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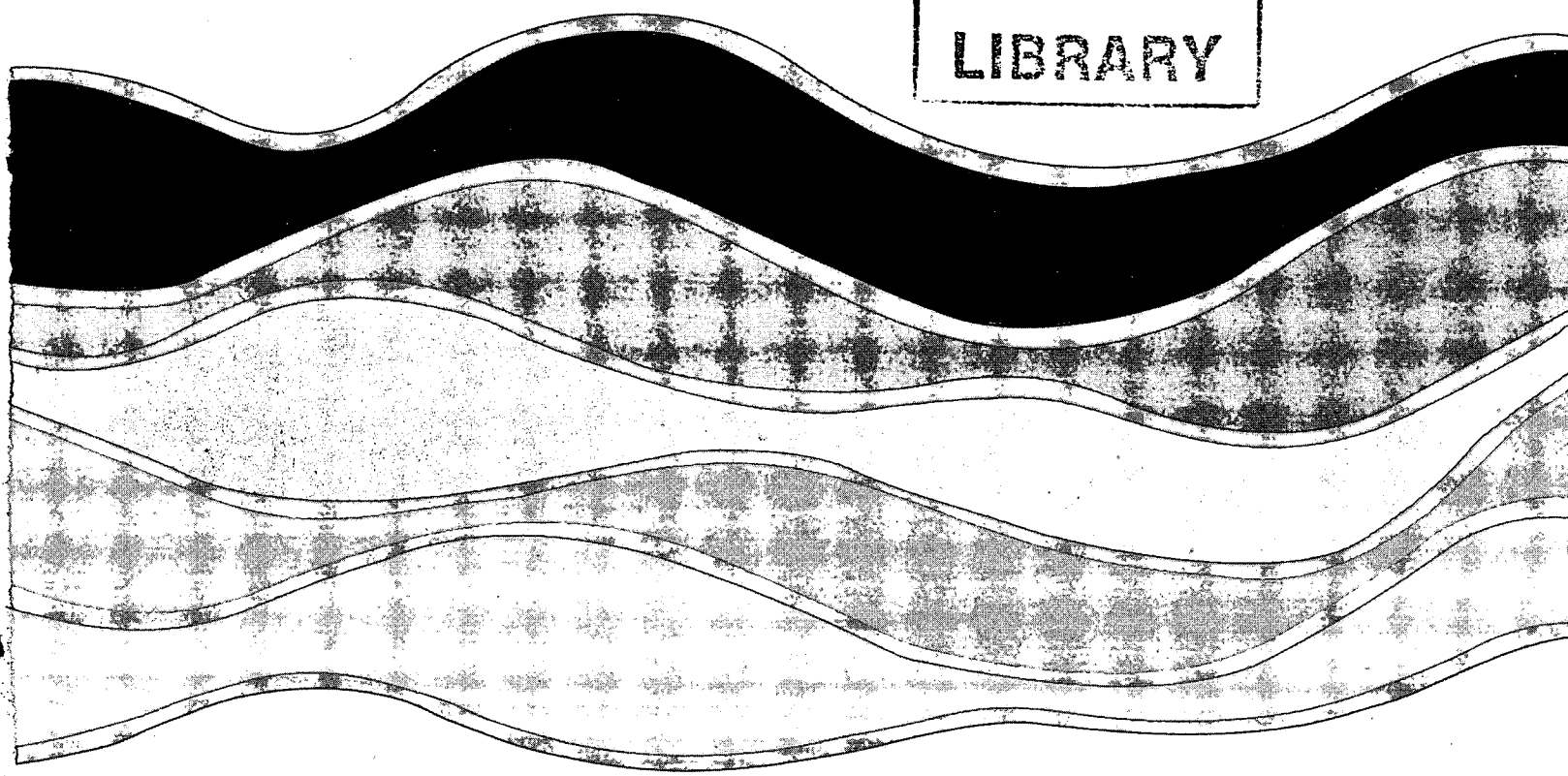

PERSISTENCE OF CYANAZINE IN THE AQUATIC ENVIRONMENT

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NWRI Contribution No. 98-005

Persistence of cyanazine in the aquatic environment

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MANAGEMENT PERSPECTIVE

The herbicide cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5,-triazin-2-yl]-amino]-2-methylpropanenitrile) is a selective triazine herbicide used for annual broadleaf and grass weed control in corn, sorghum, potatoes, soybeans and triazine-resistant canola. It is one of the most heavily used agricultural pesticides in Ontario and yet there is virtually no information on its aquatic fate. Both the U.S. Environmental Protection Agency and the Science Policy and Environmental Quality Branch of Environment Canada have noted that information is required on the occurrence, persistence, fate and toxicity of cyanazine.

This report describes experiments carried out to investigate the volatility of cyanazine from the aqueous phase and its adsorption by bottom-attached biofilms. It was found that cyanazine in the aquatic system can be considered as non-volatile up to temperatures as high as 35 °C. Cyanazine was adsorbed by algal as well as bacterial biofilms but could not be readily biodegraded as no degradation products were found. There is strong evidence that biofilms can take up cyanazine and form bound residues which cannot be extracted. Therefore, routine analysis of field samples can significantly underestimate the level of cyanazine in the environment. The results of this work will be useful for determining the hazards posed by cyanazine to the aquatic environment.

SOMMAIRE À L'INTENTION DE LA DIRECTION

L'herbicide cyanazine (2-[[4-chloro-6-(éthylamino)-1,3,5,-triazin-2-yl]-amino]-2-méthylpropanenitrile) est un herbicide sélectif de type triazine utilisé pour la lutte contre les latifoliées et les graminées annuelles dans les cultures de maïs, de sorgho, de pommes de terre, de soja et de canola résistant à la triazine. C'est l'un des pesticides agricoles les plus utilisés en Ontario et malgré cela, il n'existe presque pas d'informations sur son devenir en milieu aquatique. L'Environmental Protection Agency et la Direction de la qualité de l'environnement et de la politique scientifique d'Environnement Canada ont noté un besoin d'informations sur l'occurrence, la persistance, le devenir et la toxicité de la cyanazine. Ce rapport décrit des expériences effectuées afin d'étudier la volatilité de la cyanazine en phase aqueuse et son adsorption par des films biologiques fixés au fond. On a constaté que dans un milieu aquatique, la cyanazine peut être considérée comme non volatile jusqu'à une température de 35 °C. La cyanazine était adsorbée par les films biologiques algaux et bactériens, mais elle ne pouvait être biodégradée facilement car on n'a pas trouvé de produits de dégradation. Il existe de bonnes raisons de croire que les films biologiques peuvent absorber la cyanazine et former des résidus liés qui ne peuvent être extraits. Par conséquent, les analyses habituelles des échantillons prélevés sur le terrain peuvent sous-estimer de façon significative les teneurs en cyanazine dans l'environnement. Les résultats de cette étude pourraient être utiles pour déterminer les dangers de la cyanazine pour l'environnement aquatique.

ABSTRACT

The persistence of cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5,-triazin-2-yl]-amino]-2-methylpropanenitrile) in the aquatic environment was studied through experiments on volatility and uptake by bottom-attached biofilms. The results indicate that cyanazine can be considered non-volatile up to 35 °C. Cyanazine was lost from the water in the presence of algal or bacterial biofilms. All evidence points towards uptake by the biofilm as the cause of the disappearance of cyanazine. However, no degradation products were found showing that the cyanazine could not be biodegraded or biotransformed. Cyanazine formed bound residues within the biofilm which could not be extracted, thus increasing its persistence in the aquatic environment.

RÉSUMÉ

On a étudié la persistance de la cyanazine (2-[[4-chloro-6-(éthylamino)-1,3,5,-triazin-2-yl]-amino]-2-méthylpropanenitrile) dans l'environnement aquatique à l'aide d'expériences portant sur sa volatilité et sur son absorption par des films biologiques fixés au fond. Les résultats obtenus indiquent que la cyanazine peut être considérée comme non volatile jusqu'à une température de 35 °C. La cyanazine disparaissait de l'eau en présence de films bactériens algaux ou bactériens. Tout semble indiquer que l'absorption par le film biologique est la cause de la disparition de la cyanazine. Toutefois, on n'a noté aucun produit de dégradation, ce qui indique que la cyanazine n'était ni biodégradée, ni biotransformée. La cyanazine formait à l'intérieur du film biologique des résidus liés qui ne pouvaient pas être extraits, ce qui accroissait sa persistance dans l'environnement aquatique.

INTRODUCTION

Cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5,-triazin-2-yl]-amino]-2-methylpropanenitrile) is a selective triazine herbicide used for annual broadleaf and grass weed control in corn, sorghum, potatoes, soybeans and triazine-resistant canola (Worthing and Walker, 1987; Smith *et al.*, 1982). It is one of the most heavily used agricultural pesticides in Ontario and approximately 215,480 kg of this chemical were used in 1993, mainly for corn production (Hunter and McGee, 1994). The widespread use of cyanazine has led to the contamination of Ontario river and well waters (Frank and Logan, 1988; OMOE, 1987a,b), as well as surface water, ground water and drinking water in the United States (Cohen *et al.*, 1986; U.S. Environmental Protection Agency, 1987).

To determine the hazards posed by herbicides to aquatic environments, information is required on their occurrence, persistence, fate and toxicity to aquatic organisms. In spite of its wide usage, cyanazine has not been studied as extensively as other triazine herbicides such as atrazine and simazine and there is little information available on its aquatic fate. The U.S. Environmental Protection Agency (1988) indicated that the persistence of cyanazine in water was unknown. Consequently, a rigorous environmental hazard assessment for cyanazine, and the setting of water quality guidelines, was not possible. Generally speaking, pesticides and other anthropogenic contaminants, once introduced into the environment, are inevitably subjected to biological and non-biological transformation processes (Bollag and Liu, 1990). It is conceivable that the aquatic fate of cyanazine would be influenced by various biological, chemical, and physical processes in the system. We therefore initiated a study to identify the major processes responsible for the control of cyanazine's aquatic fate and to address the knowledge gap identified by both the U.S. Environmental Protection Agency (1988) and the Science Policy and Environmental Quality Branch of Environment Canada (Pauli *et al.*, 1991).

Preliminary studies (Lau *et al.*, 1996) had shown that cyanazine was non-volatile at room temperature. Its concentration in still or running water remained constant for more than one hundred days. There was also some evidence that cyanazine was adsorbed by biofilms. When cyanazine was added to a beaker of water which contained glass slides with attached biofilms, its concentration in the water decreased by more than ten percent in just a few days. In this report, the results of further experiments to investigate the persistence of cyanazine are summarized, including tests of volatility at a higher temperature and uptake by algal and bacterial biofilms.

EXPERIMENTAL METHOD

Chemicals

Analytical grade cyanazine and pesticide grade organic solvents were obtained from Caledon Laboratories, Georgetown, Ontario. The sodium sulfate used for drying organic extracts was

heated to 500 °C for 24 h before use. All glassware was rinsed with pesticide grade solvents before use. All other chemicals used in the experiments were reagent grade or better.

Volatility Experiment

Two beakers of distilled water were spiked with cyanazine to a concentration of approximately 6 mg/L and the solutions were sampled periodically to measure any changes in concentration. One beaker was immersed in a water bath to maintain its temperature at 35 ^{0}C. The other beaker was left at room temperature of about 20 ^{0}C. Before each sampling, the beaker and contents were weighed and distilled water was added to account for water loss through evaporation. After a brief mixing period, samples were taken for chemical analysis. Methods for sample handling and chemical analysis are described in a later section.

Biofilm adsorption experiments

Biofilm was developed on the bottom of an outdoor flume which was lined with glass. In the middle section, the glass cover consisted of 15 x 25 mm glass slides which could easily be removed for analysis. Water was pumped from Hamilton Harbour into the upstream end of the flume (200 mm x 200mm x 2 m long). While the biofilm was being developed, the water flowed down the flume into a tank and was then discharged back into the harbour. After a biofilm had developed on the flume bottom, the discharge from the tank was fed back into the upstream end of the flume, making it a re-circulating system. The water was then spiked with cyanazine and water and slide samples were then taken periodically for analysis of cyanazine content. The flow velocity in the flume was approximately 20 cm/s and the water depth was about 10 cm. All surfaces in contact with the water were either glass or stainless steel.

An identical flume was used as a control for this experiment. Both flumes were filled with the same quantity of water and flow conditions were identical. However, mercuric chloride was added to the control flume to a concentration of 100 ppm in order to kill off all organisms and to inhibit any biofilm growth. This flume was also spiked with cyanazine and water samples were taken at the same intervals. Prior to sampling, point gauge reading of the water depth was taken and water was added to the system to account for loss due to evaporation.

In another experiment, bacterial biofilm was grown on glass slides indoors in the same re-circulating flume filled with harbour water. A solution of sodium acetate was added continuously to provide nutrient for biofilm development. The solution was delivered via a peristaltic pump at a rate which provided about 1mg of sodium acetate per litre of water per day. Once the biofilm had developed, two sets of eighteen slides were removed. Each slide was placed into an individual test tube containing 15ml of harbour water which had been spiked with cyanazine to about 10 ppm. Nutrient was added to one of those sets of test tubes in the form of 15µg of sodium acetate per day. A third set of test tubes containing only distilled water with 10 ppm cyanazine and no biofilm was also prepared as a control. Three test tubes from each set were taken periodically for analysis.

Sample preparation and chemical analysis

For the volatility experiment and the outdoor flume experiment, three 10 mL samples and three biofilm slides, where applicable, were withdrawn from the system at each sampling and then 10 mL of dichloromethane (DCM) were added to each sample. For the indoor bacterial biofilm experiment, three test tubes were taken from each set and the slides were transferred into individual test tubes and 10 mL of DCM were added. 10 mL of DCM were also added to the water remaining in each test tube. The samples were extracted with 3 x 10 mL aliquot of DCM. All resulting DCM extracts were dried through anhydrous sodium sulfate. A toluene keeper was added to each and the entire extract was concentrated to 5 mL on a rotary evaporator. Further concentration and solvent exchange into a final volume of 0.5 mL of toluene was performed under a stream of nitrogen.

The toluene extracts were analyzed on a Hewlett Packard 5890-II gas chromatograph with a single splitless injector - dual column - nitrogen-phosphorus/flame ionization detector (NPD/FID) technique. Both columns were DB-5 [polymethyl(5% phenyl)siloxane] (J & W Scientific - Chromatographic Specialties Inc., Brockville, Ontario.), 0.25 mm i.d. x 30 m length, with 0.25 μm film thickness. Injector and detector temperatures were 200 °C and 300 °C, respectively. The initial column temperature was 80 °C for 2 minutes, and the program rate was 10 °/minute to 150 °C, then 4 °/minute to 280 °C, and then 8 °/minute to 300 °C with no final hold. The constant helium carrier gas flow rate was 1.0 mL/min. The gas flow rate for air and hydrogen was based on the type of detector used (FID, air - 400 mL/min, hydrogen - 30 mL/min ; NPD, air - 120 mL/min, hydrogen - 4 mL/min). All subsequent mass spectral analyses were performed using the same temperature program and column stationary phase with a Hewlett Packard 5971A gas chromatograph - mass selective detector.

RESULTS AND DISCUSSION

Data from the volatility experiment are shown in Figure 1. The cyanazine concentration stayed constant for 56 days for both room temperature and 35 °C, indicating that there was no loss from volatility or from adsorption to the beakers. Certain chemicals, such as metolachlor, are essentially non-volatile at room temperature but become increasingly volatile at higher temperatures because of the dependence of Henry's law constant on temperature (Lau *et al.*, 1995). Such is apparently not the case with cyanazine. The present results reinforce the conclusion of Lau *et al.* (1996) that cyanazine can be considered as non-volatile.

Data from the outdoor biofilm experiment are summarized in Table 1 and the cyanazine concentration is plotted against time in Figure 2. There was no decrease in concentration in the control flume. In fact, there was a slight increase which might have been caused by the fact that insufficient water was added to make up for the evaporation. In the flume with biofilm, the cyanazine concentration decreased by about fifteen percent over five days. This is very similar to the results obtained by Lau *et al.*(1996) using biofilm slides in a beaker of

water spiked with cyanazine. As cyanazine is non-volatile and is resistant to photodegradation, the most likely cause for its disappearance is through adsorption by the biofilm.

Cyanazine was found in all the biofilm slides but only in very small quantities, of the order of 1 μg per slide. At this concentration, the cyanazine in all the biofilms in the flume would comprise only a few percent of the cyanazine that had disappeared. This is not entirely unexpected and is probably the results of cyanazine forming bound residues. It is known that many pesticides leave non-extractable residues in soil or sediment even after exhaustive solvent extraction (Khan and Hamilton, 1980; Jones *et al.*, 1982). Liu *et al.*(1996) found that cyanazine in contact with bacterial biomass also formed bound residues. It is quite likely that the same process had occurred with the biofilm and the cyanazine in this flume.

No degradation products were found in any of the samples from the outdoor flume. As the biofilm consisted mainly of algae, it would have been killed very rapidly by the cyanazine. It was therefore unrealistic to expect any biodegradation of the cyanazine by the outdoor biofilm. For this reason, the indoor bacterial biofilm experiments were carried out to test whether bacterial biofilms were capable of degrading cyanazine.

Figure 3 gives the results from the experiment with bacterial biofilm. It shows the quantity of cyanazine remaining in the water in each test tube after various periods of contact with the biofilm slides. Once again the cyanazine disappeared from the water, likely as a result of adsorption by the biofilm. The adsorption was fairly rapid as most of the decrease in cyanazine had taken place after only two days. The addition of nutrient in the form of sodium acetate did not appear to have much influence as the decrease was between fifteen to twenty percent in both cases.

Figure 4 shows the quantity of cyanazine extracted from the bacterial biofilm slides after various periods of contact. The quantity increased in the first few days and then became rather constant. The data more or less mirror the pattern shown by the loss of cyanazine in the water in Figure 3. Thus it is quite evident that there was uptake of cyanazine by the bacterial biofilm. However, it appears that cyanazine in this case also formed bound residues which could not be extracted as the quantities extracted from the biofilm slides were quite small compared with the amount which had disappeared.

No cyanazine degradation products were detected in any of the samples with the bacterial biofilm. Therefore it appears that cyanazine is a very persistent chemical which cannot be readily biodegraded. This is in agreement with Pacepavicius *et al.* (1996) who found no apparent biodegradation or biotransformation of cyanazine in a test using mixed cocktails of farmland runoff and soil leachate after an incubation period of 98 days. However, the evidence here strongly suggests that biofilm is capable of taking up cyanazine and rendering it non-extractable.

CONCLUSIONS

Results from this study confirm that cyanazine can be considered as non-volatile, even up to temperatures of 35 °C.

Biofilms can irreversibly adsorb cyanazine and form bound residues which cannot be extracted. However, neither the outdoor algal biofilm nor the indoor bacterial biofilm appear to be capable of biodegrading cyanazine

Routine analysis of field samples can significantly underestimate the level of cyanazine in the environment because of the failure to detect its presence in the bound residues.

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Table 1. Cyanazine concentrations in outdoor flume

Time (days)	Cyanazine in biofilm flume (ppm)	Cyanazine in control flume (ppm)	Cyanazine per biofilm slide(μg)
0	13.73	9.58	
1.00	12.75	9.95	1.34
1.17	12.49	N.A.	1.11
4.42	11.74	10.11	0.56
5.42	11.58	10.36	0.40

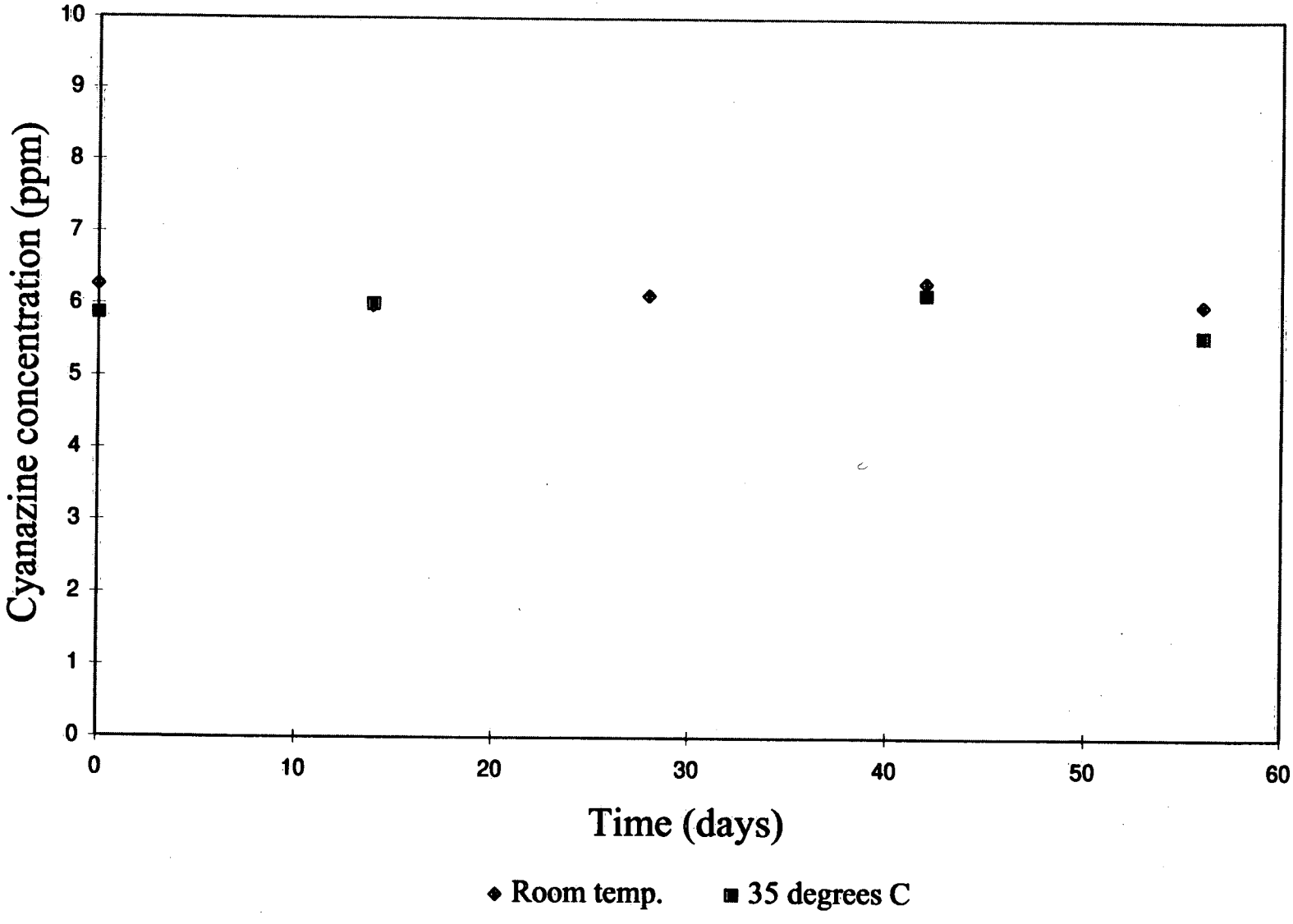


Figure 1. Cyanazine concentration - volatility experiment.

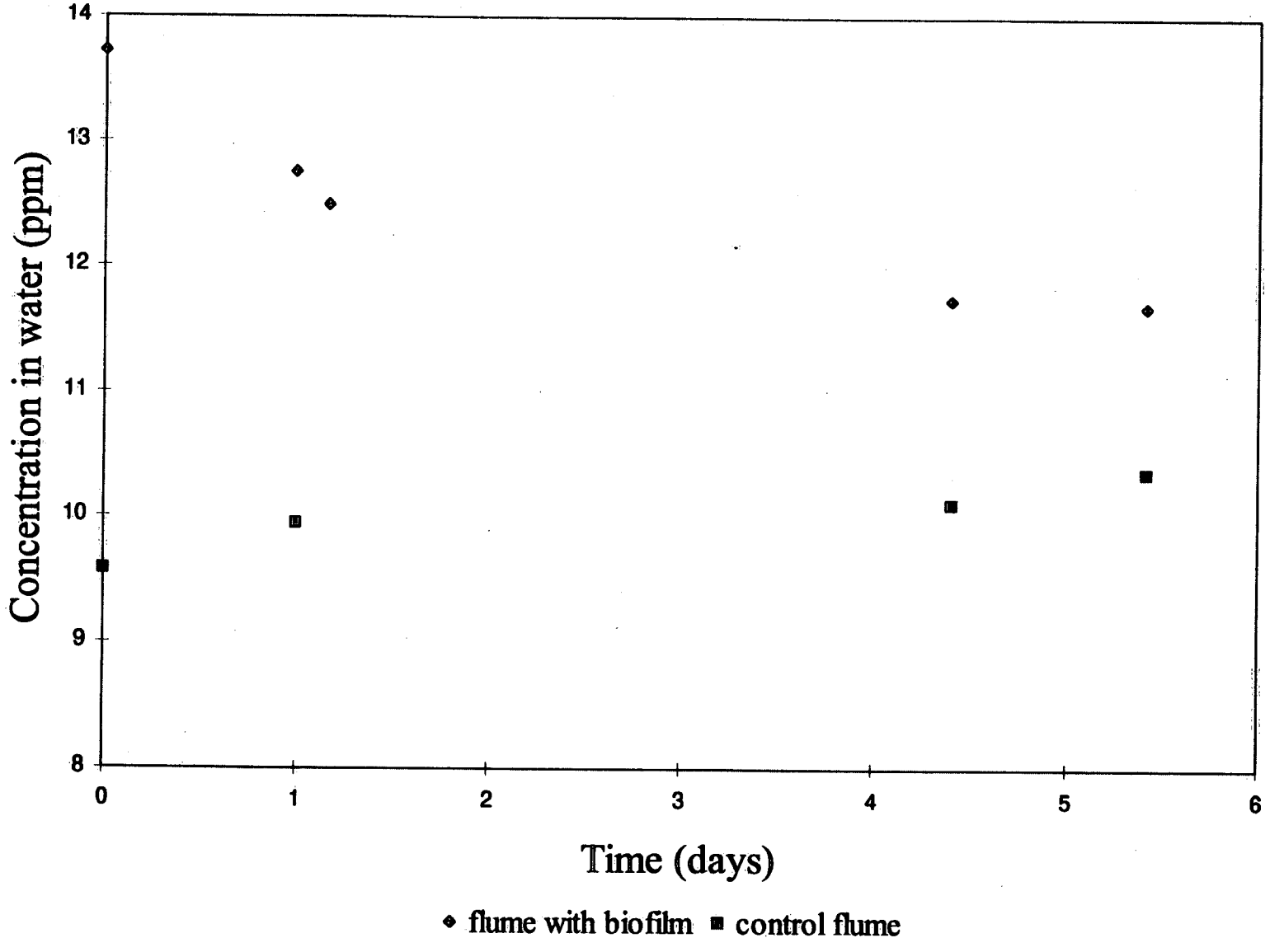


Figure 2. Cyanazine concentration versus time - outdoor flumes.

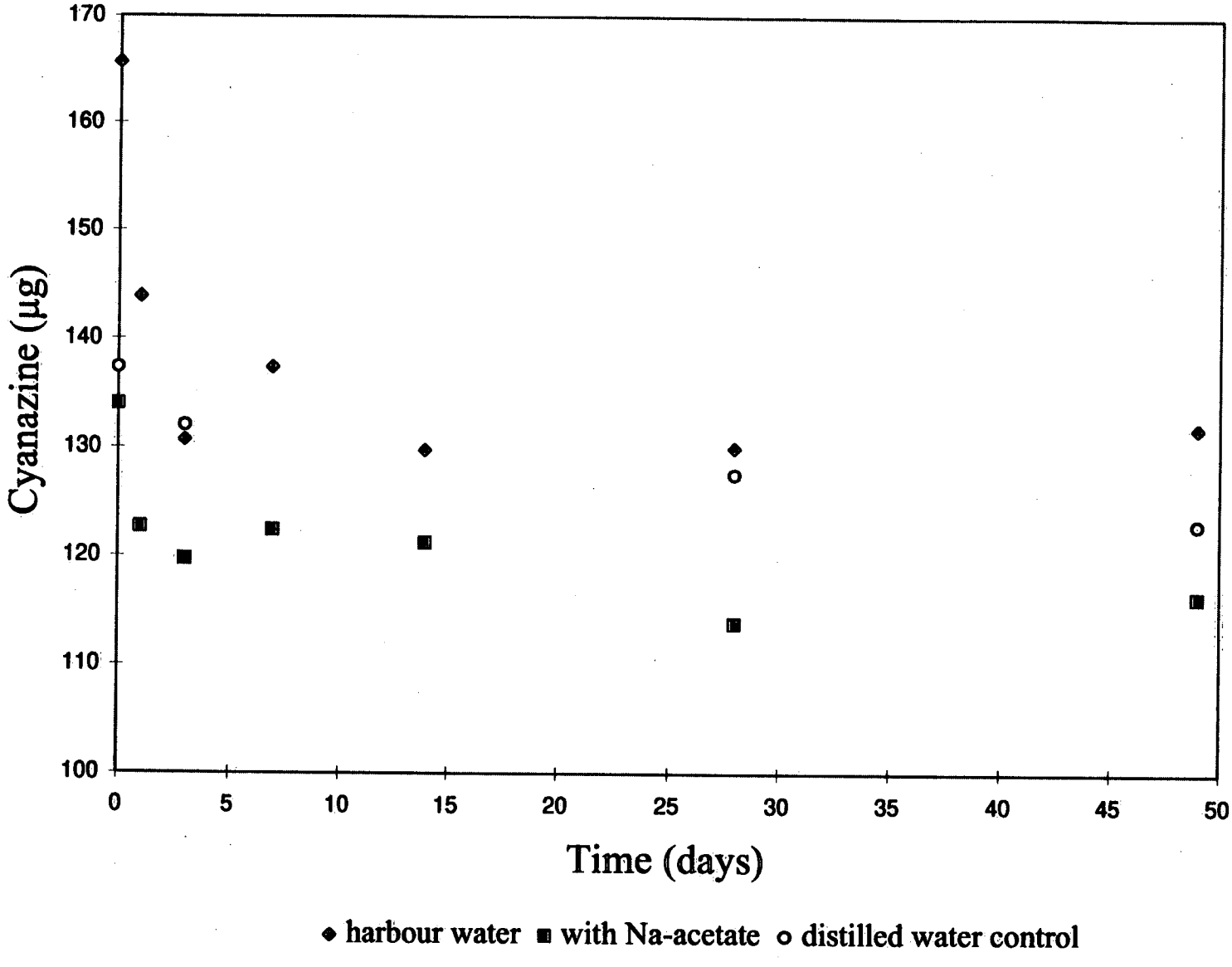


Figure 3. Cyanazine disappearance - water with bacterial biofilm.

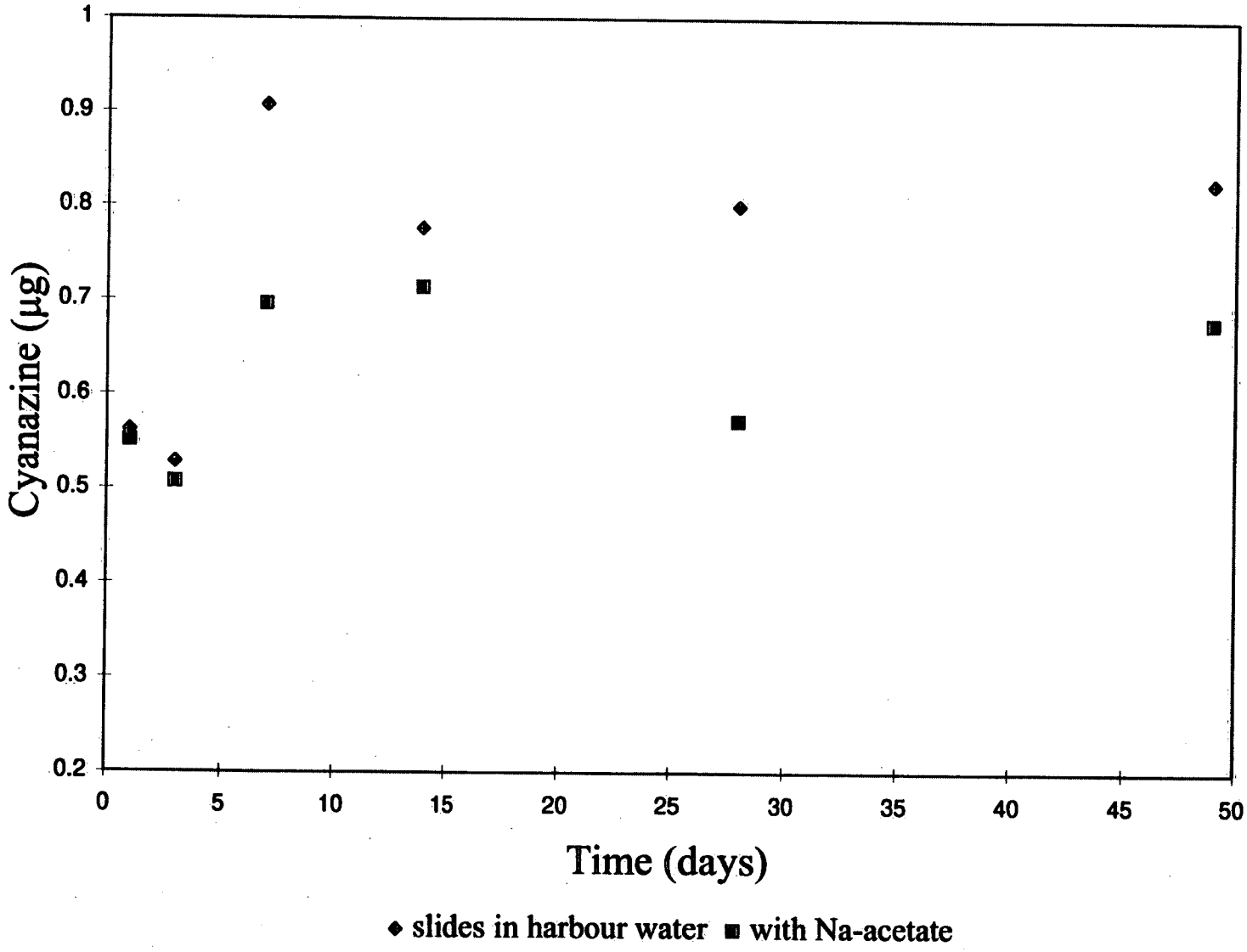
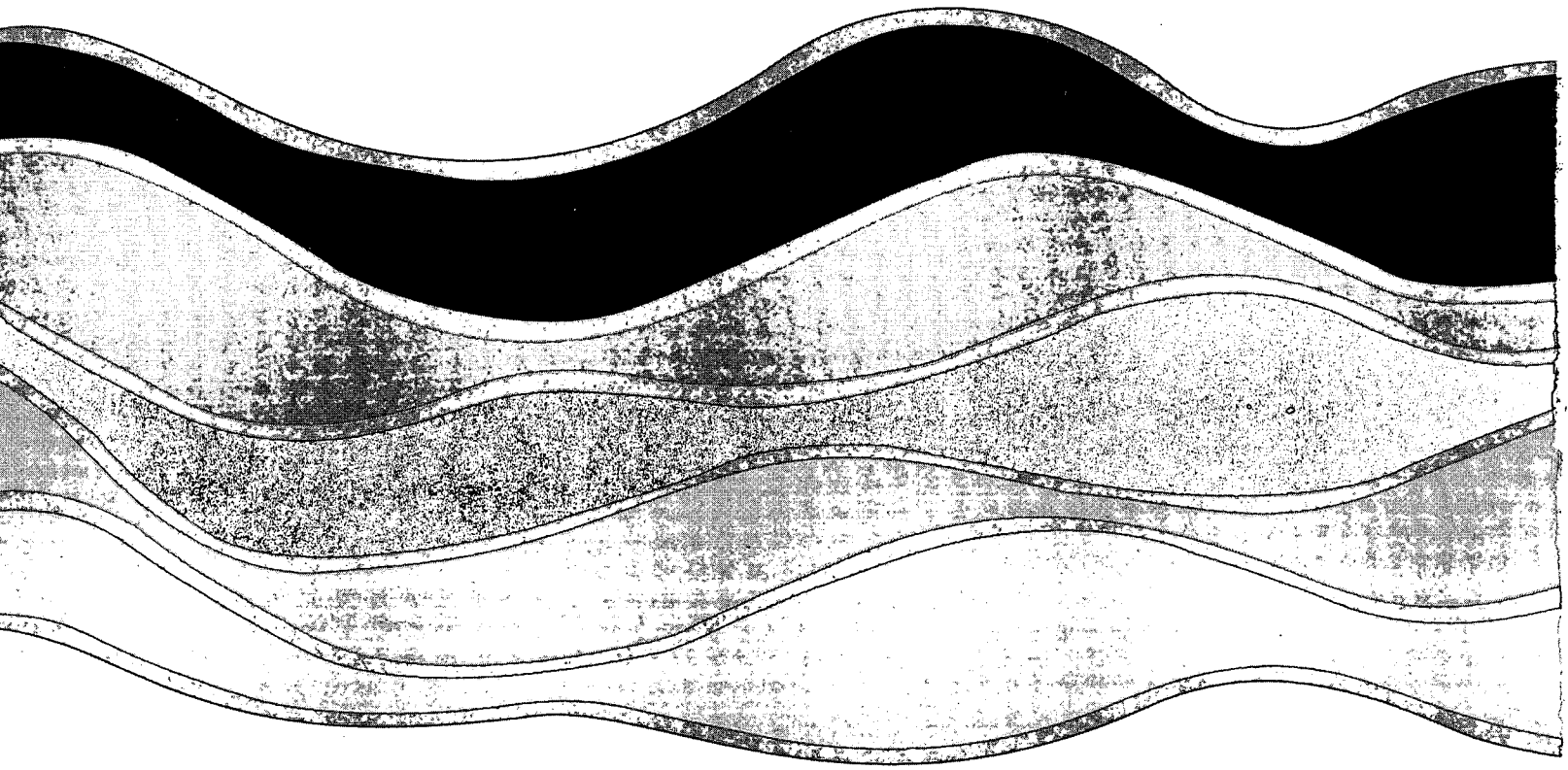


Figure 4. Cyanazine in biofilm slides.



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