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Absorption of Fenitrothion by Plankton and Benthic Algae

J. S. S. Lakshminarayana and H. Bourque
Department of Biology, Université de Moncton, Moncton, New Brunswick, Canada E1A 3E9

Fenitrothion, O, O-dimethyl O-(4-nitro-m-tolyl) phosphorotioate, an organophosphate insecticide, is used selectively for the protection of forests against the spruce budworm, Christoneura fumiferana (Clemens). Direct spraying, accidental spillages or the careless disposal of surplus concentrates or diluted spray liquids result in occasional discharges which enter streams, rivers, lakes, ponds and other aquatic environments. The fate of fenitrothion, in water (EIDT & SUNDARAM 1975), on benthos (EIDT 1975a, 1975b, 1976), on aquatic macrophytes (MOODY et al. 1978), on fish (SYMONS and HARDING 1974), on lobsters and their larvae (McLEESE 1974a, 1974b; WELLS 1975) and on crayfish (McLEESE 1976) have been presented. Studies of WURSTER (1969) on the effects of DDT and other insecticides on phytoplankton, BUSH and BATTERTON (1972) on the effects of aldrin, dieldrin and endrin on the growth rates of Anacystis nidulans and Agmenellum quadruplicatum, MAGNAMI et al. (1978) on the effects of chlordane and heptachlor on the marine dinoflagellate, Exuviella baltica Lohman, and STOCKNER and ANTIA (1976) amply illustrate the probable presence of algal sinks for pesticides in aquatic environments. Fenitrothion absorption by phyto- & zoo- plankton and benthic algae has not received much attention. The present study is a preliminary survey on the response of plankton and benthic algae of Peabody Lake, N.B. to fenitrothion spray formulations.

MATERIALS AND METHODS

Peabody Lake is situated north-west of Newcastle (47°08' and 66°10' N) and in the N.B. block-30 of the Forest Protection Ltd. This is one of the sites of study chosen by the Water Quality Branch, Environment Canada, Moncton, with which this program collaborated.

The active ingredient was applied at the rate of 3 oz/acre (210 g/ha) using T.B.M. Avenger aeroplanes flying about 35 to 40 m above the tree tops. The spray formulation contained 11 percent fenitrothion with Altox 3409 (1.5%), Arotex 3470 (1.5%) and water (86%). The spraying was carried out on the 3rd and 19th of June 1978. During the first spraying the sampling could be started immediately and continued over a 24 h period. Due to insufficient notice, the sampling was carried out only on the 21st of June 1978, nearly 48 h after the second spraying.

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Surface water samples were collected in clean B.O.D. bottles for dissolved oxygen (D.O.) and in clean Nalgene bottles for nitrate and nitrite nitrogens, phosphate, calcium, magnesium and total hardness. Samples for chlorophyll a, phaeophytin, and plankton were collected on clean and sterile 0.45 µm porous glass fibre filters using portable all-glass filter apparatus, 47 mm in diameter, under vacuum pressure and stored in individual glazed envelopes under dry ice. Phytoplankton, zooplankton and benthic algal samples were taken by using 20 and 25 µm mesh bolting silk nets respectively and immediately they were transfered into clean glass (Pyrex) bottles and preserved by adding 20 ml of A.C.S. reagent grade chloroform. All the samples were transported in a cooling chest with dry ice. With the help of environmental microbiological kits (millipore) using Chu-10, Erdschreiber and Miquel Allen culture media some of the phytoplankton was inoculated into the media for subsequent culturing and isolation in the laboratory. Twenty-four microscope slides were suspended in the surface waters (about 15.5 cm depth from the surface) by means of Kartell round slide carriers to find out the benthic surface component and its capacity to absorb fenitrothion. All the samples were stored at -60^{o} in the dark till they were tested.

Chlorophyll a and phaeophytin were determined on a Turner model III fluorometer after acetone extraction (HOLMES-HANSEN <u>et al</u>. 1965). Fenitrothion was determined by a gas chromatographic method on a Tracor 560 with NPD detector (BERKANE <u>et al</u>. 1977). Samples on filters were extracted with chloroform in an ultrasonic bath and dried with Na_2SO_4 and filtered on a Whatman no. 1 filter. Cellular and extracellular content of phyto- and zoo- plankton and benthic organisms were extracted with chloroform and tested in a similar way as other samples for fenitrothion.

Using the A.P.H.A. standard methods for the examination of water and wastewater (1971) temperature (mercury thermometer), pH (Beckman pH meter), dissolved oxygen (azide modification of Winkler), phosphate (ascorbic acid), nitrate (brucine), nitrite (diazotization), chloride (potentiometric), calcium and hardness (E.D.T.A. titrimetric), magnesium (deduction from Ca and hardness values) and suspended solids or matter (0.45 µm glass fibre filters and all pyrex glass assembly) were determined. The wet weights for suspended solids were taken after 24-48 h during which time the filters were kept dry on silica gel at -40^{o}C. Duplicate samples of water, plankton and others were taken and tested for verification. The testing was carried out by the Water Quality Branch, Moncton.

For identifying the algal species the following main sources have been employed: CLEVE (1894, 95), CLEVE-EULER (1915, 1951-5), GEITLER (1932), HUBER-PESTALOZZI and HUSTEDT (1942), HUSTEDT (1927-30, 1930a), IRENEE-MARIE (1938), PATRICK and REIMER (1966), PRESCOTT (1970), SMITH (1853-56), and TILDEN (1910). The original references were also consulted as and when necessary.

RESULTS AND DISCUSSION

Table 1 gives the water quality characteristics of the Peabody Lake and Brook. The waters of Peabody Lake are very soft with a hardness of 9.4 to 12.7 mg/l as $CaCO_3$, low alkalinity (1.0-3.7 mg/l), calcium (1.0 to 1.8 mg/l), magnesium (0.2-0.35 mg/l) and pH (6.6 to 6.8). The water temperatures on June 3rd, 1978 varied from 60 to 63°F (15° to 17°C with dissolved oxygen concentrations ranging from 8.6 to 9.1 µg/l which represent near saturation levels. The waters were clear and transparent at the sampling station. The chloride, nitrite and nitrate nitrogens represented below permissible limits of raw waters for drinking purposes. Chlorophyll a gradually increased from the end of May to the end of June indicating thereby the production of algal biomass of the waters to be poor in May and increasing in June 1978. The brook waters generally conform with the characteristics of Peabody Lake.

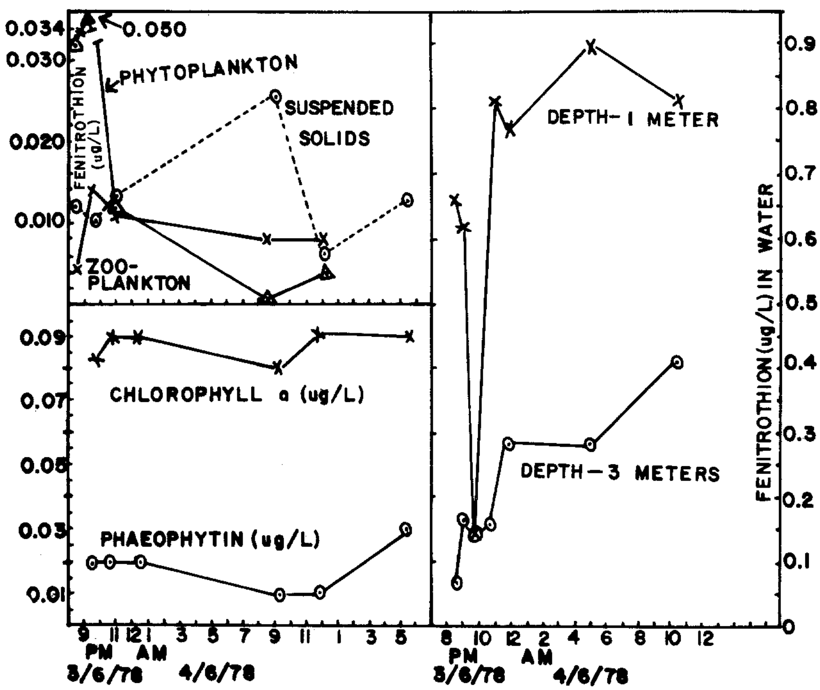

Fig. 1- Plankton pigments and fenitrothion in plankton, suspended solids and water of Peabody Lake.

Fenitrothion in water, suspended solids, phytoplankton, and zooplankton along with changes in chlorophyll a and phaeophytin during the first spray are shown in Fig. 1. Fenitrothion in water ranged between 0.06 to 0.09 µg/L. In the surface water the highest concentration of 0.9 µg/L was found on the 4th of June, eight and one half hours after spraying of the block. At 3 m depth the concentration of fenitrothion continued to rise till after thirteen and

Table 1 - Water quality characteristics of Peabody Lake and Brook

	pH	Temp	D.O. (mg/L)	Alkalinity (mg/L)	PO_4 (mg/L)	NO_3 (ug/L)	NO_2 (ug/L)	Chl a (ug/L)	Phaeophytin (ug/L)	Chloride (mg/L)	Ca (mg/L Ca)	Hardness (mg/L)	Mg (mg/L Ca)
31-5-78													
Lake	6.6	19°	8.9	2.6	.16	2.8	.1	.06	.03	0.6	1.6	9.6	0.30
Brook	6.6	19°	7.7	2.3	.18	4.0	T	.06	.04	0.6	1.6	9.8	0.30
3-6-78													
Lake													
8:30 p.m.		17°		3.6						0.5	1.0		0.20
9:40 p.m.	6.6	17°	9.1	2.0	.16	3.4	.70	.08	.02	0.5	1.6	9.5	0.28
10:40 p.m.	6.6	17°	8.6	1.8	.16	2.8	.50	.09	.02	0.4	1.6	10.6	0.32
4-6-78													
Lake													
12:30 a.m.	6.6	17°	9.5	2.1	.10	2.9	T	.09	.02	0.5	1.8	10.2	0.30
9:00 a.m.	6.6	16°	9.0	2.3				.08	.01	0.5	1.5		0.25
12:00 a.m.	6.7	17°		1.1				.09	.01	.07	1.5		0.30
6:00 p.m.	6.6	17°		2.2				.09	.03	0.5	1.5		0.27
3-6-78													
Lake	6.7	17°		1.9	.20	2.2	.10			0.4	1.7	9.8	0.28
Brook		17°		2.8						0.5	1.0		0.2
14-6-78													
Lake	6.8	18°		2.4	.16	1.2	T	.13	.10	0.6	1.2	9.4	0.2
21-6-78													
Lake	6.7	18°		1.1	.20	0.7	.1	.12	.05	0.6	1.6	12.7	0.35
Brook		18°		2.3	.14	2.8	.5			0.6	1.5	9.8	0.3

half hours after spraying and the highest concentration was 0.42 µg/L. Samples of water, suspended solids, phytoplankton and zooplankton taken during the second spray showed only traces of fenitrothion. EIDT and SUNDARAM (1975) concluded that in New Brunswick streams,where water temperatures and pH values varied from 11^{o} to 15^{o}C and 6.1 to 7.2 respectively, when sprayed at 2-3 oz/ac (140-210 g/ha) most peak concentrations of fenitrothion will be lower than 15 µg/L and diminish very rapidly assuming about 80 h half life with doubling of the rate of hydrolysis with a 10^{o}C rise in temperature. The results of our first and second survey seem to be in agreement with the conclusion that the fenitrothion in water diminishes rapidly under similar conditions given by EIDT and SUNDARAM (1975).

The following organisms were present in the Peabody Lake.

Algae: *Dictyosphaerium ehrenbergianum* Naegeli, *Scenedesmus bijuga* (Turp.) Kützing, *Chlorococcum* sp., *Stigeoclonium* sp., *Zygnema* sp., *Spirogyra* sp., *Closterium setaceum* var. *vittatum* Grönbel, *Closterium braunii* Reinsch, *Closterium* sp., *Dinobryon bavaricum* Imhof., *Stylochrysalis parasitica* Stein, *Pseudokephyrion entzii* f. *granulata* Bourr., *Cyclotella meneghiniana* Kützing, *Melosira italica* (Ehrenberg) Kützing, *Tabellaria fenestrata* (Lyngbye) Kützing, *Tabellaria flocculosa* (Rothest) Kützing, *Diatoma hiemale* (Rothest) Heibourn, *Diatoma hiemale* var. *mesodon* (Ehrenberg) Grunow, *Fragilaria brevistriata* Grunow, *Fragilaria crotonensis* Kitton, *Fragilaria* sp., *Synedra splendens* Kützing, *Syndra ulna* (Nitzsch) Ehrenberg, *Eunotia* sp., *Frustulia rhomboides* (Ehr.) De Toni, *Diploneis elliptica* (Kützing) Cleve, *Gomphonema* sp., *Navicula cryptocephala* var. *veneta* Kützing, *Navicula* sp., *Navicula minima* Grunow, *Surirella splendida* (Ehrenberg) Kützing, *Ceratium* sp., *Agmenellum quadriduplicatum* var. *glauca* Palmer, *Oscillatoria* sp., *Anabaena* sp., and *Batrachospermum* sp.

Zooplankton:

Kellicoltia sp., Nauplii of *cyclops*, *Calanus* sp., Mermithidae (Rainworms).

Before spraying, the suspended solids of the lake waters showed fenitrothion (0.005 to 0.025 µg/L). During spraying the concentration increased 0.01 to 0.26 µg/L by 9:00 a.m. on the 4th of June 1978 and thereafter it decreased (Fig. 1). The aerial drift could be responsible for the presence of low concentrations of fenitrothion in the suspended solids prior to the 1978 operations. The planes were operated from the Sevogle Air Base which is very near to the Peabody Lake.

Phytoplankton net samples did not show any fenitrothion before spraying. All the samples during and after spray showed fenitrothion. The highest concentration detected was 0.05 µg/L (cf. Fig. 1) showing thereby the uptake of fenitrothion by the algae. Zooplankton showed a maximum of 0.014 µg/L of fenitrothion. The presence of phaeophytin indicated the probable zooplankton grazing over phytoplankton.

Of the two samples of Batrachospermum one gave positive (0.07 ng/µL) and the other negative result for fenitrothion. Similarly only one of the two microscopic slide samples showed 0.3 ng/µL of fenitrothion. The suspended slides under microscope revealed several diatoms and few filaments of zygnema along with fine sediment.

From the above results the percentage of the fenitrothion associated with the algae in the water column varies from 0.24 to 5.45%. Using an unialgal culture of Chilomonas marina Braarud Halldal, which was isolated from the phytoplankton of the coastal waters of Northumberland Strait, a laboratory test was conducted to determine the capacity of this alga to absorb fenitrothion. One hundred and 500 µg of fenitrothion were used on 500 ml cultures respectively. The algal weight recovered from these cultures was 1.2081 g and 1.3507 g with fenitrothion recoveries of 11 µg and 18 µg respectively. This preliminary test indicated the capacity for absorption of this pesticide.

The present studies showed that fenitrothion will be absorbed or actively taken up by plankton, particularly the phytoplankton. The degree of absorption depends on various environmental conditions particularly their population numbers, seasonal variations, bloom formation, growth rates etc. It is not always possible to spray the forests when there are no planktonic growths in the waters. Surface drainage and aerial spray could carry fenitrothion into the water bodies. The analysis of accumulated fenitrothion in algae might be a feasible way to detect its contamination of aquatic systems when the concentration in water itself was too dilute to be analyzed.

The absorbtion or accumulation of fenitrothion by algae in water could contribute to the rapid diminution of fenitrothion in water itself, as well as to the transfer of residues to shellfish and other animals which consume algae. The algal species found in the Peabody Lake are known to occur in clean and unpolluted waters and their presence does not suggest any toxicity or pollution by fenitrothion.

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REFERENCES

BERKANE, K., G.E. CAISSIE and V.N. MALLET: Jour. chromatography 139, 386 (1977).

BOUSH, G.M., and J.C. BATTERTON: Ecological aspects of pesticide-microbial relationships in Environmental Toxicology and Pesticides, edited by MATSUMURA, F., G.M. BOUSH and T. MISATO, Academic Press, N.Y., 401-422 (1972).

CLEVE, P.T.: Synopsis of the Naviculoid Diatoms. Kongliga Svenska Vetenskaps - Akademiens Handlinger 26, 1-1974; 27, 1-219 (1894 and 1895).

CLEVE-EULER, A.: Archiv für Botanik 14, 1 (1915).

CLEVE-EULER, A.: Die Diatomeen von Schweden und Finland. Kongliga svenska Vetenskaps-Akademiens Handlinger, Ser. 4 I. 2: 1-163 (1951); V. 3, (3), 1-153 (1952; II 4, (1); 1-158 (1953a); III 4 (5), 1-255 (1953b); IV 5 (4), 1-232 (1955).

EIDT, D.C.: Can. Ent. 107, 743 (1975a).

EIDT, D.C.: Maritimes Forest Res. Centre, Info. Rep. M-X-49, 1-9 (1975b).

EIDT, D.C.: Maritimes Forest Res. Centre Info. Rep. M-X-65, 1-17 (1976).

EIDT, D.C. and K.M.S. SUNDARAM: Can. Ent. 107, 735 (1975).

HUBER-PESTALOZZI, G. and F. HUSTEDT: Die Keiselalgen. In: A. THIENEMANN (ed.), Das Phytoplankton des susswassers, Die BINNENGEWASSER, Band XVI, Teil II, Halfte II. E. Schweizerbartsche Verlagsbuch-handlung, Stuttgart (Stechert, N.Y., reprinted 1962), 365-549 (1942).

HUSTEDT, F.: Die Kieselalgen Deutschlands, Osterreichs und der Schweiz mit Beruschsichtigung der ü brigen Länder Europas sowie der argenzenden Meeresgebiete, in Rabenhorst, L. Kryptogamen - Flora 7. Pt. I, 1-272; 274-464 (1928); 465-608 (1929); 609-784 (1930); 785-925 (1930).

HUSTEDT, F.: Bacillariophyta In: Pascher, A., Die Susswasser-Flora Mittleleuropas. ed. Pascher, 10, 1-466 (1930).

HOLM-HANSEN, O., C.J. LORENZEN, R.W. HOLMES and J.D.H. STRICKLAND: J. Cons. Perm. Int. Explor. Mer 30, 3-15 (1965).

MAGNAMI, B.,C.D. POWERS, C.F. WURSTER and H.B. O'CONNORS Jr.: Arch. Environ. Conta. Toxicol. 20, 1 (1978).

MALONEY, T.E. and W.E.MILLER: In water Quality Parameters, S.T.P. 573, A.S.T.M., 344-355 (1975).

MCLEESE, D.W.: J. Fish. Res. Bd. Can. 31, 1127 (1974a).

MCLEESE, D.W.: J. Fish. Res. Bd. Can. 31, 1556 (1974b).

MCLEESE, D.W.; Bull. Environ. Contamin. Toxicol. 16, 4, 411-416 (1976).

MOODY, R.P., R. GREENHALGH, L. LOCKHART, and P. WEINBERGER.: Bull. Environ. Contamin. Toxicol. 1, 1 (1978).

SMITH, W.M.: A Synopsis of the British Diatomaceae, 1, 1-89; 2, 1-104; ed. JOHN VAN VOORST, Paternoster Row, London.

STANDARD METHODS FOR THE EXAMINATION OF WATER, SEWAGE & INDUSTRIAL WASTES: 13th ed., A.P.H.A., A.W.W.A., and W.P.C.F., 1-874 (1971).

STOCKNER, J.G., and J. ANTIA.: J. Fish. Res. Bd. Can. 33, 2089 (1976).

SYMONS, P.E.K. and G.D. HARDING. Fish. Res. Bd. Can. Tech. Rept. 432, 1-47 (1974).

TILDEN, J.: Minnesota Algae, Vol. I, The Myxophyceae of North America and adjacent regions including central America, Greenland, Bermuda, the West Indies and Hawaii, University of Minnesota (Reprinted 1969, in Bibliotheca Phycologica, 4, J. Cramer, Lehre, Germany) 1910.

WELLS, P.G.: In Proc. Aquatic Toxicity Workshop, Ontario Ministry of Environment, Nov. 4-5, Toronto, 1-339 (1975).

WURSTER, D.F.: BioScience 19, 809 (1969).