Effects of the insecticide Deltamethrin on benthic macroinvertebrates – field and laboratory studies

Master´s thesis, 20 credits.

by

Christina Arbjörk

Department of Environmental Assessment
Swedish University of Agricultural Sciences
Box 7050, SE 750 07 Uppsala

Supervisors: Nina Åkerblom, Willem Goedkoop

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Abstract

Pesticides are widely used to among others protect crops, industrial materials and humans against vermin. Substances may reach surrounding waterbodies and effects of leakage may lead to damage to non target organisms, consequently damaging ecosystem structure and function. Benthic fauna is considered to be an adequate indicator of aquatic ecosystem health. Single species laboratory toxicity tests have been commonly used to determine effects of toxic chemicals on organisms. However the application of these results on natural ecosystems may be questionable as conditions in laboratory tests differ significantly from natural conditions and for instance inter species relationships not can be assessed. In multi species tests on the other hand assessment becomes difficult as complexity increases.

In this study the effects of the insecticide Deltamethrin on benthic macro invertebrates were investigated using a multi species field study and additionally a single species toxicity test with Deltamethrin and the chironomid *Chironomus riparius.* The field study was performed in four natural fresh water ponds and the sediment used in the laboratory test was natural sediment collected from one of the ponds. The combination of the two tests will give a holistic view of the effects of Deltamethrin in this particular case. The field study was carried out in a closed down limestone quarry on the island Gotland outside the Swedish East Coast. Of the four ponds two served as control ponds and two were treated with Deltamethrin. In the laboratory test Deltamethrin was added to either the sediment or directly into the water column and the effects of the different treatments were studied.

The result of the field study showed marked effects of the Deltamethrin treatment effecting species distribution and the occurrence of species sensitive to pollution. In the laboratory test however no significant effects of Deltamethrin on survival or development rate of the midges, could be seen. The conclusions of this study are that Deltamethrin is very toxic to benthic macro invertebrates causing damage to natural ecosystem function and composition. The results of the laboratory toxicity test however showed that laboratory toxicity test results might not respond to the effects of chemicals in natural conditions as so many factors may cause variations in the results. In this study conditions in the natural sediment as large quantities of different organic material, high pH and high levels of calcium may be the reason for the low toxic effects of Deltamethrin in the laboratory study.

1. Introduction

The assessment of environmental status has become an important issue in the striving for a sustainable society and use of natural resources (Swedish Environmental Protection Agency, 1999). Pesticides which are widely used throughout the world to meet demands of enhanced food productivity, safer productions and human health, pose a threat to environmental sustainability through their unwanted effect to cause damage to non-target organisms. Consequently ecosystems are affected, threatening biodiversity and possibly humans, while wanted effects work to meet economical demands and strengthen support for a growing world population (Swedish Board of Agriculture, 2002; Heudorf and Angerer 2001; Larsson, 2000; Kacew et al., 1996).

The amount of pesticides used in Sweden has decreased more than 50% in the last 30 years (Swedish National Chemicals Inspectorate, 2001). Increased information and guidance to users of pesticides is a probable reason for significantly lower levels of pesticide residue in waters since 1994. This points to increased precautions among users and more efficient use of pesticides through the increased knowledge obtained (Swedish Board of Agriculture, 2002). Systematical environmental monitoring of pesticides has not been done before 2001 and basic data to describe effects on among others benthic organisms is considered to be missing by the Swedish Board of Agriculture and the Swedish National Chemicals Inspectorate. A national environmental monitoring program is however in progress, and since the year 2001 continuous testing of pollution in Swedish waters is carried out. The purpose of the program is to achieve a clearer view of quantities of pesticide residues in Swedish waters (Sundin et al., 2002). The testing is performed by the Department of Environmental Assessment at the Swedish University of Agricultural Sciences commissioned by the Swedish Environmental Protection Agency.

The spreading of pesticides to surrounding waterbodies may occur through leakage during runoff or drift, evaporation and soaking or by intentional spreading (Dabrowski et al., 2002; Maguire, 1992) and have been found in surface water on several different locations in Sweden (Swedish Board of Agriculture, 2002). The amount of runoff-related pesticides that enter surface waters depends on various conditions such as soil texture, content of organic material in the soil, amount of rainfall, landscape topography, size of buffer strips, chemical nature of the pesticide and time of year i.e. climate (Dabrowski et al., 2001; Swedish Board of Agriculture, 2002).

The use of benthic fauna as indicators of aquatic ecosystems' health is considered to give a good indication of water and sediment quality, as benthic invertebrate communities comprise a wide range of species from pollution sensitive to pollution tolerant, and occupy multiple trophic levels and niches in the ecosystem (Johnson et al., 1993). Benthic fauna is an important part of biodiversity, constitutes an important food resource for fish and has an important role in biological degradation (Swedish Environmental Protection Agency, 1999).

Single-species toxicity tests have been widely used to determine ecological effects of toxic chemicals on natural communities and ecosystems. However single-species tests are not always considered adequate for assessment of the long-term effects of pollutants. On the other hand in multi-species tests the assessment of effects becomes more difficult as complexity increases (Woin, 1998). In this study an attempt to combine the results of a short-term single species laboratory test with a long term field study is made to determine the effects of a pesticide on benthic fauna in fresh water ponds.

The objective of this study is to determine effects of the insecticide Deltamethrin on fresh water ecosystems, in particular benthic fauna. A field study of the effects on a multi-species community, using samples of benthic fauna collected from 4 ponds, where 2 were treated with Deltamethrin and 2 served as control ponds was performed. Additionally a toxicity test with the chironomid *Chironomus riparius* was run. In the toxicity test natural sediment from one of the ponds investigated in the field study was used. This method, combining a single species test with a multi species test, will hopefully give a more holistic view of the effects of a pollutant including the effects on a natural ecosystem with its larger complexity. Major issues addressed are how communities of benthic invertebrates are affected by Deltamethrin treatment, and if different species are affected differently, giving shifts in community after the treatment compared to before the treatment. Further the similarity of treated ponds to control ponds, with respect to species distribution and occurring species, before and after the treatment and how rapid treated ponds were recolonized by different species of benthic invertebrates was investigated.

2. Material and Methods

2.1 Test substance

The insecticide Deltamethrin is used mainly for industrial and agricultural purposes (Swedish Board of Agriculture, 2002). It is a synthetic pyrethroid (*$C_{22}H_{19}Br_2NO_2$; (S)-_-cyano-3-fenoxibensyl-(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimetylcyclopropankarboxyl*) (*figure 1*) used in agriculture, forestry, horticulture, against pests on animals, and vermin on wooden materials and plants in Sweden. It is also used for insect control of for instance mosquitoes or locusts in infested areas (Lahr et al., 2000; Yáñez et al., 2002). Deltamethrin affects the nervous system and has a high toxicity for benthic arthropods and fishes, but is less toxic for mammals and birds (Swedish National Chemicals Inspectorate, 1997). Effects of exposure on humans and mammals include effects on the nervous systems and skin irritation. Exposure can occur mainly through the lungs or intestinal canal. The substance has a low mobility in soils and plants, rapidly transforming to less toxic substances under oxygen rich conditions and in coarse-textured soils with low content of organic material. Deltamethrin has been shown to remain in surface water and leak to surrounding waterbodies in less favourable conditions (Dabrowski et al., 2002). The substance is degraded by microorganisms (Swedish National Chemicals Inspectorate, 1997). Furthermore it has a tendency to accumulate, as its K_{ow} value is high (Log K_{ow} 5.4) which means that the substance is hydrophobic. In benthic conditions bioaccumulation could cause problems as oxygen poor conditions, and thereby slow degradation of Deltamethrin, creates longer periods of exposure to benthic organisms. With present methods of analysis Deltamethrin can not be detected at levels lower than 0.01µg/g in dry matter and 0.03µg/l in water. The limit value for Deltamethrin in drinking water in the EU is 0.1µg/l.

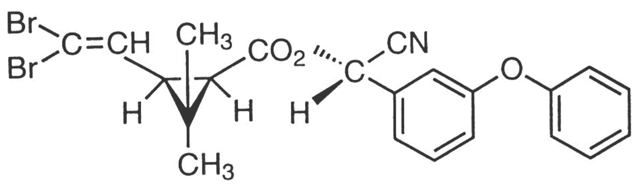
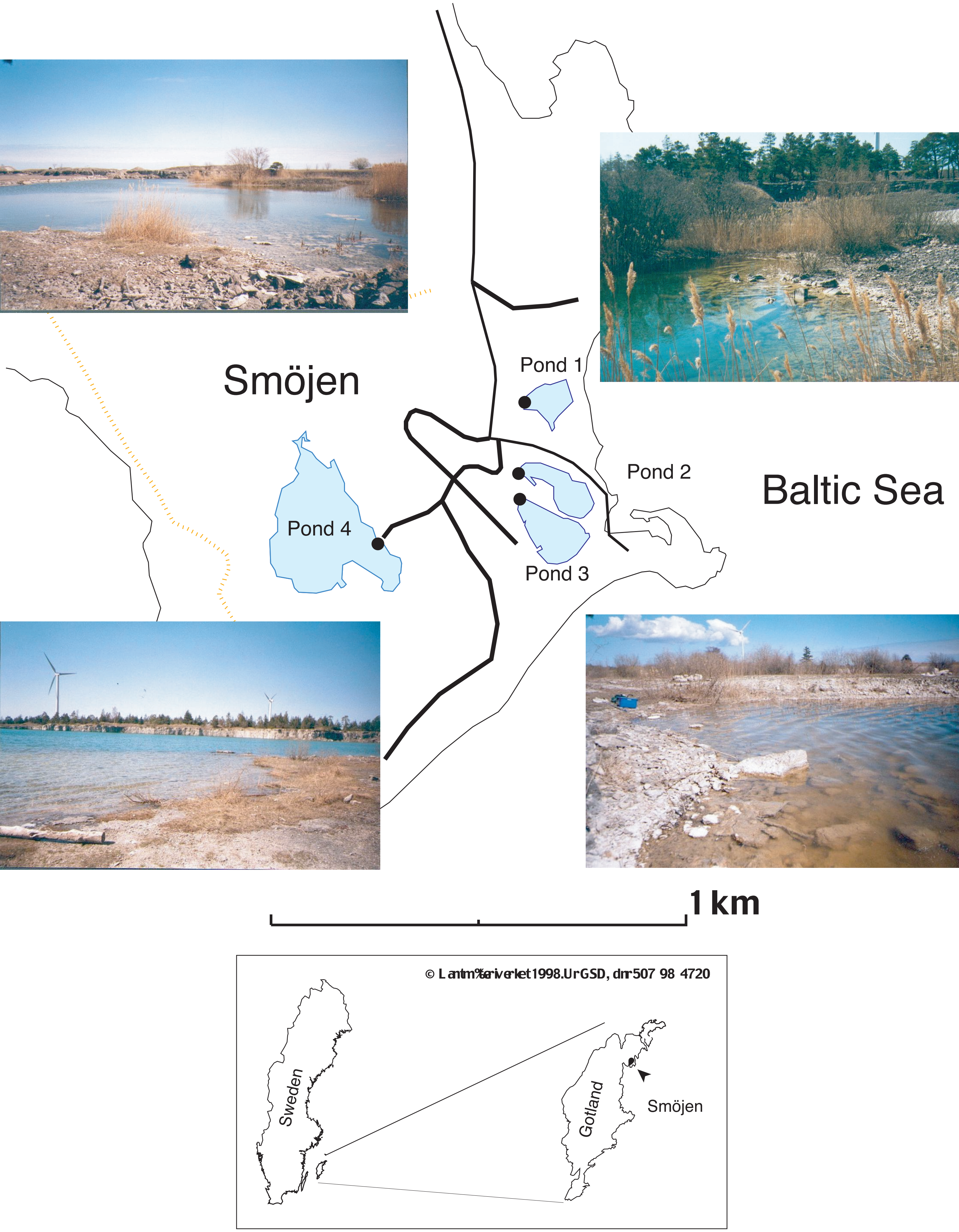


Figure 1. *Chemical structure of Deltamethrin.*



Smöjen
Pond 1
Pond 2
Pond 3
Pond 4
Baltic Sea
1 km
© Lantm%teriverket1998.UrGSD, dnr507 98 4720
Sweden
Gotland
Smöjen

The application of Deltamethrin was performed on September 19th 2001 by adding the insecticide Decis micra® in small portions from a boat. The propeller of a 1,5 horsepower boat engine was used to facilitate the diffusion of the insecticide into the water.

The four Smöjen ponds have earlier, on several occasions, been subjected to illegal or unintentional treatments with poison, presumed to be an insecticide as dead fishes and crayfishes have been found in the litoral zones and following analyses of water, sediment, fishes and crayfish showed content of Deltamethrin (Gydemo, 1995).

2.2.2 The ponds

The four ponds differ in size and are surrounded by sparse vegetation, mainly reed (*Phragmites australis*) and osier (*Salix cinerea*). They are all filled by groundwater and situated within 100 meters of the coastline of the Baltic Sea (*figure 2*). Vegetation in the ponds was dominated by the alga *Chara aspera*. Gravel and small stones cover the bottom sediment in the litoral zones of the ponds. The bedrock in the area consists of limestone. Apart from the bentic fauna, perch (*Perca fluviatilis*) and tench (*Tinca tinca*) have been observed in the ponds (Nina Åkerblom, personal communication). Pond characteristics are given in table 1. Some water chemistry data from the Smöjen ponds are collected in table 2.

Table 1. *Summary of the characteristics of the Smöjen ponds.*

Name	Control/ Treated	Sampling site location (according to Swedish national grid RT90)	Size (m^2)	Treatment (μg Deltamethrin/l water) measured on 19/9-01
Pond 1	control	O 168 74 10 N 640 51 26	7 682m^2	------
Pond 2	treated	O 169 73 97 N 640 49 68	10 717m^2	0,5μg/l
Pond 3	treated	O 168 73 96 N 640 49 08	15 019m^2	0,3μg/l
Pond 4	control	O 168 70 14 N 640 48 81	63 113m^2	------

The shores surrounding pond 1 are covered by short grass and a few osier bushes. Some reed grows in the pond. It measures 7682m^2 in size. Samples of benthic fauna were taken where the bottom was soft and covered by sediment and some small stones. The bottom slopes gently. The pond was illegally treated with Deltamethrin in 1993 and presently only contains the domestic crayfish. Therefore it serves as one of two control ponds.

Pond 2 was populated by both domestic crayfish and North American crayfish prior to the treatment and was treated with Deltamethrin in the project. The pond's estimated volume is 32 500m^3 and the concentration of Deltamethrin after addition was measured on September 19th 2001 to 0.5µg/l. It measures 10717m^2 in size. The shores are covered by grass and some osier bushes and a little reed grows in the pond. Samples of benthic fauna were taken at a site where the bottom was covered mainly by small stones and gravel and slopes steeply about 1 meter from the shore.

Pond 3 is situated close to pond 2 and was populated both by domestic crayfish and North American crayfish and therefore treated with Deltamethrin in the project. The estimated volume of the pond is 48 000m^3 and it measures 15019m^2 in size. Deltamethrin concentration after treatment was measured on September 19th 2001 to 0.3 µg/l. The shores of the pond are covered by grass with small thorn and osier bushes. Reed grows in the pond. At the benthic fauna-sampling site the bottom substrate was dominated by small stones and slopes steeply about 2 meters from the shore.

Pond 4 is the largest of the four ponds and measures 63113m^2 in size. The pond was illegally treated in 1994 and is presently populated only by domestic crayfish and not by North American crayfish. Therefore it serves as a control pond. Surrounding vegetation is mainly grass and some reed. Samples of benthic fauna were taken on a site where the bottom slopes gently and consists of flat rock with small stones and a thin layer of sediment.

2.2.3 Sampling and data treatment

Samples of benthic fauna from the four different ponds were taken before and regularly after the Deltamethrin treatment on September 19th 2001. The samples were taken one day before the treatment (i.e on September 18th 2001), 2 weeks after the treatment (i.e on October 4th 2001) and 7 months after the treatment (i.e on April 24th 2002). The samples were collected according to the "Manual for state inventory of lakes and watercourses 2000" (Department of Environmental Assessment, 2000) which follows the "Manual for environmental supervision" by the Swedish Environmental Protection Agency (2000). The collection of the samples was done using a hand bag net with a mesh size of 0.5 mm. 5 kick samples from each pond were

Table 2. *Water chemistry data tested in the water of the Smöjen ponds on September 18th 2001 (the day before the Deltamethrin treatment). Tests were analysed by Scancem Research AB, Slite, Sweden (County administrative board of Gotland).*

Variable	**Test method**	**Pond 1**	**Pond 2**	**Pond 3**	**Pond 4**
Colour (mg Pt/l)	SS 02 81 24	15	15	15	10
Turbidity (FNU)	SS 02 81 25	1.5	0.95	1.3	2.7
COD Mn (mg/l)	SS 02 81 18	6.2	3.3	4.8	4.5
pH	SS 02 81 22	8.2	8.2	8.3	8.4
Alkalinity, HCO3 (mmol/l)	SR 9712	2.25	2.46	2.38	1.89
Conductivity (mS/m)	SS EN 27 888	34.3	33.5	31.8	27.6
Ca (mg/l)	SR 9901	53	55	54	47
NH4-N (mg/l)	SS 02 81 34	0.026	0,054	0.092	0.027
Nitrogen, N, total (mg/l)	SR 9724	0.76	0.35	0.59	0.43
PO4-P (mg/l)	SS 02 81 26	<0.005	<0.005	<0.005	<0.005
P, total (mg/l)	SS 02 81 26	<0.005	<0.005	0.005	<0.005
NO3-N (mg/l)	SR 9723	0.28	<0.05	0.10	<0.05

collected at each sampling occasion. At 0-1m depth, the bag net with a long handle was swept across the bottom of the pond on a 1 x 1 m area, for 20 seconds, while the bottom sediment was stirred up by the sampler. The samples were then sieved trough a 0.5 mm mesh size sieve, and stored in plastic jars with alcohol (70%). When examining the samples an even, representative subsample was sorted under a microscope at 60x magnification.

To evaluate the samples the invertebrates were determined to species and counted, and biotic indices were calculated according to routine quality assurance at the Department of Environmental Assessment at the Swedish University of Agricultural Sciences.

The evaluation of the samples was made according to the "Environmental Quality Criteria"(Swedish Environmental Protection Agency, 1999). The evaluation gives an assessment of an ecosystem's chemical or biological status and also an estimation of the deviation from comparative values, i.e unimpacted natural conditions. Parameters used in the manual are estimated to represent the most important measures of water quality. Comparative values are based on observations made in areas where the effects of human activities are considered to be negligible. The classification of environmental quality and deviation from comparative values is divided into five different classes where indices calculated on the basis of occurring species, taxon richness (*number of species*) and abundance (*number of individuals*) represent the different classes. The different classes (based on the EU water framework directive (Nixon et al., 1996)) are: Class 1 – very high index means no or insignificant effects of disruption to benthic fauna community. These communities show no or insignificant effects of human influence and resemble fauna found under undisturbed conditions. Class 2 – high index, indicating moderate effects of disturbance. Class 3 – moderately high index showing significant effects of disturbance. Benthic communities differ moderately from undisturbed conditions. Class 4 – low index, showing strong effects of disturbance with benthic fauna significantly differing from undisturbed conditions. Class 5 – very low index, indicating very strong effects of disturbance with only few tolerant species present.

Indices used for assessment of environmental quality in this study are based on different aspects of quality and consist of measurement of diversity as Shannons diversity index (Shannon, 1948). It grades diversity as high if a community consists of many different species and several species are in a dominant position regarding abundance, and as low if few species occur and one or few species are very dominant. Further, water and ecological quality is determined by the ASPT index (Armitage et al., 1983) and the Danish fauna index (Skriver et al., 2000). The ASPT index regards the presence of species that are tolerant (low value) or sensitive (high value) to polluted water. Danish fauna index measures the influence of organic pollution and/or euthrophication and regards the presence of different key species. The presence of tolerant species result in low values while sensitive species give high values. Apart from indices, taxon richness, and abundance were used in the assessment of the ponds.

2.3 Laboratory toxicity test

The laboratory toxicity test was performed by spiking either sediment or water with Deltamethrin and expose the chironomid C*hironomus riparius* during 28 days to the treatment. The purpose of the test was to determine effects on survival and development rate of the midges. The sediment used in the study was natural sediment collected from one of the control ponds (Pond 4) at Smöjen. In order to study differences in the toxicity of Deltamethrin in water and sediment exposures, Deltamethrin was supplied to either the sediment or into the water column. The tests were performed following proposals for new OECD guidelines for the testing of chemicals (OECD, 2001a; OECD, 2001b).

The sediment from Pond 4 was collected by a diver using a small bag net. It was then stored for three days before used and during this time aerated in a vessel with a carefully added overlying M7-phase. An aquarium air pump was used for the air supply to keep the sediment oxidised and to ensure that ammonium levels caused by decomposition were not increased during storage. To remove coarse organic material the sediment was then sieved through a 0.5-mm mesh size net. Water content, organic content and density of the sediment were determined before the start of the test (*see table 3*).

Selection of exposure concentrations of Deltamethrin used in this study was based on a previous study with artificial sediment (Hedlund, 2002). The concentrations used in the test with spiked sediment were 2.6 µg/kg (dry weight); 10.4 µg/kg; 41.6 µg/kg and 166.4 µg/kg plus a control treatment. In the test with spiked water concentrations used were 6 pg/l; 12 pg/l; 24 pg/l and 48 pg/l dissolved in acetone plus control treatments. Each concentration was run in three replicates. In the spiked water test a control treatment with acetone in three replicates was also used to determine any possible effects of the acetone used as a solvent for the Deltamethrin. In the spiked sediment test the acetone was left to evaporate in a fume hood as prescribed in the guidelines and therefore no acetone control treatment was needed. The highest concentration of spiked sediment was made in two additional replicates to use for analyses of remaining concentration of Deltamethrin in the sediment after the stabilisation period of ten days, i.e at the start of the toxicity test.

The test water used in the lab study was M7 medium. It was prepared on day –20 following the procedure in the proposed OECD guidelines, which follow the procedures of Streloke and Köpp (1995). This was achieved by mixing stock solutions of trace elements and nutrient solutions with deionised water. The M7 medium was then left to stabilise for one week at 3°C and was properly aerated during this time by the use of an air pump. A vitamin stock solution was added shortly before use.

On day –13 sand (150-300 µm; Merck) was spiked by adding the Deltamethrin solutions with a pipette to 4 vessels withholding 7g sand each (concentrations 2.6 µg/kg, 10.4 µg/kg, 41.6 µg/kg plus control) and 1 vessel containing 11 g of sand (highest concentration 166.4 µg/kg) in order to get a more even distribution of the Deltamethrin solution in the sediment. The vessels containing the spiked sand, one for each concentration and one for control, were stored in a fume hood for 24 hours in order to enable evaporation of the acetone before the sediment was added to the sand. The amount of spiked sand was intended for 100 g of sediment per replicate, plus an additional 50 g of sediment, to cover for mistakes like spilling the sediment. All concentrations were made in three replicates, except the highest concentration, which was made in 5 replicates to be able to leave 2 test vessels for analysis of Deltamethrin levels after the stabilisation period (on day 0). The amount of sand used was only 2% (2 g) of the quantity of sediment per vessel and possible effects of mixing sand into the sediment were estimated as negligible due to the small amount of sand. To properly mix the sediment with the spiked sand the vessels were left to roll on a stirrer for 24 hours at low temperature (3°C) to prevent biological actions during rolling.

On day –11, 100 g properly mixed sediment was transferred to each test vessel (1000 ml glass jars, 10 cm in diameter). 420 g M7-medium were then carefully added to each test vessel, using a 10-liter water container with a tap in the bottom. The tap was connected by a plastic hose to the top part of a Duran Schott gas-washing bottle attached to a rack, to allow for the filling of the M7-medium without disturbing the sediment surface (*figure 3*). The speed of the water flow was controlled using a hose clip.

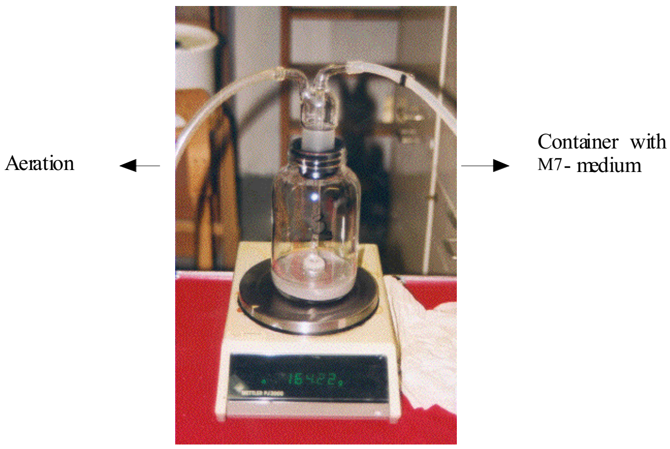


Figure 3. *Set-up used to fill up test vessels using the top part of a Duran Schott gas-washing bottle. For further description see text.*

The test vessels were then left to stabilise for 10 days in order to reach equilibrium between pore water and overlying water, in accordance with the OECD guidelines. During stabilisation the same temperature, air and light conditions were applied as during the rest of the experiment. This was achieved in climate chamber (21 – 21.5°C), where the test vessels were placed randomly, with a 16 hour photoperiod (16:8 hours light:dark). Aeration of the vessels was supplied through a glass pipette, at approximately 2-3 air bubbles per second. The speed

of the aeration is higher than prescribed in the OECD guidelines and was used to avoid high concentrations of ammonium in the vessels caused by the decomposition of organic matter in the natural sediment. On day 0 larvae of the test organisms were added to the test vessels.
In the spiked water test the sediment from pond 4 was transferred to test vessels from the storage vessel with M7-medium, as described above, directly after sieving. Preparations then followed the above description. Deltamethrin solved in acetone was added to the test vessels with a pipette the day after adding the larvae (on day 1).

The organisms used in the experiment were *Chironomus riparius* reared at The Department of Environmental Assessment at the Swedish University of Agricultural Sciences in Uppsala. The larvae were fed Tetra Phyll® fish food as ground powder during rearing. In natural conditions the larvae feed on decomposing plant material and small alive or dead invertebrates in the bottom sediment of running or standing water (Beckman, 1997). In the sediment they build tube-like structures made of secretion from the salivary glands, small plant particles, sand and algae. The larvae transform into pupal stage after approximately 3 weeks (at 20°C in culture), depending on temperature, and hatch as an adult after a few days as a pupa. Their plumose antennae easily distinguish males from females.

On day –4 and day –3, 8 egg ropes from the culture were placed in petri dishes with aerated water, one rope in each dish, to await hatching after 2-3 days. On the day of application of larvae to test vessels (day 0), 4 petri dishes with larvae in similar developmental stages were selected and five first instar larvae from each dish (resulting in a total of 20 larvae per vessel) were added to each test vessel using a glass pipette. When adding the larvae, the air supply was shut down for 10 hours allowing the larvae to find its way down and burrow into the sediment without being swirled up by the air bubbles. Shutting off the air supply for 10 hours is a shorter time than prescribed in OECD guidelines, but was chosen as high levels of different organic material were expected to exist in the natural sediment. Switching off the aeration for too long could consequently cause high levels of ammonium and potentially kill the larvae as organic material decomposed.

The larvae were fed ground TetraPhyll® fish food, 0.25 mg per day and larva for the first ten days (days 1-10) of the test period and after that (days 11-28) 0.5 mg TetraPhyll® fish food per larva and day was added. The ground fish food was sprinkled on the water surface. When hatching of adult midges began, hatched midges were counted and determined to sex daily. At the end of the test on day 28, the test vessels were emptied and the sediment carefully sought trough for remaining unhatched larvae by sieving and rinsing the sediment trough a 0.5 mm mesh size net. Development rate was calculated as portion of larval development per day. The mean development rate per vessel ($\bar{x}$) is calculated according to:

$$\bar{x} = \sum_{i=1}^{m} \frac{f_i x_i}{n_e}$$

where:

$\bar{x}$: mean development rate per vessel
i : index of inspection interval
m: maximum number or inspection intervals
f_i: number of midges emerged in the inspection interval i
n_e: total number of midges emerged at the end of experiment ($=\sum f_i$)
x_i: development rate of the midges emerged in interval i

$$x_i = 1/\left(\text{day}_i - \frac{1_i}{2}\right)$$

where: day $_i$: inspection day (days since application)
1_i : length of inspection interval i (days, usually 1 day)

2.3.1 Physico-chemical analyses

Before spiking the sediment organic matter of content in the sediment was determined by loss on ignition (20 minutes at 400°C and 140 minutes at 550°C) (*table 3)*. Water content (105°C for 12 hours) and density (comparing volume and weight with water with estimated density 1.0 g/cm^3) were also determined. pH and oxygen levels were measured in test vessels on day 0, regularly once a week during the test period, and finally at the end of test period on day 28. The pH measurements were conducted with a DG 115-SC electrode (Mettler Toledo, Stockholm, Sweden) and the oxygen with a YSI oxygen meter, model 51B, and a model 5739 electrode (YSI Scientific, Yellow Springs, OH, USA). Ammonium concentrations were measured on day 14 to ensure that levels were not high enough to contribute to larval mortality. These levels where very high but a speed up of the aeration in the test vessels from 1 air bubble per second as prescribed in the OECD guidelines, to 3 air bubbles per second was made to take care of the problem. No effects on the larvae could be seen, as survival in control treatment was higher than 80%. At the end of the test alkalinity was measured.

Further, the concentration of the Deltamethrin stock solution used as a base for the concentrations used in the test was measured, to ensure that concentrations used were correct as calculated. Concentrations of Deltamethrin in the highest concentration of the spiked sediment test were analysed on day 0. The result showed a reduction with half of the Deltamethrin concentration after the 10 days stabilisation period. The chemical analyses were carried out by the Department of Environmental Assessment.

Table 3. *Physico-chemical analyses in toxicity test with Chironomus riparius exposed to sediment and water spiked with Deltamethrin.*

Variable	***Spiked sediment***	***Spiked water***
pH (range)	7.70-8.11	7.37-8.05
(mean; stdev)	7.91; 0.11	7.79; 0.21
O_2 content		
(range) (mg/l)	7.2-9.0	7.9-9.0
(mean; stdev)	8.22; 0.42	8.48; 0.32
Ammonium		
(range) (µg/l)	100-4800	47.9-75.4
Alkalinity		
(range) (mekv/l)	1.9-2.2	1.8-2.1
Water content in sediment (%)	82.1	82.1
Sediment organic content (%)	12.5	12.5
Sediment density (g/ml)	1.12	1.12

2.3.2 Statistical methods

In accordance with the OECD guidelines ANOVA was used for statistical analyses of the toxicity test results. Values for survival were transformed by the arcsin function $X=\arcsin(\sqrt{X})$. Bonnferroni-Dunn post hoc test was used for comparison in pairs of ANOVA results.

3. Results

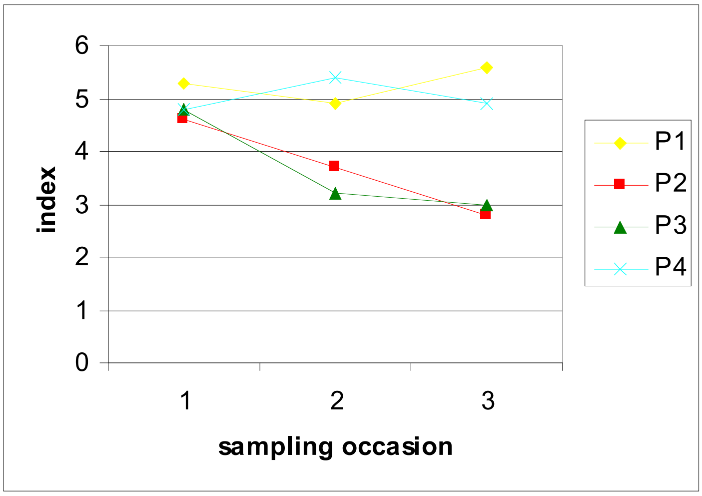
3.1 Field study

Initially, before Deltamethrin treatment, all four ponds at Smöjen were relatively similar in water quality, measured as ASPT and Danish fauna indices, (between low and moderately high) (*figure 4*). However, Shannons diversity index varied from moderately high in one control pond (P1) and low in the other control pond (P4). In treated ponds diversity was high in one of the ponds (P2) and very low in the other (P3).

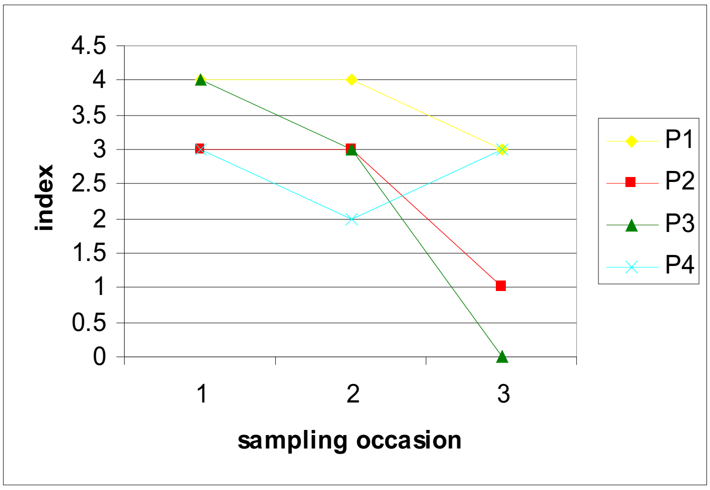
After Deltamethrin treatment ponds 2 and 3 showed a decrease in water quality. In control ponds (P1 and P4) a less marked decrease or the same values as before treatment were found. For the diversity index, however, a distinct difference between treated and control ponds could not be seen (*figure 4)*.

The most obvious difference, between treated and control ponds, was a strong decrease in taxon richness and abundance per sample in treated ponds which was not found in control ponds (*figure 5*). Taxon richness decreased with 66-74% of the species occurring before treatment (from 12 different species in pond 2 to 4 species and from 19 species in pond 3 to 5 species), and abundance per sample with 94-98%. This decrease remained seven months after Deltamethrin treatment. Species occurring in the samples after treatment (*see appendix 1*) had also shifted towards species tolerant to pollution in treated ponds while those sensitive no longer existed. Before treatment the mayfly *Caenis luctuosa* was the dominant species in all four ponds. After treatment no caddisflies or mayflies remained in treated ponds while they were still present in the control ponds. In the treated ponds mainly oligocheates and chironomids were found after treatment while control ponds had the same distribution of species as before treatment all through the sampling period, although they were fewer in numbers six months after treatment. Environmental quality measured as ASPT index and Danish fauna index showed a continued deterioration in treated ponds, which was not visible in control ponds. General trends can be seen in a decrease or increase in index in figure 4, indicating a decrease or increase in water quality or diversity. Taxon richness and abundance is illustrated in figure 5.

a)



b)



c)

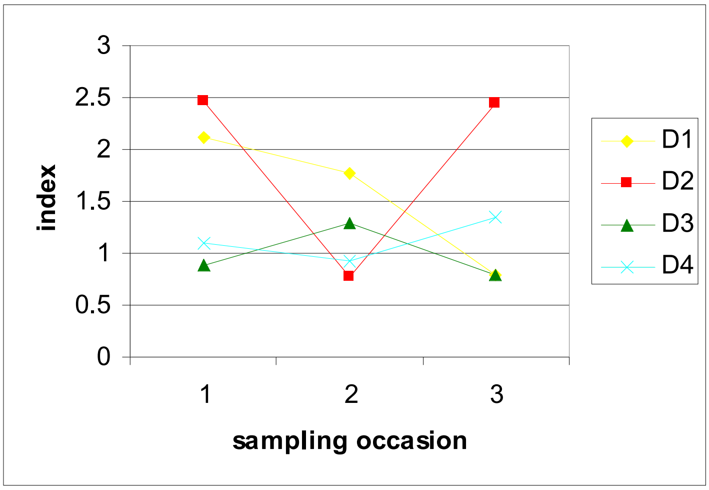
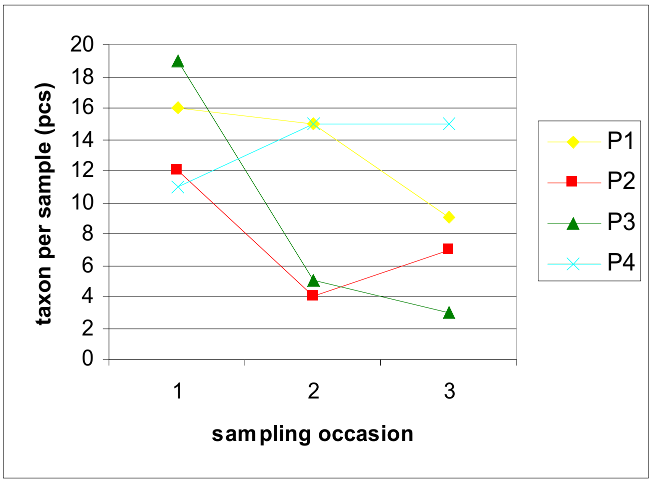


Figure 4. *ASPT index (a), Danish fauna index (b) and Shannons diversity index (c) in the four Smöjen ponds. 2 treated ponds depicted as P2 and P3 and 2 controls depicted as P1 and P4. Sampling occasion 1: 18th of September 2001 (the day before Deltamethrin treatment), occasion 2: 4th of October 2001(2 weeks after treatment) and occasion 3: 24th of April 2002 (7 months after treatment).*

a)



b)

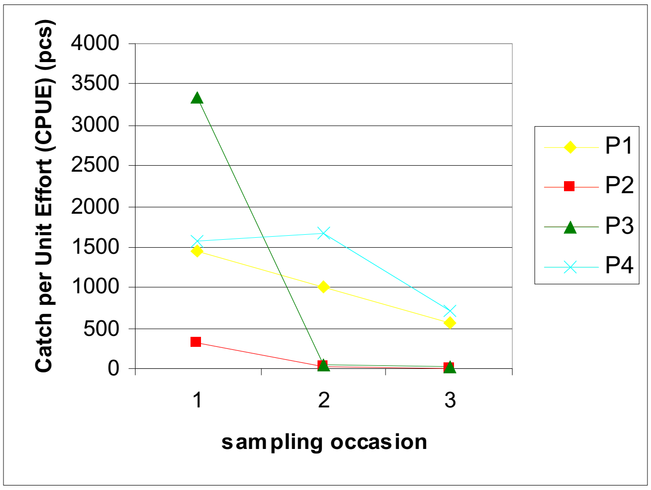


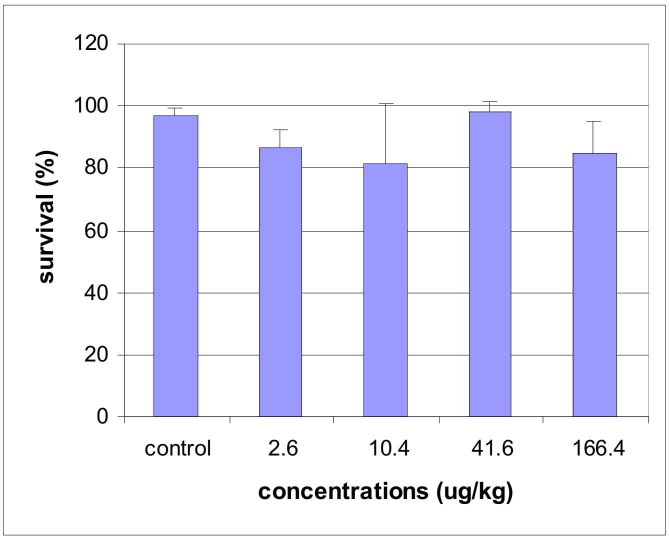
Figure 5. *Taxon richness (a) and abundance per sample (b) in the four Smöjen ponds. Sampling occasion 1: 18th of September 2001 (the day before Deltamethrin treatment), occasion 2: 4th of October 2001(2 weeks after treatment) and occasion 3: 24th of April 2002 (7 months after treatment).*

No deviations from natural conditions (i e observations from areas with no or small effects of human activities so called background values) were found for ASPT values at starting point. After Deltamethrin treatment, treated ponds showed strong deviations, which remained seven months after treatment. In control ponds values remained at natural conditions. Also Danish fauna index showed a strong decline in treated ponds, which was not seen in control ponds. In Shannon’s diversity index no distinct difference could be seen between treated ponds and control ponds.

3.2 Laboratory toxicity test

Both in the test with spiked sediment and in the test with spiked water the survival in all treatments was higher than 80 percent. Survival in the different treatments did not decrease with increased Deltamethrin levels (*figure 6*). There was no significant difference among treatments with spiked sediment (ANOVA, p> 0,05; Bonferroni/Dunn, p>0,005) or those with spiked water (ANOVA, p> 0,05; Bonferroni/Dunn, p>0,0033). As no live or dead larvae were found in the sediment at the end of the test period, survival equals emergence ratio.

a)



b)

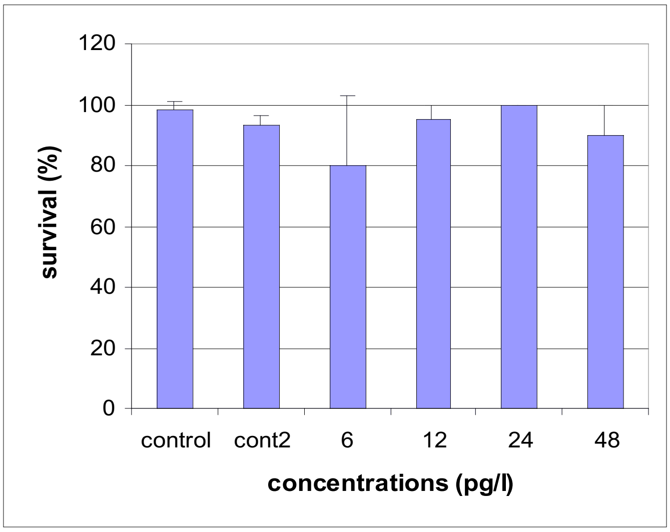
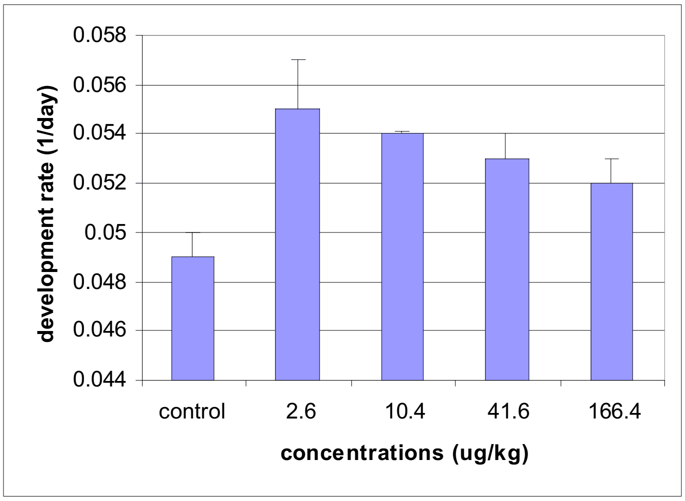


Figure 6. *Survival of Chironomus riparius (in %) in Deltamethrin toxicity test with spiked sediment (a) and spiked water(b). Cont 2 represents control treatment with acetone.*

The highest development rate, 0.064 per day, occurred in the lowest concentration of Deltamethrin treatment (6 pg/l) in the spiked water test. However, development rate did not decrease significantly with increased concentrations of Deltamethrin neither in the test with spiked sediment nor in the spiked water test (*figure 7*). Surprisingly, development rate in the treatment with spiked sediment was significantly lower in controls than in treatments with Deltamethrin (Bonferroni/Dunn, p<0,005).

a)



b)

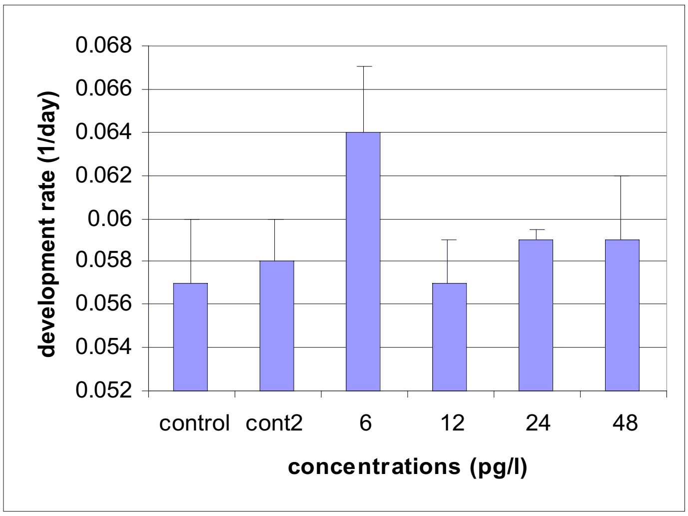


Figure 7. *Development rate (as number per day) in the Deltamethrin toxicity test with Chironomus riparius. Different concentrations of spiked sediment (a) and spiked water (b). Cont 2 represents control treatment with acetone.*

4. Discussion

Water quality in treated ponds, measured as ASPT index and Danish fauna index decreased markedly after Deltamethrin treatment. This result remained seven months after treatment. Further, bentic fauna community in treated ponds contained few species, few individuals, and those occurring consisted of pollution-tolerant species (*appendix 1*). This shows a clear effect of Deltamethrin treatment, including a shift in community composition towards dominans in numbers of tolerant species (*see appendix 1*). Seven months after treatment mainly oligocheates and chironomids, which are tolerant to pollution, remained in treated ponds while control ponds also contained several species of mayflies and caddisflies who are sensitive to pollutions (Swedish Environmental Protection Agency, 1999). Before Deltamethrin treatment all four ponds were relatively similar in species distribution all though the dominans of some species was more significant in some ponds (P3 – low index) resulting in the different values of Shannons diversity index.

In the toxicity test survival did not differ among different treatments, neither in the spiked sediment test nor in the spiked water test. This means that no evidence of difference in bioavailability of Deltamethrin if it was added to water or to the sediment, was established in this test. However concentrations of Deltamethrin used in the toxicity test were significantly lower than those used in the ponds. The reason for the lower concentrations used in the toxicity test was the follow up of an earlier study discussing values for MPC (Minimal Permissible Concentration) (Hedlund, 2002). In that study artificial sediment was used. The results of that study showed a significant decrease in survival in treatments with Deltamethrin concentrations higher than 2.6μg/kg in spiked sediment and higher than 12pg/l in spiked water. In this study natural sediment from one of the control ponds in the field survey was used. The use of natural sediment has been dealt with in several other studies which have discussed that toxic response is lower in natural sediment than in artificial sediments (Crane et al., 1999; Fleming et al., 1998; Conrad et al, 1999; Burton, 1991).

Many different factors may contribute to a lower toxic response in natural sediments. Natural sediments probably contain a far greater diversity of organic material than can be simulated in an artificial sediment (Fleming et al., 1998). One natural sediment however, can not be expected to give the same response as another (Fleming et al., 1998; Suedel & Rodgers, 1996). For instance type of organic material will influence toxicity. In artificial sediments toxic response was shown to be lower in peat based sediment than in α-cellulose based (Fleming, 1998). Further bioavilability of hydrophobic substances have been shown to depend on several different factors. Substances with high octanol-water partitioning like Deltamethrin with log K_{ow} =5.4 (hydrophobic) are likely to remain in water column for relatively short period before adsorbed onto surfaces (Crane et al., 1999).

The bioavailability of test compounds depends among other factors on the sorption to organic carbon (Fleming et al., 1998). It is reduced once bound to sediment or other surfaces (Reynoldsson, 1987). Surface area, molecular structure and physical structure of different forms of organic carbon in sediment may influence bioavailability (Conrad et al., 1999).

The removal of Deltamethrin through adsorption to suspended particles can also occur (Lahr et al., 2000). Deltamethrin has been shown to rapidly adsorb to suspended particles in overlying water, thereby drastically reducing toxic effect on fishes (Swedish National Chemicals Inspectorate, 1997). As test vessels in this study were left to stabilise for ten days, organic particles in the sediment would have dispersed into the overlying water column and the test substance probably been adsorbed to particles in the sediment and dispersed particles. Another aspect influencing removal of Deltmethrin is by hydrolysis (Lahr et al., 2000). As pH and calcium content increases, toxicity is significantly lowered (Ghillebaert et al., 1996). In the sediment used in this study calcium levels would have been high as the bedrock in the area consists of limestone consequently a possible reason for a decrease in toxicity in the laboratory test with Deltamethrin causing no effects on survival. This would indicate that liming could be a possible treatment of natural waters damaged by Deltamethrin.

Lower toxicity has also been shown to occur in sediments with increased clay and carbon content (Fleming et al., 1998). The natural sediment used in this study had a 12,5% content of organic material which is much higher than in Hedlund's study (2002) using artificial sediment (4,1-4,8%). The high water content in the sediment used in this study (82,1%), also points to a very fine-particulate sediment (previous study 30-34%). Mortality will also be decreased as contents of DOC (amounts of dissolved organic carbon) is increased (Fliedner, 1997).

As significant deterioration in treated ponds was found in this study, a further investigation of the ponds would give increased knowledge of the long-term effects. Recolonisation of the ponds will probably be possible after the warm season when the insects fly, as this is the immigration route for many of the invertebrates found in the ponds. In this study the first sample was taken in the autumn and the last in spring while the ponds were covered by ice during part of the winter. Further samples during the following summer and autumn will give a perspective of long term effects.

In the toxicity test chironomids were used as test organisms. Chironomids are often used as they are easy to rear and develop to full grown midges in an appropriate time period if study of long-term effects in a laboratory test is desired (OECD, 2001). However chironomids are considered to be relatively non-sensitive to pollutions according to the Environmental Quality Criteria. The advantages of multi-species tests have been discussed by Woin (1998). However measurement of community-level responses becomes much more difficult as complexity increases. Secondary effects of pesticides can be expected even if primary effects can not be seen, as affected waters often are exposed to annual pollution if they are close to treated areas. This can lead to effects in community structure if interspecies relationships are altered (Woin, 1998). A possible secondary effect is changes in food supply. If for instance sensitive species are strongly decreased in numbers the food supply for predatory species might change drasticly. The loss of herbivorous species could possibly also cause an increased growth of plant matter. The primary route of exposure to pollution is the water-column (Conrad et al., 1999) but in poorly water-soluble substances other exposure routes exist, once bound to sediment particles, toxic substances can be ingested through the digestive system (Fliedner, 1997). Substances can further be accumulated in benthic organisms and transported further in the food-web. Damaged aquatic communities can consequently generate damages to other parts of an eco-system effecting for instance fishes and birds (Kukkonen & Landrum, 1996; Brock & Budde, 1994).

The agricultural use of Deltamethrin can be considered to be economically important as it increases harvests and quality in crops such as protein content (Swedish Board of Agriculture, 2002). Residue of pesticides found in Swedish waters during a screening investigation, lead by the Swedish Environmental Protection Agency, were too high to be considered as negligible according to the Dutch limit values (Crommentuijn et al., 2000) in any of the waters investigated. The Dutch limit values were furthermore determined to be far too high in the previous study by Hedlund (2002). Hedlund found that mortality was 100% in a test with spiked water in treatments with concentrations corresponding to MPC-values. Survival occurred in treatments at MPC/12.5 in Hedlund's study, and were higher than 80% in treatments with concentration lower than MPC/25. In a test with spiked sediment the survival in Hedlunds study was 80% at concentrations corresponding to MPC-values. This showed that toxicity appeared to be higher in the water-phase than it is when adsorbed to the sediment. Today useful analytical methods are considered to be missing to determine effects and make large-scale control of pesticides used (Swedish Board of Agriculture, 2002) and further investigations will need to be done as negative effects on humans and sustainability might exist. The conclusions of this study are that Deltamethrin is very toxic to benthic macro invertebrates as the result of the field study shows. The effects on benthic ecosystems and community compositions are distinct. In the laboratory toxicity test however survival was not significantly affected by the Deltamethrin treatment either in the spiked water test or in the spiked sediment test, in contradiction to the results in Hedlund's study. In the laboratory toxicity test, where natural sediment was used, over 80% survival occurred at concentrations of Deltamethrin which in Hedlund's study (using artificial sediment) gave no survival. In this case natural sediment accordingly gave lower toxic response than artificial sediment. One natural sediment however can not be expected to resemble another. The results in the laboratory study were surprising and show that the results of toxicity test of chemicals might not be easily applied on natural ecosystems, as so many factors will cause variations in the results in natural conditions, and these variations are impossible to imitate in laboratory tests. Thus the assessment of effects in natural conditions have proven to be very difficult. The effects on one single ecosystem may be assessed in field studies, but the problem lies in applying the results on others. This study points to that high organic content, high pH and Ca-content in the sediment reduced the toxicity of Deltamethrin.

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Appendix 1

Benthic fauna, Smöjen, Gotland

Date: 010918
Depth: 0-1 m
Number of samples/pond:5

	Pond 1 Control (ind./sample)	**Pond 2 Treated** (ind./sample)	**Pond 3 Treated** (ind./sample)	**Pond 4 Control** (ind./sample)
TOTAL	**1453,2**	**329,8**	**3344,6**	**1581,2**
GASTROPODA				
Gyraulus sp.	37,6	25,6	110,2	–
Radix peregra	12	1,6	–	–
OLIGOCHAETA	60,4	89,4	123,6	39,8
HIRUDINEA				
Helobdella stagnalis	–	–	18,8	0,4
HYDROCARINA	1,6	–	7,8	–
ARANEAE				
Argyroneta aquatica	–	–	0,2	–
CRUSTACEA				
Asellus aquaticus	28,4	34,8	5	6
EPHEMEROPTERA				
Baetis sp.	1,2	–	–	–
Caenis luctuosa	797,8	122,6	2932	1206,6
Centroptilum sp.	71,6	14,4	22,6	4
Cloeon sp.	–	–	2,2	–
Cloeon simile group	–	–	0,2	–
Leptophlebia vespertina	146	3,4	44	–
PLECOPTERA				
Leuctra fusca	1,2	–	–	–
ODONATA				
Anisoptera	4,8	–	–	4
Platycnemis penn.-Pyrrhosoma nymph.	7,2	–	–	–
Other Zygoptera	1,6	2,2	2,8	0,2
COLEOPTERA				
Dytiscidae	–	–	–	–
Oulimnius sp.	–	–	0,8	–
HETEROPTERA				
Corixidae	–	–	–	–
TRICHOPTERA				
Agraylea sp.	–	–	–	0,2
Athripsodes sp.	–	–	–	40,8
Cyrnus trimaculatus	–	–	12,8	2
Ecnomus tenellus	5,6	1,8	0,4	–
Holocentropus sp.	–	–	0,8	–
Oxyethira sp.	–	–	–	–
Polycentropus sp.	1,2	–	6	0,4
LEPIDOPTERA	–	–	0,8	–
DIPTERA				
Ceratopogonidae	–	1,6	–	–
Chironomidae	273,8	30,2	53,6	276,8
Tipula sp.	1,2	2,2	–	–

Benthic fauna, Smöjen, Gotland

Date: 011004
Depth: 0-1 m
Number of samples/pond:5

	Pond 1 Control (ind./sample)	**Pond 2 Treated** (ind./sample)	**Pond 3 Treated** (ind./sample)	**Pond 4 Control** (ind./sample)
TOTAL	**1009.2**	**21.2**	**52.4**	**1668,8**
GASTROPODA				
Gyraulus sp.	27,2	2,4	24,4	2,4
Radix peregra	–	–	–	–
OLIGOCHAETA	30	18	25,6	10
HIRUDINEA				
Helobdella stagnalis	2	–	0,4	3,4
HYDROCARINA	1,2	–	–	–
ARANEAE				
Argyroneta aquatica	–	–	–	–
CRUSTACEA				
Asellus aquaticus	31,6	–	–	–
EPHEMEROPTERA				
Baetis sp.	–	–	–	–
Caenis luctuosa	688	0,4	0,8	1349,2
Centroptilum sp.	26	–	–	6,4
Cloeon sp.	1,6	–	–	1,2
Cloeon simile group	–	–	–	–
Leptophlebia vespertina	96	–	–	0,8
PLECOPTERA				
Leuctra fusca	–	–	–	–
ODONATA				
Anisoptera	2	–	–	4,4
Platycnemis penn.-Pyrrhosoma nymph.	1,6	–	–	0,8
Other Zygoptera	4	–	–	–
COLEOPTERA				
Dytiscidae	–	–	–	0,2
Oulimnius sp.	1,2	–	–	–
HETEROPTERA				
Corixidae	–	–	–	–
TRICHOPTERA				
Agraylea sp.	–	–	–	–
Athripsodes sp.	–	–	–	18,2
Cyrnus trimaculatus	–	–	–	–
Ecnomus tenellus	8,4	–	–	4,6
Holocentropus sp.	–	–	–	–
Oxyethira sp.	–	–	–	2,4
Polycentropus sp.	1,6	–	–	–
LEPIDOPTERA	–	–	–	–
DIPTERA				
Ceratopogonidae	–	0,4	–	0,2
Chironomidae	86,8	–	1,2	264,6
Tipula sp.	–	–	–	–

Benthic fauna, Smöjen, Gotland

Date: 020424
Depth: 0-1 m
Number of samples/pond:5

	Pond 1 Control (ind./sample)	**Pond 2 Treated** (ind./sample)	**Pond 3 Treated** (ind./sample)	**Pond 4 Control** (ind./sample)
TOTAL	**560.8**	**3.8**	**23,6**	**703,2**
GASTROPODA				
Gyraulus sp.	–	0,6	–	0,8
Radix peregra	–	–	–	–
OLIGOCHAETA	–	1,4	19,6	4
HIRUDINEA				
Helobdella stagnalis	–	0,2	0,8	1,6
HYDROCARINA	0,8	–	–	0,8
ARANEAE				
Argyroneta aquatica	–	0,2	–	–
CRUSTACEA				
Asellus aquaticus	3,2	0,2	–	3,2
EPHEMEROPTERA				
Baetis sp.	–	–	–	–
Caenis luctuosa	76,8	–	–	501,6
Centroptilum sp.	–	–	–	27,2
Cloeon sp.	0,8	–	–	1,6
Cloeon simile group	–	–	–	–
Leptophlebia vespertina	–	–	–	–
PLECOPTERA				
Leuctra fusca	–	–	–	–
ODONATA				
Anisoptera	1,6	–	–	1,6
Platycnemis penn.-Pyrrhosoma nymph.	–	–	–	–
Other Zygoptera	–	–	–	–
COLEOPTERA				
Dytiscidae	–	–	–	–
Oulimnius sp.	–	–	–	–
HETEROPTERA				
Corixidae	1,6	0,8	3,2	–
TRICHOPTERA				
Agraylea sp.	–	–	–	–
Athripsodes sp.	4	–	–	18,4
Cyrnus trimaculatus	–	–	–	–
Ecnomus tenellus	0,8	–	–	3,2
Holocentropus sp.	–	–	–	–
Oxyethira sp.	–	–	–	0,8
Polycentropus sp.	–	–	–	0,8
LEPIDOPTERA	–	–	–	–
DIPTERA				
Ceratopogonidae	–	–	–	–
Chironomidae	471,2	0,4	–	137,6
Tipula sp.	–	–	–	–