	0.3×10^3	0
Hantzschia	6.9×10^3	0.3×10^3	0.1×10^3
Navicula	1.2×10^3	0	0
Oscillatoria	86.7×10^3	0	0
Stichococcus	5.3×10^3	0	0
All 5 genera	130.3×10^3	0.6×10^3	0.1×10^3

a. 10 separate slides from 5 separate cups examined for each treatment.

Numbers of algal cells on the slides at depths greater than 10 mm from the soil surface are not shown since, even on control slides, colonization was sporadic in this zone.

Diuron at a theoretical concentration throughout the soil of 1 ppm (Table 2) prevented colonization of the slides by all of the genera listed except *Chlorella* and *Hantzschia*. *Hantzschia* was also present in low numbers on slides removed from soil treated with 5 ppm diuron, but colonization by this diatom was completely prevented in soil treated with 10 ppm diuron. Diuron at 1 ppm reduced the total recorded algal population by greater than 99% in the 0 to 10 mm depth. The need for conducting large numbers of replicates at each treatment level should be emphasized. This is caused by the clumped growth patterns of most of the algae observed. *Hantzschia*. with the most uniform dis-

tribution, was present on 100% of control slides in the form of small groups of cells or single cells scattered over the slide surface. However, *Navicula* was present on only 80% of control slides, and frequently was observed to form large groups of cells. *Chlorella* and *Oscillatoria*, present on 50% of the control slides, and *Stichococcus*, present on only 20% of control slides, were observed almost exclusively in large clumps. The pattern of growth of the algal population on the slides therefore influences the reproducibility of the results.

Since algal colonization of the implanted slide may be assumed to be dependent upon either the presence of the alga in the vicinity of the slide following implantation, or upon movement (active or passive) of the alga towards the slide surface, the data provided by the technique are a function of the ability of the alga firstly to reach the slide surface; secondly, to adhere to the slide; and thirdly, to grow. Consequently, reduced colonization in the herbicide-treated soil may indicate interference with these functions, and may not necessarily be indicative of death of the algae. The results presented in this paper, therefore, do not necessarily indicate that the application of 1 ppm diuron had a lethal effect on the algal population, but rather that the herbicide exerted some influence upon algal colonization in soil up to 30 days after treatment. The colonizing flora was reduced to approximately 1% of that observed in the untreated soil. Further experiments are being conducted to evaluate the exact nature of the effect of diuron on the ability of soil algae to colonize glass slides, to monitor the longterm effects of the herbicide on the soil algae beyond the 30 day maxima reported in this paper, and to evaluate more precisely the distribution of the herbicide in the top few millimetres of soil.

In conclusion, the implanted slide technique provides an efficient method for direct observation of soil algae in the arrangement in which they probably exist in the soil. It also has a potential use in providing a simple means for the routine examination of pesticides to determine any effects which these might have on the algal population of the soil.

ACKNOWLEDGEMENTS

Research was supported by the Saskatchewan Research Council by means of a graduate award (A.E.P.), and also by the Natural Science and Engineering Research Council of Canada, (grant-in-aid, D.R.C). Advice and assistance of A.E. Smith and B. Rainey are gratefully acknowledged.

REFERENCES

ALEXANDER, M.: Introduction to Soil Microbiology. John Wiley and Sons, Inc. 1961.

ALI, S. & G.R. SANDHU: Oikos 23, 268 (1972).

ANANTANI, Y.S. & K.V. MARATHE: Bombay Univ. Journal 41, 88 (1972).

BROWN, M.E. & D. HORNBY: Trans. Brit. Mycol. Soc. 56, 95 (1971).

BURGES, A. & F. RAW (Eds.): Soil Biology. Academic Press 1967.

CHOLODNY, N.: Arch. Mikrobiol. 1, 620 (1930).

CULLIMORE, D.R. & A.E. McCANN: Plant Soil 46, 499 (1977).

FOGG, G.E., W.D.P. STEWART, P. FAY & A.E. WALSBY: The Blue-green Algae. Academic Press Inc. (London) Ltd. 1973.

GOCHENAUR, S.E. & G.M. WOODWELL: Ecology 55, 1004 (1974).

GROVER, R.: Can. J. Soil Sci. 55, 127 (1975).

HANCE, R.J. & C.E. McKONE: The determination of herbicides. In L.J. Audus (Ed.), Herbicides: physiology, biochemistry, ecology. Academic Press 1976.

HAWKER, L.E., A.H. LINTON, B.F. FOLKES & M.J. CARLILE: An Introduction to the Biology of Micro-organisms. Edward Arnold (Publishers) Ltd. 1960.

JOHNSON, A.: Gardens' Bull. Singapore 19, 379 (1962).

JURGENSEN, M.F. & C.B. DAVEY: Can. J. Microbiol. 14, 1179 (1968).

KATZ, S.E.: J. Assoc. Offic. Anal. Chem. 49, 452 (1966).

NICHOLS, H.W. & H.C. BOLD: J. Phycol. 1, 34 (1965).

PANTERA, H.: Meded. Fac. Landbouwet. Rijksuniv. Gent. 35, 847(1970).

ROSSI, G.: Die direkte bakterio-mikroskopische Untersuchung des Ackerbodens. Festschrift Jul. Stoklasa, Berlin 1928.

SANBORN, J.R., B.M. FRANCIS & R.L. METCALF: The degradation of selected pesticides in soil : a review of the published literature. U.S. EPA - 600/9-77-022 (1977).

SCHWOERBEL, J.: Methods of Hydrobiology (Freshwater Biology). Pergamon Press 1970.

SHUBERT, L.E. & T.L. STARKS: Algal succession on orphaned coal mine spoils. In M.K. Wali (Ed.), Ecology and Coal Resource Development. Pergamon Press 1979.

SINGH, R.N.: Role of Blue-Green Algae in Nitrogen Economy of Indian Agriculture. Indian Council of Agricultural Research, New Delhi 1961.

TCHAN, Y.T.: Proc. Linn. Soc. N.S.W. 77, 265 (1952).