# Impacts of cypermethrin and deltamethrin's use on aquatic invertebrates in commercial aquaculture ponds

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Abstract. Agricultural pyrethroid insecticides especially cypermethrin and deltamethrin are being used regularly for the control of aquatic insects in commercial carp aquaculture ponds in northwest Bangladesh. Thisstudy was conducted in a farmer's ponds under commercial aquaculture to know the impact of cypermethrin and deltamethrin on aquatic invertebrates (insects, zooplanktons and benthos). Commercial aquaculture ponds were treated with cypermethrin and deltamethrin @ 5  $\mu$ g (active ingredient)/liter pond water in presence of fish. Required amount of each of the insecticides were diluted with water in big aluminium pots and broadcasted over the pond water. Water quality parameters of the experimental ponds were measured at pretreatment stage. Sampling of aquatic invertebrates was done pretreatment and after 1 day, 2 days, 5 days, 10 days, 15 days, 21 days and 28 days of the application of insecticides. No fish mortality was recorded after the treatment. Aquatic insect population was found to be most affected;number count declined89.87% and 86.24% for cypermethrin and deltamethrin respectively within one day after treatment. The insect count recovery began after day 10 and day 5 of cypermethrin and deltamethr in treatment respectively. Loss of insect diversity was observed in cypermethrin treatment and diversity count did not recover within observation period of 28 days.Zooplankton populationwas relatively less impacted (21.10% and 26.33% declined respectively in cypermethrin and deltamethrin treatment). Benthos population count and diversity was not affected, possibly due to the high organic carbon content and clay soil of the pond bottom. Key words: Pyrethroids, Cypermethrin, Deltamethrin, Aquatic invertebrates

## Introduction

Aquatic insects are a common part of any aquatic ecosystem. Nasiruddin et al. (2014) reported higher abundance (number) of aquatic insects in pond system compared to the lake in Chattogram, Bangladesh. Tidwell et al. (1997) found macro-invertebrate densities to be significantly higher in fed and fertilized ponds than in unfed ponds. Aquatic insects are also common in commercial carp aquaculture ponds of northwest Bangladesh due to intensive feeding and fertilization. Kashyapet al. (2013) recorded the presence of aquatic insects in various fishponds including nursery, rearing and stocking ponds of northern and central states of India, many of which were detrimental to aquaculture.Dragonfly nymph/larvae were found to be a most effective common carp fry predator by Gonzalez and Leal (1995). Corbet (1980) described odonate (dragonfly) larvae as the exclusive predator in the aquatic environment. Marco et al. (1999) found that medium size dragonfly species were more abundant and potential predators of fish fry in aquaculture ponds of south-east Brazil. Additionally, backswimmer (Anisops sp.)was found to be causing predation-related mortality of carp fry in Lao PDR by Sano et al. (2011). Increased size of backswimmers increased their predation potential (Gonzalez and Leal 1995) and can prey onup to 46.7% carp fish larvae within 24 hours in nursery ponds (Sano et al. 2011). Water scorpions (Nepa sp. and Ranatra sp.) also attack living insects and fish fry (Kashyapet al.2013). Predation of fish fry by water insects was found to be proportionate to the size ratio of water insects and fish fry:larger insects could capture larger fish fry (Gonzalez and Leal

1995). Predation of fish fry by aquatic insects is primarily limited in nursery ponds but and not in culture ponds of table fish.

Despite having no predation risk, commercial carp farmers of northwest Bangladesh consider aquatic insects hazardousfor table fish in aquaculture ponds due to the potential competition of supplied feed for fish and disturbances posed to fish by large number of aquatic insects. Therefore, carp farmers of northwest Bangladesh were found to be using pyrethroid insecticides (cypermethrin and deltamethrin) once in a monthat low concentrations in aquaculture ponds to keep the aquatic insect population under control. This study was conducted to understand the impact of cypermethrin and deltamethrin's use on aquatic insect, zooplankton and benthos populations of ponds in northwest Bangladesh as practiced by the farmers.

# **Materials and Methods**

The study was conducted in ponds under commercial aquaculture in Hatgodagari area of Poba Upazilla of Rajshahi district. Farmers were asked to inform the author before using pesticides in their ponds so that the author could conduct the study. Accordingly, a farmer informed the author prior to using cypermethrin (brand name Ripcord, BASF Bangladesh ltd., distributed byPadma Oil Company limited) and deltamethrin (brand name Desis, marketed by Bayer Crop Science limited Bangladesh) in commercial aquaculture ponds measured over 2 acres in size (water area) respectively in November 2018 and March 2019  $@5\mu g$  (active ingredient)/liter pond water for both insecticides.Before the use of the insecticides water quality parameters of the treatment ponds were measured. Soil samples from the bottom of both experimental ponds were collected at pretreatment stage, sundried, grounded, and tested for organic carbon content by the Soil Resource Development Institute (SRDI) laboratory in Rajshahi. Required quantity of both insecticides were diluted with water in a large aluminium pots and manually broadcasted along the water's edge of the whole pond. The farmer made sure that no supplementary feed was supplied to the fish on the treatment day and the following day. The sampling of zooplankton, aquatic insects and benthos was done before the treatment and on days 1, 2, 5, 10, 15, 21, 28 following the cypermethrin and deltamethrin treatment.

**Sampling of aquatic insects:** A one square meter fine meshed net fitted in a bamboo frame was used for samplingof aquatic insects. The net was towed in the water along the edges of the pond for a three-meter distance. Then the accumulated insects from the net were transferred to a plastic containercontaining water and 10 ml of formalinwas added. The sampling process was repeated three times to be considered as three replications for sampling of both treatments.

**Sampling of zooplankton:** A funnel shaped plankton net with specificationsof75 to 85 microns mesh sizewas fitted with a collecting bottle on the tapering end anda round metal frame on the open end. During each sampling the plankton net was towed for a 13-meter distance in the water, one foot below the surface. Then the sample was transferred to a plastic bottle and 5 ml formalin was added. The collected samples were transferred to the lab, where the total volume was measured, representing the amount of zooplankton per volume of water the net was traversed through and calculated using following formula: Water volume in liter = ' $\pi$  (3.14)' multiplied by the'square radius of the plankton net metal frame in meters' multiplied by the'distance the net traversed through in meters' multiplied by1000. Zooplankton were identified and counted using a Sedgewick rafter cell counter (Welch 1948) and microscope.

**Sampling of benthos:** A metal scoop with a 38.7 square centimeter opening was used to collect the soil samples from the bottom of the pond at 30-centimeter depth from the surface of the water. The process was repeated three times during each sampling to be considered as three replications for both treatments. In the lab, the collected soil samples were put under running tap water on a fine meshed sieve, through which all soil particle was washed away and the separated benthos samples were collected.

## Results

Water pH in both treatmentswas found to be around 7.5. Water turbidity was higher (43.19 ntu) in the deltamethrin treated pond than in cypermethrin treated pond (28.05 ntu). Electrical conductivity was also higher in deltamethrin treatment (516  $\mu$ S/cm) than in cypermethrin treatment (310  $\mu$ S/cm). Organic carbon content of the pond bottom soil was 2.19 and 3.40% (dry weight basis) respectively for cypermethrin and deltamethrin treatment (Table I).

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Treatment	Water	Water	Water turbidity	Water electrical	Organic carbon content of the
ponds	pН	temp (°C)	(ntu)	conductivity ( $\mu$ S/cm)	pond bottom sludge (%)
Cypermethrin	7.41	23.1	28.05	310	2.19
Deltamethrin	7.70	30.4	43.19	516	3.40

Within one day after use of cypermethrin and deltamethrin, the total insect count went down (90 and 86% respectively for cypermethrin and deltamethrin) for both treatments to reach to their lowest point of the study period (Fig. 1). The total insect count for deltamethrin treatment went up sharply above the pretreatment levelfive days after the treatment and continued to beat a near static level until the end of the observation period. Increase of the insect-count in cypermethrin treatment levelswithin the observation period. Insect diversity went down after use of cypermethrin in the aquaculture pond. Insect diversity was lowest at day-2 after the use of cypermethrin (Fig. 1). The diversity gradually increased up to six types of insects at the end of the observation period of 28 days, while eight types of insect were recorded at pretreatment stage.



**Fig. 1.**Total count of aquatic insects (nos/3 sq meter) and their diversity before and after use of cypermethrin and deltamethrin in commercial aquaculture ponds.

## IMPACTS OF CYPERMETHRIN & DELTAMETHRIN'S USE ON AQUATIC INVERTEBRATES

One day after the use of deltamethrin, the population of both boatmen and backswimmer declined with the decline of backswimmer deeper (92.59%) that that of boatman (86.36%) population. The backswimmer population continued to remain at a low level until the  $10^{th}$  day after the treatment and then increased slowly. On the contrary boatman population sharply increased at day 5 after the treatment above the pretreatment level and maintained that density until the end of the observation period of 28 days (Fig. 2).



Fig. 2.Aquatic insect count (nos/3 sq meter) at various points of deltamethrin treatment @  $5\mu g$ /liter of water in commercial aquaculture pond.

Backswimmer, damselfly nymph and water boatman were the most dominant water insects in the pretreatment stage of the cypermethrin application. A decline of count by 93.22%, 85.9% and 96.42% was recorded respectively for damselfly nymph, backswimmer and boatman within 24 hours of the use of cypermethrin (Fig. 3). Boatmen count increased sharply at 15 days after the treatment while the backswimmer population first rose and then declined at day10 after the treatment. All other types of insects except *Nepa* sp., dragonfly nymph and creeping water beetle showed slow recovery 15 days post treatment. *Nepa* sp. and dragonfly nymph was observed on day1 after the cypermethrin treatment and did not return until the end of observation period.



Fig. 3. Insect count (nos/3 sq meter) at various points before and after use of cypermethrin @5  $\mu$ g (active ingredient) /liter in commercial aquaculture ponds.

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The decline of the zooplankton count was 26.33% on day 2 after the deltamethrin treatment and 21.10% on day 5 after the cypermethrin treatment, reaching their lowest level compared to the pretreatment level (Fig. 4).Recovery of the zooplankton population occurred earlier and more rapidly in deltamethrin treatment than in cypermethrin treatment. Zooplankton count exceeded the pretreatment level at day 5 and day 10 respectively afterdeltamethrin and cypermethrin treatment. The higher count of zooplankton for both treatments (after recovering from their lowest point) was maintained until the end of the observation period on28<sup>th</sup>dayafter the treatment.



**Fig. 4.** Zooplankton count (nos/liter) before and after use of cypermethrin and deltamethrin in commercial aquaculture pond.

Zooplankton diversity showed little change in both the cypermethrin and deltamethrin treatments (Table II). The count of *Asplanchna* sp., *Daphnia* sp. and *Filinia* sp. increased towards the end of the observation period of 28 days after cypermethrin treatment, however without any specific evidence it is hard to attribute that increase to any specific factor. *Trichocerca* sp. is the only zooplankton that was found in small numbers in the pretreatment stage of cypermethrin experiment but not found again during thepost treatment observation period of 28 days.

Total benthos counts for both cypermethrin and deltamethrin treatmentchanged little from their pretreatment level and almost remain static till the end of the observation period of 28 days after treatment (Fig. 5). Like the benthos count, benthos diversity was not affected by both treatments (Table III).



**Fig. 5.** Benthos count (nos/38.7 sq. cm. pond-bottom) before and after use of different insecticide in ponds under commercial aquaculture.

Insecticide	Benthos	Pre-	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
		treatment							
	Brachionus sp.	$995 \pm 269$	$751\!\pm\!104$	$803 \pm 32$	$546 \pm 32$	$653 \pm 183$	$907 \pm 48$	$756 \pm 12$	$727 \pm 71$
	Keratella sp.	$333 \pm 204$	$391 \pm 140$	$428 \pm 21$	$510 \pm 56$	$602 \pm 224$	$1037 \pm 177$	$727 \pm 53$	$679\pm78$
	Cyclops sp.	177±59	$69 \pm 23$	$14 \pm 12$	$60 \pm 26$	$140 \pm 46$	$150 \pm 89$	$241 \pm 118$	$145 \pm 36$
	Nauplius	$622 \pm 183$	$232 \pm 39$	$184 \pm 67$	$249 \pm 54$	$248 \pm 95$	$339 \pm 92$	$326 \pm 58$	$435 \pm 27$
	Polyarthra sp.	$148 \pm 181$	$263 \pm 117$	$198 \pm 110$	$53 \pm 58$	$159 \pm 112$	$228 \pm 159$	$214 \pm 67$	$392 \pm 93$
-	Diaptomus sp.	$7 \pm 12$	$7 \pm 13$	-	$22\pm 22$	$7 \pm 13$	$23\pm 23$	$14 \pm 12$	$23 \pm 23$
Li	Asplanchna sp.	$7 \pm 12$	$147 \pm 103$	89±16	$175 \pm 55$	$323 \pm 158$	$445 \pm 116$	$473 \pm 70$	$321\pm32$
leth	<i>Daphnia</i> sp.	$14 \pm 25$	$15 \pm 27$	$52 \pm 14$	$83 \pm 11$	$27 \pm 16$	$86 \pm 17$	$53 \pm 32$	$53\pm36$
ern	Moina sp.	$7 \pm 12$	-	-	$7 \pm 12$	-	-	-	-
Cyp	Filinia sp.	$125\pm126$	$108 \pm 81$	$141 \pm 104$	195 <u>+</u> 87	$220 \pm 63$	$282 \pm 92$	$183 \pm 34$	$260\pm75$
	Trichocerca sp.	$14 \pm 25$	-	-	-	-	-	-	-
	Brachionus sp.	$936 \pm 80$	$718 \pm 8$	$591\pm32$	$1628 \pm 161$	$1133\pm220$	$1040 \pm 179$	$1123\pm\!117$	$1151\pm96$
	<i>Keratella</i> sp.	$720 \pm 34$	$520\pm73$	$432\pm42$	$570 \pm 108$	$1321\pm61$	$1125\pm183$	$1004\pm67$	$1159\pm53$
	Cyclops sp.	$263\pm104$	86±34	98±66	$192 \pm 41$	$121 \pm 47$	$210 \pm 61$	$217 \pm 33$	$172 \pm 45$
	Nauplius	$632 \pm 86$	$505 \pm 35$	$439\pm81$	$529\pm126$	534 <u>+</u> 67	516 <u>+</u> 84	$531\pm100$	598±44
	Polyarthra sp.	$714 \pm 60$	$725 \pm 51$	$912\pm94$	$721 \pm 62$	$647\pm181$	$59 \pm 164$	$654 \pm 61$	$646\pm58$
	Diaptomus sp.	-	-	$7 \pm 12$	$64 \pm 14$	8±13	7 <u>+</u> 13	-	7±13
	Asplanchna sp.	$650 \pm 58$	$552 \pm 70$	$577\pm31$	$810 \pm 69$	$794 \pm 32$	$664 \pm 79$	$733\pm88$	$772 \pm 61$
eltamethrin	<i>Daphnia</i> sp.	$143 \pm 109$	$148\pm56$	$75\pm71$	$144 \pm 22$	$129 \pm 75$	$69 \pm 46$	$69 \pm 83$	$125\pm11$
	Moina sp.	-	-	-	-	8±13	-	-	-
	Diphanosoma sp.	$15 \pm 13$	$7 \pm 13$	$37 \pm 34$	-	-	-	-	-
	Filinia sp.	$182\pm158$	$23 \pm 39$	$15 \pm 26$	$16 \pm 28$	$15 \pm 27$	-	$23 \pm 41$	$39\pm26$
1	Trichocerca sp.	$65 \pm 113$	$16 \pm 27$	-	-	$56 \pm 50$	$15 \pm 13$	$31 \pm 56$	$46 \pm 22$

 
 Table II. Zooplankton count (nos/liter water) before and after of cypermethrin and deltamethrin treatment in commercial aquaculture ponds

Table III.	Benthos o	count	(nos/38.7	square	meter	bottom)	before	and a	fter o	of cyperm	ethrin
	and d	leltam	ethrin tre	eatment	in con	ımercial	aquaci	ulture	pond	s	

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Insecticide	Benthos	Pre-	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
		treatment							
	Chironomid	$27.0\pm7$	$29.3 \pm 13$	$18.7 \pm 18$	$20.7 \pm 7$	$31.0 \pm 28$	$34.3 \pm 16$	$38.3\pm7$	$38.7\pm8$
	larvae								
Cypermethrin	Tubifex	$6.0 \pm 1$	$3.7 \pm 1$	$8.0 \pm 4$	$3.0 \pm 1$	$3.0\pm 2$	$6.0\pm3$	$7.7 \pm 4$	$8.3 \pm 2$
	Leech	$0.7 \pm 0.6$	$1.0 \pm 1.0$		$1.0 \pm 1.0$		$1.0 \pm 1.0$	$0.3 \pm 0.6$	$0.7 \pm 1.2$
Deltamethrin	Chironomid	$151.3 \pm 25$	$174.3 \pm 12$	$165 \pm 25$	$171.3 \pm 25$	$186.3 \pm 8$	$193.3 \pm 9$	$190 \pm 17$	$179.3 \pm 12$
	larvae								
	Tubifex	$44.7 \pm 8.0$	$36.7 \pm 5.5$	$33.3 \pm 6.0$	$45.3 \pm 7.4$	$50.0 \pm 2.6$	$41.0 \pm 9.6$	$44.0 \pm 7.9$	$43.0 \pm 4.6$

## Discussion

No fish mortality was recorded during this study, despite of the doses (5  $\mu$ g/liter)in this study exceeding the acute toxicity concentration for silver carp (Shaluei *et al.* 2012), *Cirrhinus mrigala* (Veni and Veeraiah 2014) and *Labeo rohita* (Tiwari *et al.* 2012, Das and Mukherjee, 2003) regarding cypermethrin; common carp (Datta *et al.* 2003) and *Labeo rohita* (Suvetha *et al.* 2015) regarding deltamethrin. During this study the fish that were in the ponds were a minimum of 0.75 kg in size (mostly adults) and were less susceptible to the cypermethrin and deltamethrin than at their juvenile stages (Mohammed2013) due to their larger body size (NPIC 2010). All of lethal concentration dose-determination experiments cited above, were conducted in laboratory

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conditions (room temperature and tap water) using small individuals (juveniles and fingerling) as subject, which is different than the culture conditions (temperature, turbidity, water hardness, pH, light etc.) of commercial aquaculture ponds used in this study. Day (1991) also mentioned a lesser impact of pyrethroid pesticides in field conditions than the impact predicted by laboratory test data.

Datta *et al.* (2003) found that temperature, water hardness and turbidity profoundly impacted the toxicity of deltamethrin on common carp, where toxicity was lowest (higher  $LC_{50}$  concentration) at 30°C temperature, in hard water as compared to soft water, and in the presence of soil particles (humus, clay, organic carbon) where deltamethrin was absorbed by the soil. Cypermethrin is also hydrophobic, having low water solubility (Jones 1992) and strong absorption tendencies into soil particle. Hydrolysis is faster in a basic solution and the pesticide photodegrades rapidly with half ranges from 8 to 16 hours, with microbial degradation also possible (ETN 1996). Roberts and Hudson (1999) found that biodegradation of deltamethrin can be stalled due to its strong absorption by particulate organic matter. Compared to other pyrethroids deltamethrin has a potential of volatilization to the air from water, with an average half-life found to be 2.5 days in pH 9 solution. Microbial action, photolysis and hydrolysis also degrades deltamethrin (NPIC 2010). High turbidity, pH, electrical conductivity and favorable water temperature (Table I) of the treatment ponds have reduced the effect of both cypermethrin and deltamethrin to the level where it was nontoxic to the fish in the experimental pond.

Total insect count bounced back earlier (Fig. 1) in the deltamethrin treatment primarily due to the higher water turbidity, pH and conductivity that reduced the deltamethrin's toxicity (Table I) sooner than that of cypermethrin. Mulla*et al.* (1982) found 50 to 100% mortality of arthropod insects in field ponds caused by pyrethroid insecticides and 2 to 4 weeks after the treatment were required for their recovery of arthropod insects to the pretreatment level. In this study, the initial insect diversity was higher in the of cypermethrin treatment compared to the deltamethrin pond. This is due to the cypermethrin experiment being conducted in the month of November, at the end of monsoon when generally insect abundance and diversity are higher (Kashyap *et al.* 2013, Nasiruddin*et al.* 2014) in Indian sub-continent. Insect diversity of deltamethrin treatment was low at pretreatment level asthe study was conducted during the month of March and remained the same after day2 of the treatment. After the deltamethrin treatment, the insect diversity increased. It is within the study to the drug than the pretreatment stage when insects were likely stronger and evaded catchment.

The drastic rise of boatman population made the total insect count higher for deltamethrin treatment from day5 onwards after the treatment (Figs. 1 & 2). Gutierrez (2016) found that the 72-hour LC<sub>50</sub> values of *Buenoatar salis* and *Martarega bentoi* (backswimmers) to be 4.0 and 102.5 ng (active ingredient) /liter respectively for deltamethrin. A low insect diversity of two typesplus backswimmers' susceptibility to deltamethrin and their relative slow recovery, possibly gave boatman free space to thrive after the deltamethrin treatment. Boatman population is considered less hazardous by the fish farmers compared to backswimmer in aquaculture ponds since backswimmers are predatory to fish fry and boatman are non-predatory (Gonzalez and Leal 1995).

## IMPACTS OF CYPERMETHRIN & DELTAMETHRIN'S USE ON AQUATIC INVERTEBRATES

It seems the insects that take longer to reproduce were the most affected by cypermethrin treatment. Saha and Kaviraj (2008) found the 96-hours LC<sub>50</sub> value of *Rantrafiliformis* is  $0.06\mu g/liter$  for cypermethrin. The 24-hour LD<sub>50</sub> value of mayfly (Heptagenidae), damselfly (*Enellagma* and *Ishnura* sp.) and water scavenger beetle (*Hydrophilus* sp.) was found to be 1.3, 1.4 and  $8.3\mu g/liter$  respectively for cypermethrin (Siegfried 1993). Stephenson (1982) recorded the 24-hour LC<sub>50</sub> value of adult *Corixapunctata* (water boatman) as  $\geq 5\mu g/liter$  for cypermethrin in a static test method in the laboratory. Aquatic insects are seldom found at greater water depths, rather prefer to live in shallow waters towards the surface and upper part of the water bodies (Kashyap *et al.* 2013). The manual spray of both cypermethrin and deltamethrin in this study was done along the edges of the water of the pond, henceheavily affecting the water insects.

Compared to the aquatic insects, zooplanktons were less affected; population decline was not as deep as in the insect populationin both treatments (Figs. 1 and 4). Though *Daphnia magna* is more susceptible against deltamethrin (Xiu *et al.* 1989 and Beketov 2004) than cypermethrin (Stephenson 1982) but in this experiment it was found to be less affected in both treatments. Day (1991) found fenvalerate, deltamethrin and cyhalothrin toxicity to *Daphnia magna* decreased with the increase of dissolved organic carbon concentration in water. Quicker recovery of the zooplankton population in deltamethrin treatment can be attributed to the water quality (higher turbidity, electrical conductivity and pH) of the treatment pond. In addition to the water quality factor, the lower degree of predation from lower number of aquatic insects may have triggered the quicker recovery (5 days and 10 days for deltamethrin and cypermethrin) of zooplankton population in post treatment condition.

Due to the higher organic carbon content in the sediment (Table I), the deltamethrin treated pond fostered higher concentrations of benthos population (pretreatment stage) compared to the cypermethrin treatment-pond (Table III). Though Stephenson (1982) recorded a 24-hour LC<sub>50</sub> value of *Chironomus thummi* as  $\geq 5 \ \mu g/liter$  for cypermethrin in a static test method in the laboratory, high water turbidity (Table I) of the experimental ponds indicate the clay soil of the bottom which is rich in organic carbon has a strong absorption capacity of cypermethrin and deltamethrin, affecting the bioavailability of the insecticides; left the benthos population unaffected in this study. Akerblom et al. (2008) confirmed lower organic matter content of artificial sediments that was spiked with deltamethrin was highly toxic to Chironomus riparius larvae (the 28-day LC<sub>50</sub> value was  $11\mu g/kg$  sediments), while deltamethrin induced (@166 $\mu g/kg$ ) mortality was zero in natural sediment due to its high organic matter content. Muir et al. (1985) reported lower bioavailability of pyrethroid pesticides, including cypermethrin and deltamethrin, in silt and clay sediments and water above those sediments. This is evidenced by a 5 to 15-fold higher bioaccumulation of pyrethroids by Chironomus tenfans larvae in sand sediments containing pyrethroids as compared to that of silt or clay sediments. Moreover, there was mixing inconsistency of both cypermethrin and deltamethrin due to the large volume of water of the experimental ponds and the manual spray method along the water edges, leaving the large portion of the pond water in the middle with very little or almost no pesticide. This left enough space for fast moving fish to take refuge in water devoid of pesticides and spared some aquatic insects and zooplanktons to reproduce within the observation period.

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