Detection of pesticide residues in rivers of an Atlantic rain forest reserve in Brazil

Master's thesis 20 p.
(examensarbete)

by

Sara Elfvendahl

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

Supervisors: Henrik Kylin and Rosana Moraes (Chalmers Technical University, Gothenburg)

2000 Rapport 2000:10

Detection of pesticide residues in rivers of an Atlantic rain forest reserve in Brazil

ISSN 1403-977X

Abstract

A screening of pesticides in different environmental compartments was performed in rivers of a protected Atlantic rain forest reserve, Parque Estadual Turístico do Alto Ribeira (PETAR). PETAR is located in Ribeira Valley, which is a poor and less developed region in the southern part of the State of São Paulo, Brazil. PETAR is affected by different human activities both inside the reserve and in the surroundings. In the watershed of the rivers draining the reserve there are plantations with tomato as main cash crop. Pesticides are used to a large extent and have been detected in river water sampled in PETAR in 1998 and 1999. In addition to water, this study also includes river sediment and fish muscle tissue. A sampling survey was conducted during the wet season in Betari, Iporanga and Pilões River and small tributaries in their watershed in mid-January 2000. Altogether seven different sites were visited. Two species of bottom dwelling catfish (Order Siluriforme) were selected for the study. One is a carnivorous-insectivorous and piscivorous species called bagre (*Rhamdioglanis frenatus*, Family Pimelodidae) and the other is a primary consumer called cascudo (*Isbrueckerichthys sp.* Family Loricariidae).

The samples were analysed on GC-ECD/NPD after extraction and clean-up. Pesticides were detected in samples from all the sites examined and the fish have even accumulated some of the more persistent pesticides or metabolites. In all, 27 different pesticides or metabolites were detected. In the screening of water, 106 pesticides were included and 16 were found in the samples. A few of them are considered highly toxic to fish and other aquatic organisms. The sediment was screened for 27 organochlorine compounds (OCs) and pyrethroid pesticides and 14 OCs were included in the study of fish. Seven OCs were found in sediment and ten OCs in fish. Fish from two small streams in Iporanga and Betari watershed seemed to be more contaminated than the other sites and sediment samples from the same Betari tributary contained the highest number of pesticides found in sediment.

The results show that the aquatic flora and fauna of PETAR are exposed to pesticides both dissolved in the water and in the sediment. There may be a risk that pesticides in the water reach levels that stress the organisms and cause adverse effects on the aquatic ecosystem. However, it is difficult to quantify the risk to biota exposed since several different stress factors affect them simultaneously and synergistic or antagonistic effects cannot be excluded.

Keywords: Pesticide residues, water, sediment, freshwater fish (Order Siluriforme), muscle tissue, Atlantic rain forest, PETAR, Brazil

Abbreviations

a.i.	Active ingredient
B	Betari River
BAM	2,6-dichlorbenzamide (degradation product of dichlobenil)
CTU	Chalmers Technical University, Gothenburg, Sweden
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (degradation product to DDT)
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (degradation product to DDT)
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (organochlorine insecticide)
ERA	Ecological Risk Assessment
ESA	Department of Environmental System Analysis
GC-ECD	Gas chromatograph with electron-capture detector
GC-NPD	Gas chromatograph with nitrogen-phosphorus detector
GPC	Gel Permeation Chromatography
HCH	Hexachlorocyclohexane
HBB	Hexabromobenzene
I	Iporanga River
IB	Instituto Biológico, São Paulo, Brazil
IMA	Institutionen för Miljöanalys (Department of Environmental Assessment)
LEA	Laboratório de Ecologia de Agroquímicos, Centro Protecão de Ambiental, Instituto Biológico de São Paulo, Brazil
MFS	Minor Field Study
OC	Organochlorine
P	Pilões River
PETAR	Parque Estadual Turístico do Alto Ribeira
Sida	Swedish International Development Cooperation Agency/Agencia Sueca de Cooperacão International para o Desenvolvimento (Asdi)
SLU	Swedish University of Agricultural Sciences
SPE	Solid Phase Extraction
USP	University of São Paulo, São Paulo, Brazil

Table of Contents

Detection of pesticide residues in rivers of an Atlantic Rain forest reserve in Brazil

1. Aim

The aim of this MFS project is to determine the content of pesticide residues in water, sediment and fish tissue samples in streams of Parque Estadual Turístico do Alto Ribeira (PETAR) - a Brazilian Atlantic rain forest reserve.

2. Introduction

2.1 Background and characteristics of PETAR

Parque Estadual Turístico do Alto Ribeira (PETAR) is an Atlantic rain forest reserve in Ribeira Valley in the south-western part of the State of São Paulo (figure 1). Most of PETAR is located on a mountain ridge between the municipalities of Apiaí in the west and Iporanga in the east. Ribeira Valley has about 235 000 inhabitants and is the poorest and least developed area of the State of São Paulo (*Galetti & Fernandez, 1998*). There is a high degree of illiteracy (22%) and the infant mortality index is also high (4.2%) in the area according to the Environmental Secretary of the State of São Paulo, 1993. Agriculture and fishing are the main occupations for the people, and as in the whole country, most of the land is in the hands of only a few families. The main income is banana and tea plantations in lowland areas. Banana and tea are cultivated in large quantities throughout the year while crops like rice, beans, corn and citrus are seasonal and cultivated in lower quantities (*Almeida, 1995*). Due to the hilly topography, agriculture is less developed here than in some other regions of the State of São Paulo. This also means that the Atlantic rain forest has not been exploited to the same extent here (*Pardini, 1998*). Even though the forest in Ribeira Valley partly has been affected by human activities over the years, it still holds one of the largest remaining forested areas of the Brazilian Atlantic rain forest. Besides PETAR, there are several other state parks like Parque Estadual Fazenda Intervales, Parque Estadual Jacupiranga and Parque Estadual Carlos Botelho protecting the forest in the Ribeira region (figure 1). Together with Serra do Mar Environmental Protection Area they embrace 400 000 hectares of protected forest. (*Molander & Moraes, 1998*)

PETAR reserve is 36 000 hectares and the terrain is quite hilly with altitude variations from 100 to 1000 meters above sea level (*Molander & Moraes, 1998).* It is located in the subtropical zone and climate is classified as mesothermic humid according to the climate system of Köppen (*Liljequist, 1975*). This means that precipitation exceeds evaporation and that the temperature during the coldest month normally is above -3°C and the warmest month has temperatures above 10°C. There are three rivers, Betari, Iporanga and Pilões, draining the reserve and some of the surroundings in the west. They are all first order tributaries to the big Ribeira River in the east. In figure 2 there are pictures of Betari Valley and Betari River.

The environment of PETAR is threatened by several human activities even though the reserve has been protected since 1958. There are human settlements within and near PETAR that may release nutrients and oxygen-demanding organic compounds, which may lead to anthropogenic eutrophication of the water. In the reserve there are many old closed lead mines that may be a source of metals leaching to the surroundings. There are also many closed and a

few active limestone mines within the park. Illegal deforestation is also a problem in PETAR. An example is the illegal harvesting of palm hearts ("palmitos"), which is the edible top apical meristem of the palm tree, *Euterpe edulis* (*Galetti & Fernandez, 1998*). It is considered a delicacy and the tree has almost become extinct since the harvesting includes killing the whole tree. Mainly, the deforestation problem in PETAR is related to the gathering of firewood and the need for more arable lands. Within the limits of PETAR agriculture is restricted and only small-scale subsistence agriculture is allowed. People of PETAR mostly grow beans to cover their household needs. There are only a few guards working with the difficult task to enforce PETAR regulations.

Figure 1. Map showing the localisation of Parque Estadual Turístico do Alto Ribeira (PETAR), State of São Paulo, Brazil, and other protected areas in the Ribeira Valley. The scale is approximate. Modified after Molander and Moraes (1998).

There are some well-developed agricultural areas in the vicinity of PETAR and in the surroundings of the reserve there are extensive cultivations of mainly tomatoes and passion fruit. Tomatoes require heavy pesticide usage and throughout the growing season different pesticides are used during the different stages of cultivation: seeding, flowering, fructification, and harvest (*Molander & Moraes, 1998*). The tomato season is from September to March,

which coincides with the wet season. This means that water runoff from fields is expected to be large at the same time as pesticides are applied to a greater extent than in the dry season. Figure 3 shows the average precipitation over the 27 last years in a village located near the PETAR limit in Betari Valley.

a) BetariValley

b) Betari River

c) Fishing

d) A cascudo

Figure 2. Pictures of a) Betari Valley in Parque Estadual Turístico do Alto Riberia, State of São Paulo, Brazil,
b) Betari River,
c) Fishing with electroshocking device in Soarez Stream and
d) A Cascudo, *Isbrueckerichthys sp*. Photos by S. Elfvendahl, 2000.

The limits of PETAR do not fully include the drainage areas of its rivers and streams (see figure 5). The headwaters and tributaries of the three rivers originate outside the reserve. There are some cultivations located in the drainage areas upstream the PETAR rivers (especially in the drainage area of Pilões River), hence agricultural practices here may affect the PETAR reserve. For example, pesticides used in these areas can come to affect the aquatic environment of the reserve by run-off or leakage through the soil to water from cultivated fields. There may also be a winddrift of pesticides from the site of application, as well as several other possible routes of pesticide contamination of the environment. There are also occasions when insecticides are applied close to, or directly in the PETAR streams by the municipal authorities in order to fight mosquitoes troubling cattle and people. According to the municipal administration of Iporanga at least temephos is used for this purpose.

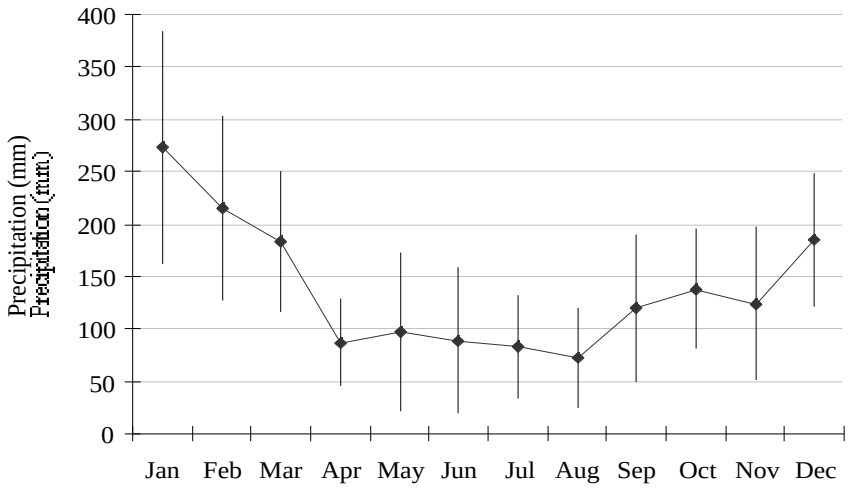


Figure 3. Average precipitation 1972-1999 with standard deviation in Bairro da Serra (Betari Valley), State of São Paulo, Brazil. R. Moraes provided the data used. Source; Water and Electrical Energy Department of the State of São Paulo (DAEE).

Sida (Asdi) has been financing an Ecological Risk Assessment (ERA) of human impacts on ecosystems in the reserve since 1997. The project was initiated by the Department of Technical Environmental Planning (since 2000 named ESA) at Chalmers Technical University (CTU), Gothenburg. The Brazilian Ph.D.-student Rosana Moraes and her supervisor Sverker Molander are in charge of the project and their collaborators are the PETAR-administration and researchers from the Department of Zoology at University of São Paulo. Biologists from the Brazilian university have performed ecological studies of different fish communities in PETAR for several years. Dr. Henrik Kylin of the Department of Environmental Assessment (IMA) at Swedish University of Agricultural Sciences (SLU), Uppsala has supervised analyses of pesticides in samples from PETAR and he also functions as an advisor for the sampling programme. The aim of the ERA project is to evaluate how the different anthropogenic stressors – such as those mentioned above - are affecting the PETAR ecosystems.

Water samples taken by R. Moraes and S. Molander from streams in PETAR in November 1998 and March 1999 were analysed at IMA, SLU, Uppsala. Pesticides like *p,p'*-DDE, permethrin, malathion, metazachlor and chlorprofam were found dissolved in the water (see

appendix 1 for full list of pesticides found). This means that the aquatic biota in PETAR is exposed to pesticides and that aquatic organisms may accumulate pesticides from the water.

Very little is known about the impact of human activities in the tropical and sub-tropical ecosystems. Most of the ecotoxicological research is focusing on effects in the temperate zones and it is of interest to develop and modify methods that can be used when trying to do similar studies in the tropics.

Two species of catfish were selected for this study; bagre, *Rhamdioglanis frenatus* (Order Siluriformes, Family Pimelodidae) and cascudo, *Isbrueckerichthys* (previously *Pareioraphis*) *sp*. (Order Siluriformes, Family Loricariidae) both bottom-dwelling species of PETAR, but representing different trophic levels. Figure 2 d) shows a picture of the cascudo. *Rhamdioglanis frenatus* is an insectivorous and piscivorous fish whereas *Isbrueckerichthys sp.* is a primary consumer scraping and grazing microalgae on rocks and submerse plants the bottom. The latter also ingests sediment when grazing. Both species are considered stationary and they do not move long distances in the rivers and streams (*Gerhard, 1999; Buck, in prep. 2000*). Hence, it is possible to reach the assumption that they are exposed to the same amounts and kinds of pesticides. In previous studies of the fish abundance in the PETAR streams (*Molander & Moraes, 1998; Moraes & Molander, 1999*) these species occurred at several of the investigated sites. Both, or one of the species were known to occur at the sites selected for this field trip.

This study is a screening of pesticides in different environmental compartments of the PETAR rivers. Pesticide contents of water, sediment and muscle tissue from two species of catfish - expected to be exposed to pesticides - from Betari, Iporanga and Pilões Rivers (7 sites) were compared. The study was performed to obtain information on the distribution of pesticides between the different environmental compartments.

3. Literature review

3.1 The Atlantic rain forest

The Atlantic rain forest of Brazil is a unique ecosystem. The diversity of plant and animal species is high as well as the level of endemism. It holds many species threatened by extinction, for example jaguar (*Panthera onca*) and the neotropical river otter (*Lontra longicaudis*). The Atlantic rain forest is considered to be one of the most endangered ecosystems on Earth, since only 8 % of its original area remains more or less undisturbed (*Ranta et al., 1998*). Once the Atlantic rain forest stretched 4000 km along the Brazilian coast, from the present-day state Rio Grande do Norte in the north-east to Rio Grande do Sul in the south (*da Fonesca, 1985*). The distribution of the forest along the coast is caused by the gradient of decreasing rainfall from the coast to the inland. The forest used to cover a narrow continuous area of 1.0-1.5 million km^2, while today only scattered fragments of the forest remain with a total area of about 20 000 km^2. The connection to Amazonia was broken in the Tertiary and the two forests have evolved separately since then, resulting in great differences in species composition etc. (*da Fonesca, 1985; Ranta et al., 1998*).

In the early 16th century the first Europeans came to the continent and explored the Brazilian coast. They realised the great value of the natural resources and the exploitation of the Atlantic rain forest started in the north, soon after the arrival of the settlers. An intensive logging of Brazil wood, *Caesalpinia echinata* started, whereby the forest was more or less cleared in order to grow sugar and to obtain pastures and firewood. The population grew along the coast and later also the southern part of the Atlantic forest was an object for land clearing (*da Fonesca, 1985*). Today, the Atlantic rain forest is a protected part of the national heritage according to the constitution of Brazil (*Ranta et al., 1998*).

3.2 Pesticide use in Brazil and Apiaí

The major categories of pesticides used in Brazil are herbicides (25 017 metric tons a.i. 1995), insecticides (14 538 metric tons a.i. 1995) and fungicides (4719 metric tons a.i. 1995) (FAO, 2000). Triazines (37 % of the total used herbicides), organophosphorus compounds (37 % of the total used insecticides) and carbamate compounds (32 % of the total used insecticides) are the main chemical groups used. The agricultural use of organochlorine (OC) pesticides has been restricted since 1985 by the government in Brazil. Aldrine and mirex usage is allowed to control ants and dicofol is used in cotton and citrus plantations (*Polese et al., 1996*). Endosulfan is a cyclodiene OC used frequently as an insecticide and its application is restricted to plantations of sugar cane, cocoa and coffee only (*Araújo et al., 1999*). Authorities are allowed to use OC pesticides to control vector transmitted diseases. There has also been an illegal use of OC pesticides after 1985.

The Agricultural Secretary of São Paulo mapped the use of pesticides in the Apiaí region, west of PETAR in 1997 (*Molander & Moraes, 1998*). Further studies were performed by R. Moraes in 1999 (*Moraes & Molander, 1999*) by interviewing farmers in the area and staff selling pesticides at the local stores in Apiaí. Pesticides known to be used in tomato plantations and are sold in Apiaí are listed in table 1.

Table 1. Pesticides used in tomato plantation in Apiaí area, State of São Paulo, Brazil according to the Agricultural Secretary of São Paulo 1997 (Source: Secretaria de Estado dos Negócios da Agricultura e Abastecimento – Instituto de Economia Agrícola) and pesticides sold in in Apiaí according to agronomist Pedro Galvao working at a store in Apiaí. Modified from Molander and Moraes, 1998 and Moraes and Molander, 1999.

Pesticides sold in Apiaí stores	Pesticide type	Pesticides used in Apiaí area	Pesticide type
Abamectin	Insecticide/acaracide	Benomyl	Fungicide
Acefate	Insecticide	Captan	Fungicide
Benomyl	Fungicide	Cartap	Insecticide
Benzimidazole	Fungicide	Chlorothalonil	Fungicide
Captan	Fungicide	Copper oxychloride	Fungicide
Carbaryl	Insecticide	Cyanamide	
Carbofuran	Insecticide	λ-Cyhalothrin	Insecticide
Cartap	Insecticide	Cymoxanil	Fungicide
Chlorothalonil	Fungicide	Cyromazine	
α-cyfluthrin	Insecticide	Deltametrin	Insecticide
β-cyfluthrin	Insecticide	Fluazifop-p-butyl	
Cymoxanil	Fungicide	Guazatine	Fungicide
Cypermethrin	Insecticide	Magnesium phosphide	Insecticide
Deltamethrin	Insecticide	Metamidofos	insecticide/acaracide
Fenpropathrin	Acaracide	Methyl Parathion	Insecticide
Fenvalerat	Insecticide	Paraquat	Insecticide
Imidacloprid	Insecticide	Permethrin	insecticide
λ-cyhalothrin	Insecticide	Thiophanate-methyl	
Mancozeb	Fungicide		
Metalaxyl	Fungicide		
Methamidofos	Insecticide/acaracide		
Methomyl	Insecticide		
Methyl-Parathion	Insecticide		
Oxytetracyclin	Bactericide		
Oxytetramycin	Bactericide		
Permethrin	Insecticide		
Phorate	Insecticide		
Propargit	Acaracide		
Streptomycin	Bactericide		
Tetradifon	Insecticide		
Trizoid	Fungicide		

3.3 Environmental fate of pesticides

There are many pesticides in use over the world, varying widely in physical and chemical properties and belonging to different groups of pesticides. Even though a pesticide, when applied, has a certain target as a pest or weed in a certain area, it might cause effects on non-target organisms and in non-target areas through environmental transport processes. Many factors and processes affect the environmental fate of pesticides after application to a cultivated field. The properties of a pesticide will affect its behaviour in the environment and also its biological activity (*Gevao et al., 2000*). Important properties are water solubility, volatility, hydrophobicity, ionisability, polarity, polarisability, molecular size and substituents of the pesticide (*Torstensson, 1989; Gevao et al., 2000*). Characteristics like solubility, soil mobility and rate of degradation are the most important and useful when predicting their potential to contaminate waters (*Eke et al., 1996*). Also, the properties of the environment in which the pesticides are used play a role. Transport of pesticides is dependent upon climatic factors like rainfall, wind and temperature and other factors like topography of the environment (*Torstensson, 1989*). The soil structure and texture and amount of organic material are factors affecting adsorption of pesticides to soil and thereby also transportation.

In addition to transport processes, losses of pesticides from the site of application are also influenced by degradation and transformation processes – photochemical, chemical and biological – which reduce or eliminate the amount of the substances. The rate of degradation is dependent both on the properties of the pesticides as well as the environment where the pesticides are used, such as temperature, UV-radiation, precipitation and microbial biomass in the soil. Agricultural practices like soil preparation, irrigation and drainage, addition of lime and nutrients and pesticide application (techniques, amounts, and formulations) are also important for the rates of transport, degradation and transformation of pesticides (*Torstensson, 1989*).

3.3.1 Environmental transport

There are several routes for pesticides to enter aquatic environments, including spillage, inappropriate disposal of dilute pesticides and washing of equipment used for application, run-off and leaching from the agricultural field and through drainage tiles (*Torstensson, 1989*). Other possibilities are transportation by wind drift when applying the pesticides and through volatilisation of pesticides from soil and vegetation after application (*Kreuger, 1999*). Pesticides in the atmosphere can then be transported to and deposited on surface waters. Figure 4 schematically shows the different transport routes of pesticides. In summary, the main transportation routes of pesticides from the field to surrounding waters are volatilisation/deposition, run-off and leaching (*Kreuger, 1999*). Rainfall and irrigation affect the intensity of the two latter. Surface run-off occurs after storm events when the infiltration rate of the soil is exceeded and a surface flow of water occurs. Pesticides in run-off water are, depending on their water solubility, transported either dissolved or bound to suspended particles. Compounds with low water solubility are more frequently adsorbed to particles and transported in the sediment phase of run-off water (*Walker et al., 1996*). Particles in running water will tend to settle to stream or lake bottoms where water current is reduced and thus accumulate in the sediment. The adsorption of organic pollutants to particles in the sediment limits their mobility and availability to bottom-dwelling organisms. Water parameters like temperature, pH, and especially oxygen content - which determines the rate and nature of chemical and biochemical reactions - will affect the possibility of an absorbed compound to become free again (*Walker et al., 1996*).

The soil can be seen as a filter for pesticides applied on the surface. Leaching of pesticides through soil to groundwater or drainage tiles is a process driven by the water flow in the soil (*Walker et al., 1996*). The movement of water is of great importance for the mobility and persistence of compounds in the soil. The adsorption ability and water solubility of the pesticide and soil characteristics like porosity, texture and hydrology will influence the rates and pathways of the transport (*Brown et al., 1995*). Different soil types differ in adsorption capacity and it is mainly the organic matter content and clay content of the soil that governs its sorption capacity (*Brown et al., 1995; Torstensson, 1989*). Generally, hydrophilic compounds move more freely in soil than hydrophobic compounds, which often tend to bind strongly to soil clay or organic matter (*Walker et al., 1996*). This means that hydrophobic compounds are not as mobile and are not readily available to soil organisms as hydrophilic compounds are. In a field study in Germany (*Traub-Eberhard et al. 1995*), different leaching behaviour was observed for pesticides in two different soils - one was sandy and poorly structured while the other was a silt loam. Both soils had subsurface drains and the added pesticides were measured in the drainage water. The expectation is that the texture of the silt

loam will lead to a slow flow of water while it is expected to be faster in a coarse-grained soil. The study showed that fast flow in clayey soil is possible since it tends to form cracks, which may allow fast flow of water through the soil. Thereby, pesticides may move much faster under these conditions than predicted from their properties.

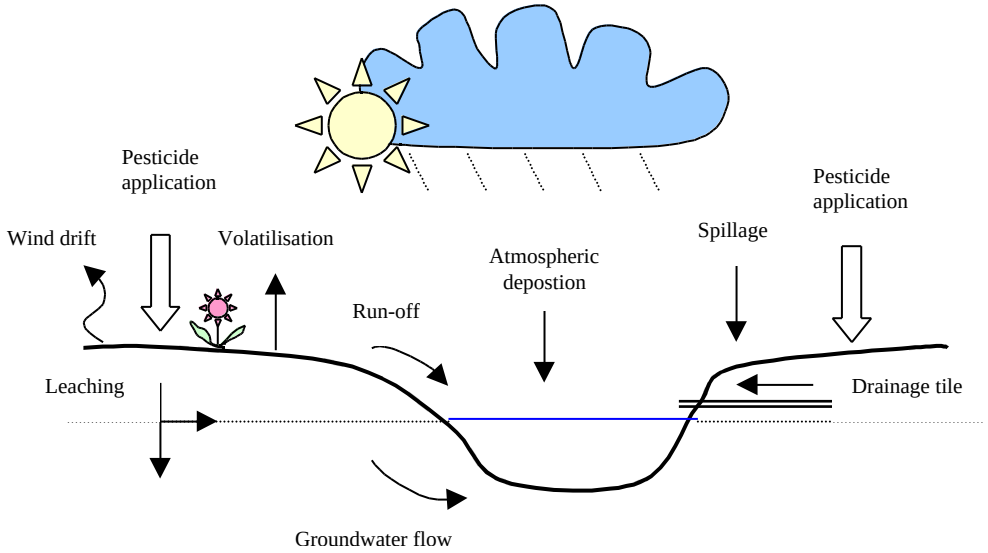


Figure 4. Possible routes for pesticides used in agriculture to enter surface water and groundwater. Modified after Kreuger (1999).

Persistence of pesticides tends to be greatest in soils high in clay and/or organic matter. Low temperatures also favour persistence since the rate of metabolism is lower under these conditions. Under tropical conditions an appropriate assumption is that losses due to volatilisation, chemical degradation and biotransformation are faster than under more temperate and cooler conditions (*Walker et al., 1996*).

There is an obvious risk that pesticides may end up in surface waters close to agricultural areas and this has been observed in many studies. Most studies performed within this field have been in the temperate region. As an example, a monitoring study by Kreuger (1998) showed that concentrations of pesticides in streams located in an agricultural landscape in the southern part of Sweden exceeded or were close to the levels where adverse effects on the aquatic flora and fauna are expected. A study made in an environment more closely related to PETAR, is the study made by Almeida (1995) in the lower parts of the Ribeira River in Brazil. This was a study in an estuary, and sediment, fish and osprey were analysed for OCs. Lindane (γ-HCH), γ-chlordane and DDT metabolites were found in the muscle tissue of a fresh water catfish. DDT metabolites were also detected in samples of fish liver. HCB, lindane, aldrin, DDT metabolites and α-chlordane were detected in sediment samples. OCs were also found in ospreys.

3.3.2 Bioaccumulation

Non-polar compounds with high hydrophobicity, e.g. PCBs and DDTs, tend to accumulate in organisms. Generally, these characteristics coincide with long biological half-life and high values for K_{OW} (partition coefficient between water and octanol for a given compound; a

measure of hydrophobicity) and they tend to bioaccumulate and possibly biomagnify in a food web. Bioaccumulation can be expressed by the bioconcentration factor, which is the ratio between the concentration in the organism and its food or the ambient medium. Bioaccumulation is affected by the rate of transformation/degradation of the compound in the organism. Biomagnification of a compound means that it is present at increased levels in organisms representing higher trophic levels in the food web. Organisms in aquatic ecosystems may take up pesticides from the food and ingested water, but a diffusion of pollutants between the organisms and the ambient water is also possible (*Walker et al., 1996*). With complex organisms like fish the bioconcentration of a pollutant can be difficult to predict since the uptake and loss are dependent upon additional factors besides diffusion. The presence of metabolising and detoxifying systems affects the rate of bioconcentration of pollutants in a fish. Aquatic organisms can take up pollutants by eating contaminated food as well as from ingested sediment particles and water (*Walker et al., 1996*).

Differences in feeding, living habits and trophic level of fish can affect their exposure to pollutants like pesticides. Predatory fish may accumulate hydrophobic compounds by eating other organisms, while bottom feeders are in constant contact with pesticides sorbed to the sediment. Herbivorous fishes grazing phytoplankton and periphyton also have a close contact with the sediment.

Hydrophobic compounds taken up by an organism are mainly distributed to tissues rich in fat and the concentrations of the compounds found are often expressed per fat weight. Concentrations of OCs in fish have been shown to vary with the lipid content of the organism, which in turn varies through the different stages of life and also shows seasonal variations (*Cullen & Connell, 1992*). Cullen and Connell found that the concentration of dieldrin and DDT with metabolites increased with the age of the fish in an Australian river as a result of bioaccumulation and bioconcentration. Non-migrating, stationary fish showed increased levels of pesticides throughout their life, while residue levels decreased in fish migrating to the open sea for spawning after having reached maturity. During the stay at sea they are exposed to clean oceanic water with lower concentrations of pesticides compared with the river water and this results in a depuration process and net loss of pesticides (*Cullen & Connell, 1992*). Pesticide residues in fish may also be lost through the lipid rich eggs. In temperate zones the fat content of fish decreases during winter and a minimum is reached after spawning (*Olsson et al., 1978*). During spawning fish spend the energy saved during the year and can contain less fat after this period. Hence, a given amount of a compound in a fish can give rise to different concentrations before and after spawning depending on the fat content of the fish being lower after spawning. The variation of fish fat content is of importance when choosing the time for sampling.

The ecological effects on aquatic organisms caused by pesticides in surface water are dependent both on the peak concentrations the pesticides may reach and the duration of exposure of organisms to pesticides. High concentrations of pesticides can cause acute effects on biota even at a short duration, while low concentrations at long duration may cause chronic effects. If treatment of fields with pesticides coincides with rainfall and susequent run-off events, high concentrations of pesticides may be found in surface water recipients. This may give adverse affects on sensitive populations of vertebrates such as fish. There is a risk that the fish community structure may change in exposed streams.

4. Materials and Methods

4.1 Sample sites

The sampling was done between 20-28 January 2000, during the wet season in Brazil. Samples were taken from the three different rivers of PETAR at 7 points (figure 5). Characteristics of the sites are summarised in table 2, where also sampling date is given. The selection of sampling sites was based on the expected impact of pesticides on the rivers of PETAR and according to the abundance of the selected fish species to be studied.

Table 2. Description of sample sites (stream name, type and location - latitude and longitude) visited in PETAR, Parque Estadual Turístico do Alto Riberia, Brazil, during January 2000 (co-ordinates are from *Moraes and Molander, 1999*).

Sample site number	Stream name	Stream characteristics	Watershed	Sampling date	Lat. 24°-S	Long 48°-W
B4	Furnas	Small stream, rocky bed	Betari	25.01.00	32.14	42.17
B9	Betari	Large stream, rocky bed	Betari	22.01.00	31.91	42.20
I2	Iporanga	Small stream, sandy bed	Iporanga	21.01.00	26.85	39.26
I4	Iporanga	Large stream, rocky bed	Iporanga	20.01.00	29.85	35.35
I5	Soarez	Small stream, rocky bed	Iporanga	24.01.00	33.24	36.10
P5	Córrego Preto	Small stream, sandy bed	Pilões	21.01.00	23.57	37.92
P9	Pilões	Large stream, rocky bed	Pilões	28.01.00	29.15	29.02

4.2 Chemicals and chromatographic conditions

Solvents used to extract water, sediment and fish tissue samples were of pesticide grade. The anhydrous sodium sulphate used was also of pesticide grade. The different brands used were Merck, Carlo Erba, Chemitest and Cinética. The chemicals used are listed in appendix 2.

All samples were analysed using a Hewlett Packard model 5890 gas chromatograph equipped with two ^{63}Ni electron-capture detectors (GC-ECD) and two columns (CP-Sil 19 CB and CP-Sil 5 CB, 20 m x 0.32 i.d. and 0.25 µm film thickness, Chrompack Sverige AB, Nacka, Sweden) attached to the same injector. Injection was splitless with 2 µl injection volume. Injector and detector temperatures were 250 °C and 300 °C respectively. Initially, the oven temperature was set for 90 °C for one minute, thereafter increasing 30 °C/min to 180 °C and then 4 °C/min to 260 °C. The temperature was then held at 260 °C for 12 min. A Varian STAR model 3400 gas chromatograph with nitrogen-phosphorus detector (GC-NPD) was used for the analysis of nitrogen-containing pesticides in the water samples. The chromatographic conditions were the same as described above.

In each series of samples run on the GC, standards were injected at the start, in the middle and at the end. In series with more than twenty samples, standards were injected five times instead of three. Calibration curves were used to evaluate the results and analyte concentration was calculated by dividing response in the sample with response of external standard and the

amount of sample and then multiplying by the standard concentration. Calculated concentrations were corrected for recoveries of the internal standard in each sample but not for recoveries of standards in fortified samples. Ethion and hexabromobenzene (HBB) were used as internal standards and their purpose is to compensate for losses during sample handling and determination.

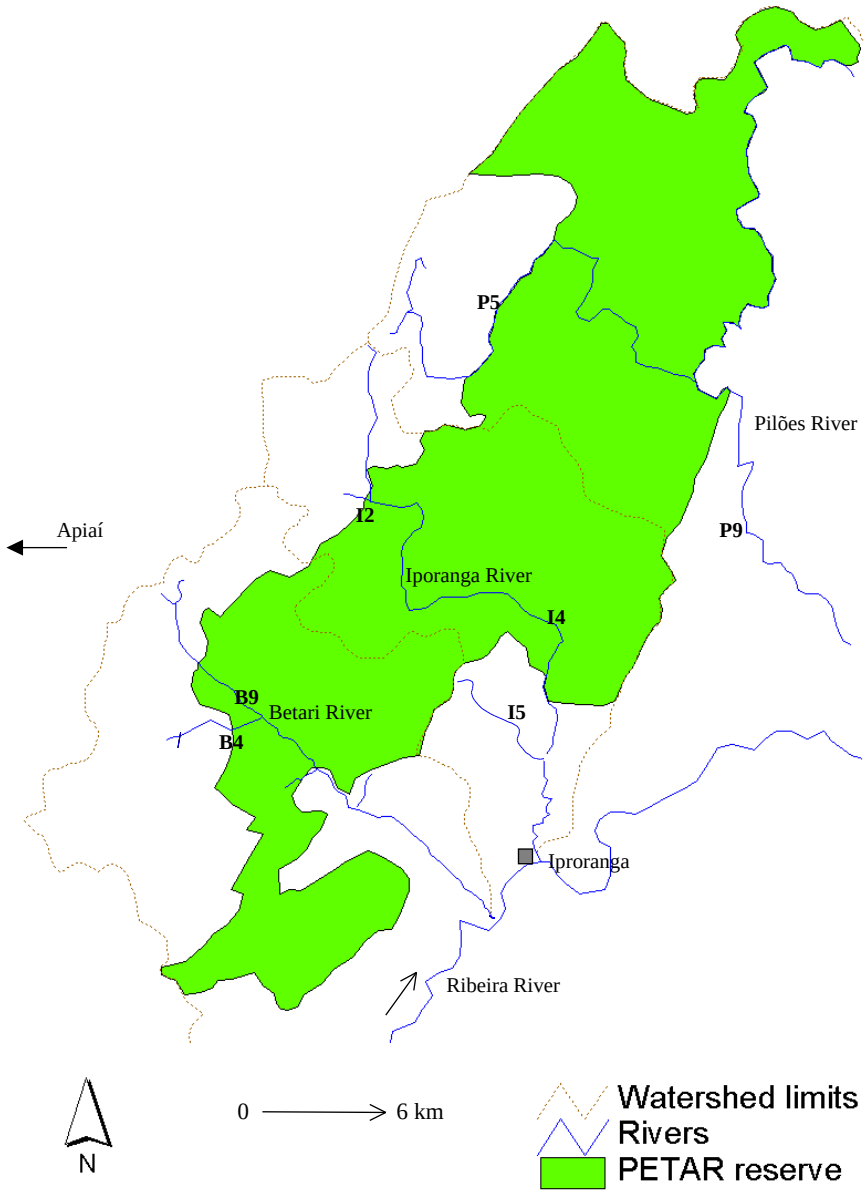


Figure 5. Map showing rivers and their watershed limits in the reserve Parque Estadual Turísticio do Alto Ribeira (PETAR), State of São Paulo, Brazil. Sampling sites visited during a sampling survey in January 2000 are also shown. B4 is a sample site in Furnas Stream, B9 is in Betari River, I2 and I4 are in Iporanga River, I5 is in Soarez stream, P5 is in Córrego Preto Stream and P9 is in Pilões River. (The map was provided by R. Moraes and thereafter somewhat modified)

4.3 Sampling and analysis

4.3.1 Water

Water samples were collected using 2-l or 5-l polypropylene bottles rinsed with stream water. Eight litres of water were taken from each site. All water samples were extracted later on the sampling day with the exception of two samples (I2:2 and P9:2). In these cases the water was kept in a refrigerator until extraction.

Out of the sample volume of 8 l four replicates of 2 l each were extracted per site. Two of the replicates were acidified to a pH of 2-3 using concentrated hydrochloric acid before extraction. pH was measured roughly with pH indicator paper. The internal standard (0.353 µg ethion, 7µl of a standard solution with 50.48 µg ethion/ml), together with approximately 100 g sodium chloride and 5 ml methanol, was added to each replicate. To extract pesticides from the water pre-filter and silica based solid-phase extraction (SPE) columns and a pressure filtration apparatus were used (figure 6). The SPE columns contain a sorbent having high affinity for non-polar compounds.

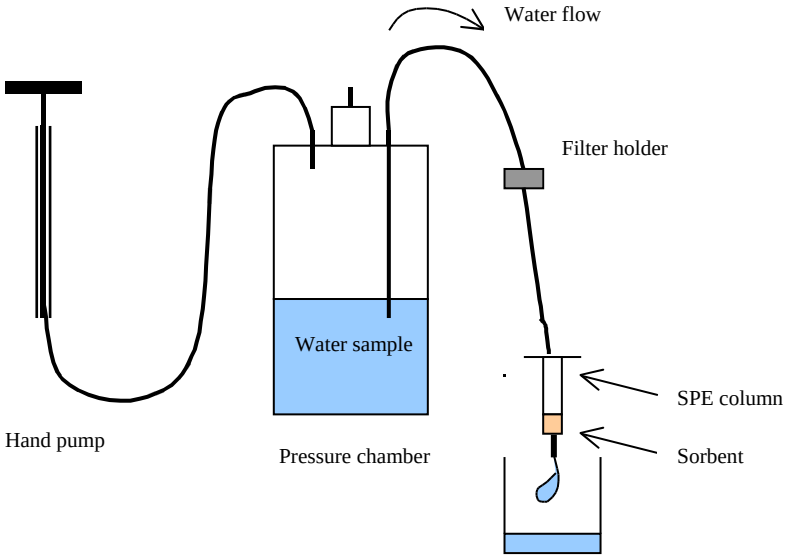


Figure 6. Pressure filtration apparatus used to extract pesticides from water in the field. Solid-Phase Extraction (SPE) columns contains a filter and a silica based sorbent having high affinity for non-polar compounds. In the filter holder a glass microfibre filter is placed to collect the particles in the water samples.

Thirty pre-filter SPE columns were pre-washed with dichloromethane (2x5 ml), methanol (5 ml) and distilled water (10 ml) and packed in aluminium foil before the field trip. The columns were dried, wrapped in aluminium foil and put in plastic bags before transport to Brazil. Six columns were pre-washed in Brazil using ethylacetate:acetone (1:1, 5 ml), methanol (5 ml) and distilled water (10 ml).

Before extraction the SPE columns were activated with a few ml of methanol and put on the outlet of the pressure filtration apparatus and the water sample was added to the pressure container. A Whatman glass microfibre filter (47 mm diameter) was placed inline in front of the column to collect the particles in the water.

Pressure was created in the container using a bicycle pump and water flowed through the system. Two pressure filtration containers were set in parallel in order to run two extractions at the same time. Four extractions per site were performed; two acidified and two non-acidified, in all cases except P5 (only two extractions of which one was acidified) and I4 (six extractions of which three were acidified). After extraction, all columns and filters were wrapped in aluminium foil and put in plastic bags and stored at -18° C in a freezer. During transport by car and plane the samples were kept on ice.

At IMA, Uppsala the extracts were eluted with dichloromethane (2x3 ml) while the columns were placed on a vacuum elution device (IST VacMaster®, Sorbent AB, V. Frölunda, Sweden). The extracts were filtered through anhydrous sodium sulphate to round-bottom flasks and cyclohexane (5 ml) was added and evaporated to 0.5 ml on a rotary evaporator. The extracts were diluted with acetone:cyclohexane (9:1) to a final volume of 1 ml. Samples were analysed on GC-ECD and GC-NPD. Pesticides included in the analysis are presented in appendix 3.

To test the recovery and the accuracy as well as the contamination risk of the extraction method spiked tests were performed with distilled water (2 l). To two samples called Std 1 0.035 µg of chlorothalonil and carbofuran respectively, were added. One of the samples was acidified. To two other samples, Std 2, 0.35 µg of each compound was added and one of the samples was acidified. These samples were also treated with internal standard (7µl), sodium chloride (100 g) and methanol (5 ml) before extraction.

Recovery tests with distilled water (2 l) and internal standard (7 µl) were also performed. In this case two non-acidified extractions were made and the samples were treated with sodium chloride and methanol as described above. As blanks two pre-washed SPE columns were wrapped in aluminium foil and thereafter treated as the columns used for extraction.

Blanks and recovery samples were then treated and eluted the same way as the other water samples.

Data on the extractions are summarised in appendix 4.

4.3.2 Sediment

Sediment was collected in polypropylene jars washed with stream water. Two replicates of approximately 100 g were taken from each site. The sediment samples were taken from the same 7 sites as the water samples. The jars were stored in a freezer and transported on ice to Sweden were they were extracted and analysed. Sediment (about 20 g) was ground and evenly mixed with Hydromatrix (10 g, Scantec, Partille). The mixture (9 g) was extracted in pre-washed cellulose thimbles with acetone:dichloromethane (1:1), using a Soxtec Avanti 2050 (FOSS Tecator AB, Höganäs, Sweden). The extraction was conducted in two steps where the sediment first was placed in boiling solvent for 2 h. Then the sediment was raised above the solvent surface to allow washing with solvent for 1 h. Solvents were then collected from the condensers for 2 min. Before extraction, ethion (0.33 µg) was added as internal standard. Extracts were dried with anhydrous sodium sulphate and filtered to round-bottom flasks. The solvent beakers from the Soxtec were rinsed with dichloromethane, which was then added to the round-bottom flasks together with cyclohexane (5 ml). Most of the solvent was then evaporated on a rotary evaporator. The volume of each extract was adjusted to 2 ml with

cyclohexane:dichloromethane (1:1), and filtered through a syringe filter (Gelman acrodisc 13 CR PFTE 0.45 µm). The extracts (1 ml) were cleaned using gel permeation chromatography and after addition of HBB (0.0665 µg), the volume of the eluate was adjusted to 1 ml with cyclohexane:acetone (9:1). Half of the purified extract was also cleaned by treatment with concentrated sulphuric acid saturated with cyclohexane. Samples were analysed for OC and pyrethroid pesticides with GC-ECD. The pesticide standards used in the analysis are listed in Appendix 3. For determination of the dry weight, approximately 20 g of sediment was dried in an oven at 105 ºC for 16 hours.

Recoveries of substances in the standards were determined by spiking four replicates of sediment from site I5 with two different standard solutions. To two replicates, 200 µl and 400 µl of a standard solution containing 0.2000 µg *p,p'*-DDE/ml, 0.3000 µg *p,p'*-DDD/ml and 0.4000 µg *o,p'*-DDT and *p,p'*-DDT/ml were added, respectively. To two other replicates, 200 µl and 400 µl of a standard solution of permethrin (2.903 µg/ml) were added. The additions were made to wet sediment (20 g) before mixing with Hydromatrix (10 g). The mixture (9 g) was then extracted. Hydromatrix was extracted as a chemical blank. These samples were all processed as outlined above.

4.3.3 Fish

Fish were caught from the streams using an electroshocker device (figure 2 c)). The largest specimens were selected for tissue sampling. They were killed on land with a blow to the head. The fish were weighed on a mechanical scale and the length (muscle length and maximum length) was measured with a ruler. Where possible, sex was also determined. The fish were cut open and the insides were removed whereby the fillets were skinned and samples of muscle tissue - as much as possible - were taken from each fish. Immediately after dissection, the muscle tissue was wrapped in aluminium foil and stored in liquid nitrogen during the days in the field and then stored in a freezer (-18 °C) in São Paulo until extraction.

Extraction of fat and fat-soluble compounds from the muscle tissue was performed at the LEA at IB, São Paulo. First, the muscle tissue was weighed. Muscle tissues from the bagres were extracted separately while the muscle samples from cascudos were pooled into four groups per site before homogenisation and extraction due to the small size of the fish. Each pooled group represented one to four samples of muscle and the samples were selected so the pools of each site were approximately of the same weight. The muscle tissue was homogenised in acetone (35 ml) and cyclohexane (10 ml) using an Ultra Turrax. The homogenate was filtered through a 150-ml separatory funnel with a sintered glassfilter and the muscle tissue homogenate in the column was rinsed twice with cyclohexane:acetone (9:1, 25 ml), for five minutes. The solvents were collected in a filterless 150-ml separatory funnel and 50 ml of a MilliQ-water solution of sodium chloride (0.2 M) and phosphoric acid (0.1 M) was added and the funnel was turned carefully a couple of times. The water phase was separated from the organic phase and washed with cyclohexane (10 ml), which was then added to the organic phase. The organic phase was dried with anhydrous sodium sulphate and filtered to a round-bottom flask and the solvent was evaporated on a rotary evaporator. The fat was then resolved in a small amount of acetone and transferred to a glass test tube with a Teflon-coated screw cap. The acetone was evaporated with nitrogen gas and the test tubes were stored in a freezer (-18 °C) for approximately four weeks in São Paulo before transportation to Sweden.

At IMA, SLU, Uppsala, the extracts were resolved in cyclohexane:acetone (9:1) and they were once again filtered through anhydrous sodium sulphate (due to problems with the humidity in Brazil) and evaporated as described above. The extracts were transferred to pre-weighed glass test tubes and the solvent was evaporated under a gentle stream of nitrogen, whereafter the test tubes were weighed and the fat content of the muscle calculated to the nearest 1/1000 g. HBB (0.0333 µg) was added as internal standard and the fat was redissolved in cyclohexane (0.5 ml). Clean-up was performed by adding concentrated sulphuric acid saturated with cyclohexane to the extracts and slowly turning the test tubes 20 times. Samples were analysed for OC pesticides with GC-ECD. The standards of OC pesticides used in the analysis are presented in Appendix 3.

Two chemical blanks, called A and B, were run following the whole procedure described above.

5. Results

5.1 Pesticide content and recoveries

5.1.1 Water

Pesticides were found in all water samples and a total of 16 different pesticides and metabolites were detected (table 3). Of the pesticides found only three (chlorpyrifos, heptachlor and malathion) have been detected in samples from PETAR taken in 1998 and 1999 (Appendix 1). As many as nine different compounds were identified in the samples from Iporanga River (I4) but the pesticides found were not present in all three samples. In the one sample taken from Córrego Preto Stream (P5), and in samples from site B9, four different pesticides were found.

Table 3. Pesticides (µg/l) detected in water samples taken from rivers in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil, January 2000. The sampling sites are Furnas Stream (B4), Betari River (B9), Iporanga River (I2 and I4), Soarez Stream (I5), Córrego Preto Stream (P5) and Pilões River (P9).

Site	BAM	Captan	Chlorfenvinfos	Chlorpyrifos	Dichlobenil	Dimethoate	Fenitrothion	Heptachlor
B4:1	0.01					0.05	0.06	
B4:2						0.03		
B9:1						0.07		
B9:2						0.03		
I2:1	0.01						0.06	
I2:2					0.01	0.02		
I4:1						0.03		
I4:2	0.03					0.06		
I4:3			0.04	0.10			0.01	
I5:1							0.05	
I5:2						0.04	0.03	
P5:1				0.42			0.05	0.01
P9:1		0.02	0.04	0.54			0.02	
P9:2			0.05				0.01	

Table 3. continued.

Site	Heptachlor epoxid*	Hexazinone	Malathion	Metamitron	Metribuzin	Parathion	Prochloraz	Quinalfos
B4:1	0.03		0.15	0.05				
B4:2	0.01		0.10	0.03			0.24	
B9:1	0.03		0.50	0.19				
B9:2			0.20	0.07				
I2:1	0.01				0.01		0.14	
I2:2	0.01							
I4:1								
I4:2			1.0	0.20				
I4:3	0.03	0.03						
I5:1				0.05		0.03	0.42	
I5:2			0.23				0.12	0.25
P5:1	0.02							
P9:1	0.04							
P9:2	0.04						0.35	

*Probable artefact

Traces of heptachlor epoxide were detected in the blanks and dichlobenil traces were found in one of the recovery test samples with distilled water and ethion and in both spiked samples.

Recoveries of ethion (Appendix 4) were low in all the samples and the mean recovery was 33 % and the standard deviation was large (13). The recoveries in the two first samples from I4 were exceptionally low (13 %).

In the test with distilled water and ethion the recoveries were 48 and 52 %. No carbofuran peaks were detected in the spiked samples and chlorothalonil could only be detected in one of the GC channels due to a disturbance peak in the other channel. The chlorothalonil recovery was low in both samples - only 29 % in the low addition and 51 % in the high addition of standard.

Samples with lowered pH were unfortunately not analysed and neither was the particulate matter on the filter paper from the extraction. The acidified water samples can be analysed further for phenoxi acid compounds and the filters for hydrophobic compounds.

5.1.2 Sediment

Different OC pesticides were found in the sediment samples. In both B4 samples high concentrations of quintozen were found, while dieldrin, heptachlor, *p,p'*-DDD and *p,p'*-DDE were found in either one or the other of the replicates. In sample P5:1 (Córrego Preto Stream) no pesticides were found, while quintozen was found in a high level in the other sample. Also, in sample I2:2 (Iporanga River) no pesticides were detected. Both samples from P9 (Pilões River) contained *p,p'*-DDE. At three sites α-HCH was found. The most frequently found pesticide was *p,p'*-DDD, which was detected at all sites but P5 and P9. In table 4 the pesticides found at the different sites are shown. Appendix 5 shows the amount of sediment extracted from each site and also % of dry weight. No pyrethroid residues were found in the screening of sediment.

Table 4. Pesticides (µg/kg d.w.) detected in sediment samples taken from rivers in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil, January 2000. The sampling sites are Furnas Stream (B4), Betari River (B9), Iporanga River (I2 and I4), Soarez Stream (I5), Córrego Preto Stream (P5) and Pilões River (P9). Two samples per site were analysed.

Site	Dieldrin (µg/kg d.w)	*p,p'*-DDD (µg/kg d.w)	*p,p'*-DDE (µg/kg d.w)	α-HCH (µg/kg d.w)	Heptachlor (µg/kg d.w)	Quintozen (µg/kg d.w)
B4:1	27		0.8			66
B4:2		1.1			0.4	24
B9:1		1.0				
B9:2		1.2				
I2:1		1.1				
I2:2						
I4:1		0.8				
I4:2		0.8		0.4		
I5:1		0.8		1.0		
I5:2				0.9		
P5:1						
P5:2				1.0		27
P9:1			8			
P9:2			13			

Recoveries of OCs in the spiked samples varied from 51 to 112%. In the sample with the lower amount of standard added, the GC response was too low to calculate recoveries except for *p,p'*-DDD, where 85% was recovered. In the case with 400 µl added, the recoveries were 51% for *p,p'*-DDT, 74% for *o,p'*-DDT, 108% for *p,p'*-DDE and 110% for *p,p'*-DDD. Since *p,p'*-DDD was found in the sediment used for the recovery tests this was subtracted from the sample before calculation of recovery. The recovery of pyrethroid compounds could not be calculated due to low response factors.

Recoveries of internal standards (Appendix 5) varied from 10-105 % for ethion with a mean of 78 % and a high standard deviation (24). Only one sample (P9:1) had such a low recovery as 10 %. The HBB recovery varied from 57-105 % and the mean was 84 % with a slightly lower standard deviation (15).

5.1.3 Fish

One or both of the species selected for the study were caught at the sampling sites except at site P5 where no fish were caught. Altogether 44 bagres were caught at five different sites; B4, B9, I4, I5 and P9 and 33 cascudos were caught at B4, B9, I4 and I5. Table 5 shows the number of bagres and cascudos caught at the different sites and also the range of weight for the fish from each site. In appendix 6 and 7, the weight, muscle weight, weight of extractable fat, length and sex for cascudos and bagres are presented, respectively. Bagre numbers 103 and 108 were not extracted and analysed.

Table 5. Number and weight of bagres (*Rhamdioglanis frenatus*) and cascudos (*Isbrueckerichthys sp.*) caught in Parque Estadual Turístico do Alto Riberia, State of São Paulo, Brazil in January 2000. Both species are catfish of the order Siluriforme. The sampling sites are Furnas Stream (B4), Betari River (B9), Iporanga River (I2 and I4), Soarez Stream (I5), Córrego Preto Stream (P5) and Pilões River (P9).

Site	Number of bagres	Range of weight – bagres (g)	Number of cascudos	Range of weight - cascudos (g)
B4	8	4.5-53.5	2	3.4-4.7
B9	8	8.5-27.5	10	2.2-9.6
I2	-		11	1.2-8-2
I4	11	2.4-33.0	8	2.2-9.2
I5	7	4.4-35.0	-	
P5	-		-	
P9	10	7.8-43.0	-	

The cascudos were pooled in four pools for each site except B4, where only two fish were caught and analysed as one sample (pool 13). A total of 13 pools with 1-4 fish in each pool were analysed and seven different pesticides were detected in the pooled cascudo samples (table 6). Heptachlor, γ-HCH and γ-chlordane were found in all pools while either one or both of the DDT metabolites *p,p'*-DDD and *p,p'*-DDE were present in all pools but number 12. The highest levels of γ-chlordane and *p,p'*-DDE were detected in the single B4 sample. It was also the only sample containing *p,p'*-DDT.

In the samples of bagres ten OC pesticides were found (table 7). In cases where α- and β-endosulfan were detected it has been marked as traces in the tables. Heptachlor concentrations

were determined from one GC column only due to a disturbance peak in the other column. In the blanks traces of γ-chlordane were found. The highest levels of γ-chlordane, *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, γ-HCH and heptachlor were detected in the fish from Soarez Stream (I5). The content of extractable fat in the muscle tissue was 0.4 % for bagres and 1.9 % for cascudos. Tables 8 and 9 show a comparison of the mean levels of pesticides in the muscle fat of bagres and cascudos based on fat weight and muscle weight, respectively.

In figure 7 the mean level of the most frequently detected pesticides in bagres and cascudos from I4 and B9 (the sites where both species were caught) are plotted and a comparison based on fat and muscle weight is made. Figure 8 is a comparison of the mean fish pesticide concentration in fish in the three different watersheds in PETAR and figure 9 shows the same results as a comparison of each site.

Recoveries of HBB (Appendix 6 and 7) varied from 59-79 % with a mean of 70 % and the standard deviation 8 for cascudos. For the bagres it varied from 45-118 % and the mean was 62 % and the standard deviation was 12. For bagres and cascudos together the mean recovery was 64 % and the standard deviation was 12.

Table 6. Pesticides and metabolites (µg/g fat) detected in muscle tissue of catfish, cascudo (*Isbrueckerichthys sp.*) from Furnas Stream (B4), Betari River (B9) and Iporanga River (I2 and I4) in Parque Estadual Turístico do Alto Riberia, State of São Paulo, Brazil, January 2000. Samples were analysed on a GC with electron capture detector.

Pool Number	Site	γ-HCH (µg/g fat)	γ-Chlordane (µg/g fat)	*p,p'*-DDT (µg/g fat)	*p,p'*-DDD (µg/g fat)	*p,p'*-DDE (µg/g fat)	Heptachlor* (µg/g fat)	α- and β-Endosulfan
1	I4	0.11	0.10		0.02	0.02	0.13	
2		0.15	0.13			0.04	0.16	
3		0.12	0.12			0.03	0.16	
4		0.11	0.08		0.03	0.02	0.15	
5	I2	0.11	0.10		0.04		0.19	Traces
6		0.24	0.21		0.07		0.24	Traces
7		0.31	0.33		0.05		0.38	Traces
8		0.03	0.03			0.01	0.04	
9	B9	0.04	0.06		0.02		0.08	
10		0.04	0.04		0.01		0.06	
11		0.04	0.05			0.01	0.06	
12		0.09	0.10				0.13	Traces
13	B4	0.13	0.58	0.32		0.14	0.26	

* Heptachlor concentrations determined from one GC column only due to disturbance in the other column.

Table 7. Pesticides and metabolites (µg/g fat) detected in muscle tissue of catfish, bagres (*Rhamdioglanis frenatus*) from Furnas Stream (B4), Betari River (B9), Iporanga River (I2 and I4), Soarez Stream (I5) and Pilões River (P9) in Parque Estadual Turístico do Alto Riberia, State of São Paulo, Brazil, January 2000. Samples were analysed on a GC with electron capture detector.

Fish number	Site	α-HCH (µg/g fat)	γ-HCH (µg/g fat)	δ-HCH (µg/g fat)	γ-Chlordane (µg/g fat)	*p,p'*-DDT (µg/g fat)	*o,p'*-DDT (µg/g fat)	*p,p'*-DDD (µg/g fat)	*p,p'*-DDE (µg/g fat)	Heptachlor * (µg/g fat)	α- and β-Endosulfan
101	I4		0.16		1.10	0.39		0.14	0.25	0.51	traces
102					1.20	0.26		0.10		0.50	traces
104					0.64	0.21		0.12	0.11	0.30	traces
105					0.15	0.07			0.07	0.08	
106					1.17	0.13			0.23	0.52	
107					1.91				0.30	0.87	
109					0.04				0.02	0.03	traces
110			0.02		0.13	0.04			0.04	0.08	
111			0.08		0.46				0.11	0.29	
112	B9		0.03	0.03	0.20				0.05	0.12	
113			0.03		0.15	0.03		0.02	0.05	0.09	traces
114			0.07		0.46			0.06	0.06	0.30	traces
115			0.03		0.20			0.03	0.05	0.11	
116			0.10		0.62				0.15	0.36	
117			0.04		0.21					0.14	
118					0.40				0.10	0.25	
119			0.04		0.20				0.05	0.13	traces
120	B4		0.16		0.80			0.18	0.19	0.57	traces
121			0.69		1.02			0.18	0.18	0.59	traces
122			0.43		0.84			0.13	0.17	0.52	traces
123			0.30		0.57			0.09	0.14	0.32	traces
124			0.33		0.60				0.11	0.30	
125			0.98		1.70			0.22	0.33	1.22	
126			0.27		0.51			0.07	0.10	0.36	
127			0.37		0.56			0.16	0.29	0.43	
128	I5		1.13		1.60			0.30	0.77	1.41	traces
129			0.74		0.99			0.19	0.35	0.89	traces
130		0.03	0.31		0.48	0.39		0.18	0.49	0.43	
131		0.06			0.89	0.40		0.19	0.41	0.75	
132			0.57		0.68	0.41		0.17	0.42	0.64	traces
133			1.23		1.84			0.22	0.60	1.37	
134			0.30		1.00	0.65		0.23	0.63	0.87	

Table 7. continued.

Fish number	Site	α-HCH (µg/g fat)	γ-HCH (µg/g fat)	δ-HCH (µg/g fat)	γ-Chlordane (µg/g fat)	*p,p'*-DDT (µg/g fat)	*o,p'*-DDT (µg/g fat)	*p,p'*-DDD (µg/g fat)	*p,p'*-DDE (µg/g fat)	Heptachlor * (µg/g fat)	α- and β-Endosulfan
142	P9	0.01	0.03		0.07	0.01		0.03	0.17	0.07	
143			0.18		0.23			0.11	0.44	0.19	
144			0.23		0.82			0.14	0.25	0.66	traces
145		0.09	0.74		1.80	0.09			0.34	1.46	
146					0.54			0.09	0.40	0.42	
147			0.47		0.69				0.22	0.59	
148		0.01	0.04		0.17	0.01	0.05	0.09	1.02	0.13	
149			0.43		0.64			0.11	0.73	0.52	traces
150					0.44			0.09	0.45	0.48	
151			0.55		1.09			0.15	0.77	1.05	traces
A (blank)					0.004						
B (blank)					0.002						

* Heptachlor concentrations determined from one GC column only due to disturbance in the other column.

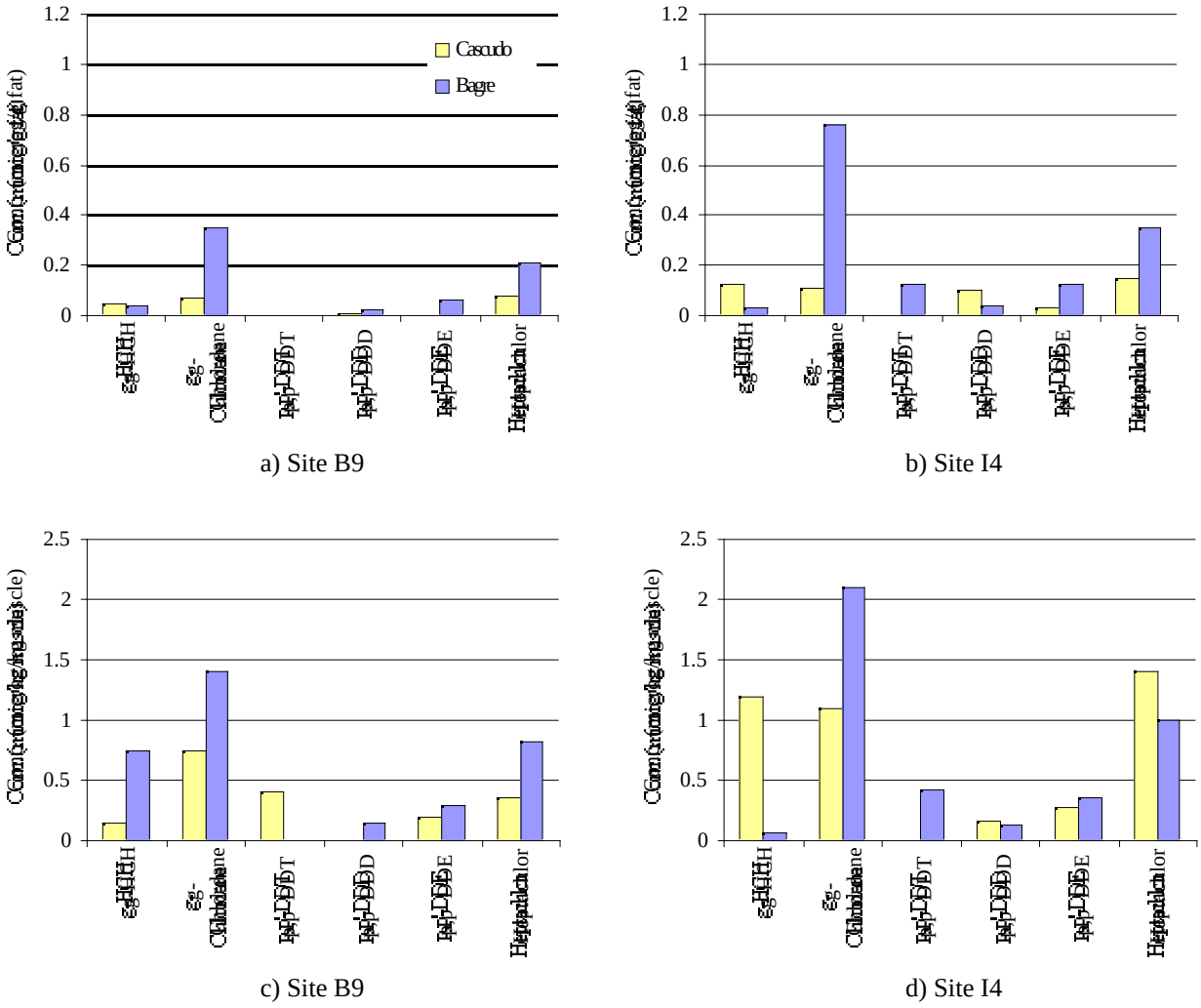


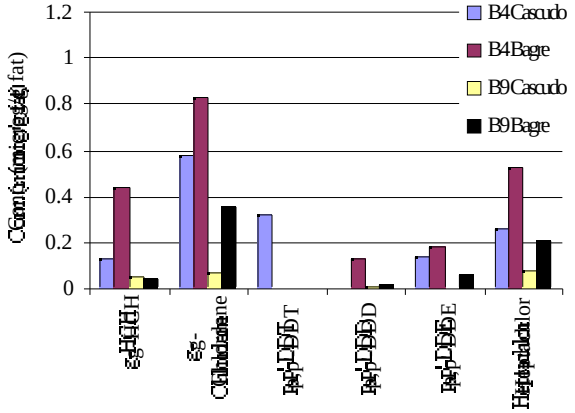
Figure 7. Mean concentrations of different pesticides and metabolites detected in fish muscle tissue of bagres (*Rhamdioglanis frenatus*) and Cascudos (*Isbrueckerichthys sp*.). Concentrations are expressed per g extractable fat in a) and b) and in c) and d) per kg muscle tissue. Samples are from Iporanga river, site I4 and Betari River, site B9 in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil January 2000.

Table 8. Mean levels of pesticides and metabolites per fish or pool (µg/g fat) detected in extractable fat in muscle tissue of bagres (B), *Rhamdioglanis frenatus* and cascudos (C), *Isbrueckerichthys sp*. caught in Furnas Stream (B4), Betari River (B9) and Iporanga River (I2 and I4) Soarez Stream (I5) and Pilões River (P9) in Parque Estadual Turístico do Alto Riberia, State of São Paulo, Brazil, January 2000.

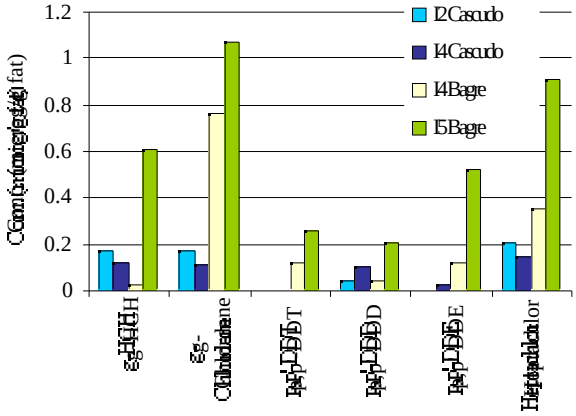
Site		α-HCH (µg/g fat)	γ-HCH (µg/g fat)	δ-HCH (µg/g fat)	γ-chlordane (µg/g fat)	*p,p'*-DDT (µg/g fat)	*o,p'*-DDT (µg/g fat)	*p,p'*-DDD (µg/g fat)	*p,p'*-DDE (µg/g fat)	Heptachlor (µg/g fat)
B4	B		0.44		0.83			0.13	0.18	0.53
	C		0.13		0.58	0.32			0.14	0.26
B9	B		0.04	0.004	0.35	0.003		0.02	0.06	0.21
	C		0.05		0.07			0.01	0.003	0.08
I2	C		0.17		0.17			0.04	0.003	0.21
I4	B		0.03		0.76	0.12		0.04	0.12	0.35
	C		0.12		0.11			0.01	0.03	0.15
I5	B	0.01	0.61		1.07	0.26		0.21	0.52	0.91
P9	B	0.01	0.26		0.65	0.01	0.01	0.08	0.48	0.56

Table 9. Mean levels of pesticides and metabolites per fish or pool (µg/kg muscle) detected in extractable fat in muscle tissue of bagres (B), *Rhamdioglanis frenatus* and cascudos (C), *Isbrueckerichthys sp*. caught in Furnas Stream (B4), Betari River (B9) and Iporanga River (I2 and I4) Soarez Stream (I5) and Pilões River (P9) in Parque Estadual Turístico do Alto Riberia, State of São Paulo, Brazil, January 2000.

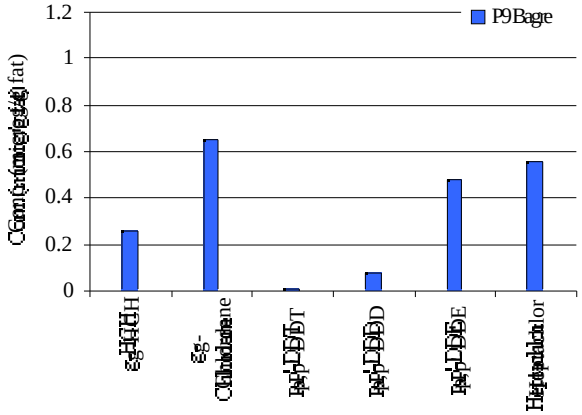
Site		α-HCH (µg/kg muscle)	γ-HCH (µg/kg muscle)	δ-HCH (µg/kg muscle)	γ-chlordane (µg/kg muscle)	*p,p'*-DDT (µg/kg muscle)	*o,p'*-DDT (µg/kg muscle)	*p,p'*-DDD (µg/kg muscle)	*p,p'*-DDE (µg/kg muscle)	Heptachlor (µg/kg muscle)
B4	B		0.74		1.4	0		0.14	0.29	0.83
	C		0.15		0.75	0.4			0.2	0.35
B9	B		0.14	0.01	1.0	0.01		0.06	0.21	0.63
	C		0.58		0.74	0		0.09	0	0.93
I2	C		2.1		2.1	0		0.41	0.08	2.9
I4	B		0.07		3.4	0.42		0.13	0.36	1.0
	C		1.2		1.1	0		0.16	0.28	1.4
I5	B	0.03	1.0		1.4	0.29		0.24	0.67	1.3
P9	B	0.02	0.42		0.79	0.33	0.02	0.11	0.82	1.3



a) Betari watershed

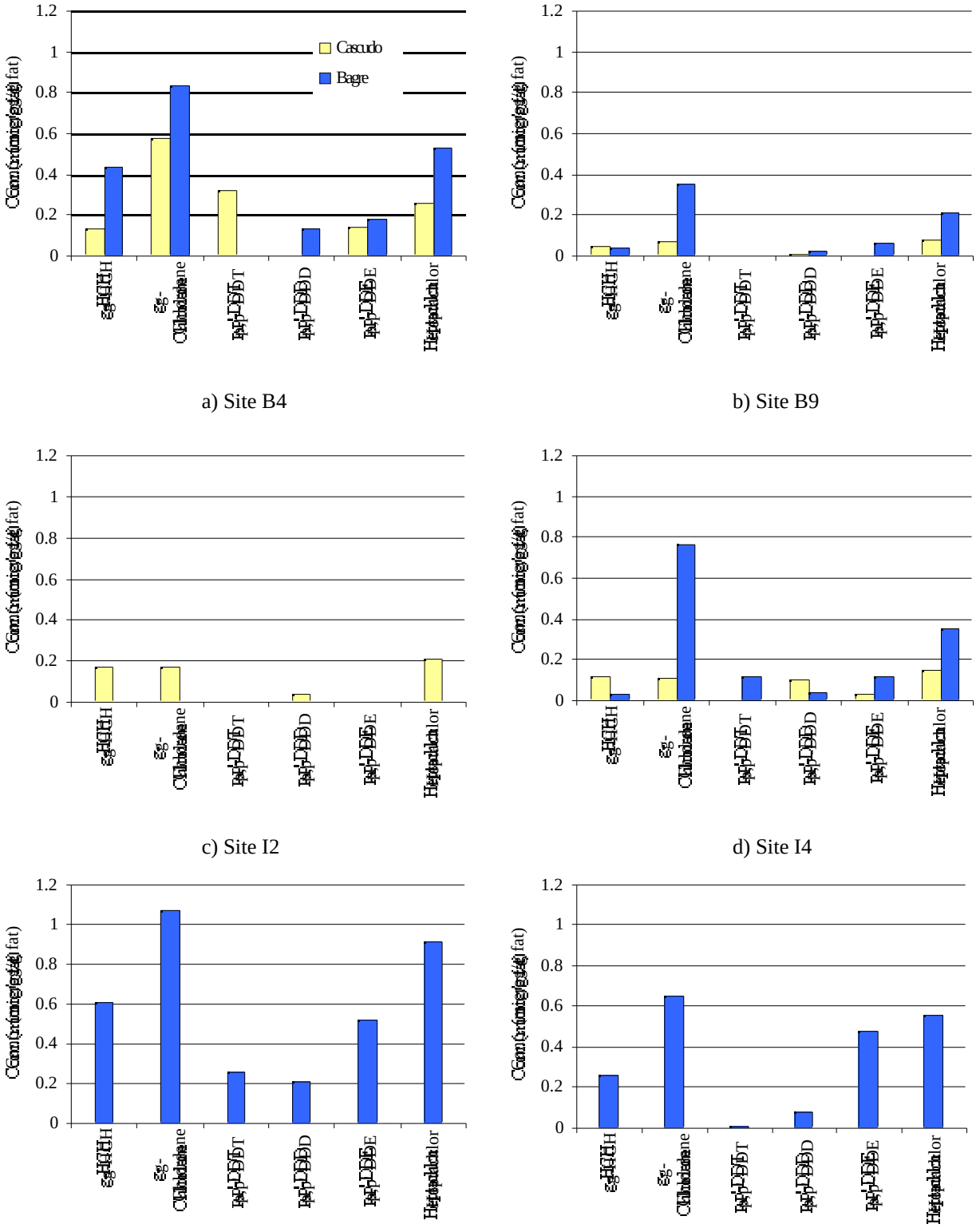


b) Iporanga watershed



c) Pilões watershed

Figure 8. Mean concentrations of pesticides and metabolites (µg/g fat) detected in fish muscle tissue of Bagres (*Rhamdioglanis frenatus*) and Cascudos (*Isbrueckerichthys sp*.) samples in a) Betari watershed (Furnas Stream (B4), Betari River (B9)), b) Iporanga watershed (Iporanga River (I2 and I4) and Córrego Preto Stream (I5)) and c) Pilões River (P9) in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil January 2000.



a) Site B4

b) Site B9

c) Site I2

d) Site I4

e) Site I5

f) Site P9

Figure 9. Mean levels of pesticides and metabolites detected in fish muscle tissue of bagres (*Rhamdioglanis frenatus*) and Cascudos (*Isbrueckerichthys sp*.) from a) Furnas Stream (B4), b) Betari River (B9), c) and d) Iporanga river (I2 and I4), e) Soarez Stream (I5) and f) Pilões river (P9) in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil January 2000. Concentrations are expressed per g extractable fat in muscle tissue.

6. Discussion

6.1 Pesticide content and recoveries

6.1.1 Water

Of the pesticides detected in water, captan, chlorfenvinfos, chlorpyrifos, heptachlor, malathion, metribuzin and quinalfos are considered highly toxic to fish and other aquatic organisms, while others are considered moderately toxic. Toxicological data for fish and Daphnia in relation to the detected pesticides are listed in appendix 8. Unfortunately, the data given are LC50 (lethal concentration for 50 % of the animals) values and the organisms are affected at far lower concentrations. For example, LOEC or NOEC (lowest/no observed effect concentration) are much more interesting when dealing with environmental samples.

In some samples heptachlor epoxide was detected but there were also traces of the same amount in the two blanks. Therefore, the heptachlor epoxide found in the water samples is probably an artefact. The spiked sample with the lower amount of chlorothalonil and carbofuran added, contained traces of malathion. Neither was the dichlobenil detected in the spiked samples. The equipment used to extract the samples was rinsed with distilled water and methanol between each sample but there is still a risk for contamination. Since the blanks were clean of both dichlobenil and malathion there must have been a contamination during the laboratory work, or possibly the distilled water was kept in a contaminated bottle. It may also be noted that the distilled water used had a pH of 6.

The recoveries of ethion were low in all samples and by far the lowest were the samples from Iporanga (I4:1 and I4:2). They were sampled and extracted on the very first field day and the extraction was not very successful due to problems with leaking. Still, the volume of water that went though the solid-phase columns was measured after extraction so there should be a correction for losses during extraction. The third replicate from I4 had a 43 % recovery. Both samples from B9 also had a very low recovery and this might result in a too high concentration value of the analytes. The low recoveries of ethion in the samples might have parallel losses of analytes, but the size of the losses is not known. It probably varies between samples and different analytes.

The sampling method itself might cause a loss of hydrophobic pesticides, which tend to absorb to glass and plastic surfaces like the sampling bottle. One way to avoid this is to use a method where water is extracted in the field by pumping it directly from the river or lake through the SPE columns. This method has been described by Arvidsson (1999).

The results from this study represent the pesticide content in random samples of water taken on one occasion. Since the standard recoveries were low and the analytes have not been confirmed on GC-MS there are some uncertainties about the reliability of the results. All pesticide concentrations reported from this study are only indications of pesticides occurring in the rivers of PETAR. It is clear from these results that the aquatic biota are exposed to different pesticides, but a more extensive sampling programme must be undertaken if we are to draw conclusions about the effects this exposure might have on the biota. Duration of exposure and peak concentration of pesticides decides the toxicity to organisms. A composite sampling over a long period of time is desirable. Techniques for time averaged composite sampling exist, e.g. the method with an automatic sampler with time-paced sampling which was used by Kreuger (1998). This method allows composite sampling of water in

programmed time intervals.

The timing of sampling is of great importance when analysing pesticides in water samples. Peak concentrations in the environment coincide with storm events and the subsequent surface run-off from agricultural fields. January 2000 was unusually dry in PETAR with only 115 mm precipitation compared with the January average, which is 266 mm (figure 3). Also, in December there was little rain compared with other years. The precipitation data are from a small village just outside the park in the south-east and the amount of rain can locally differ very much during the wet season.

6.1.2 Sediment

Samples from site B4 seem to be the most contaminated, with several different pesticide residues detected. The pesticides included in the sediment screening are hydrophobic and, generally, they are expected to be found in the sediment and bound to particles suspended in the water. The pyrethroids are readily biodegradable but show some persistence when bound to particles in soil and sediment. Still, they were not detected in the sediment. As the pyrethroid compounds added to the spiked samples were not detected on the GC there is a possibility the sediment samples contained pyrethroids that remained undetected. The pyrethroids are not very persistent compared with many OC compounds and their metabolites. Dieldrin, heptachlor and *p,p'*-DDT with its metabolites *p,p'*-DDD and *p,p'*-DDE are highly toxic, while the HCH isomers are considered moderately toxic (*Walker et al., 1996*). There is a problem with analysing *p,p'*-DDT because it tends to break down to *p,p'*-DDD or *p,p'*-DDE in the GC injector. The concentration of *p,p'*-DDT is therefore uncertain. It is necessary to run a test sample with only DDT to check how much is transformed to DDD or DDE in the GC. This might explain the 110 % recovery of *p,p'*-DDD in the spiked sample.

In many cases there are great differences in pesticide concentrations between the replicates from one site, e.g. in the samples from B4. An explanation might be that they are sampled as close as possible but still not at the very same spot and that they rather should be seen as two different samples from the same site. The most contaminated sediment seems to be from site B4, Furnas stream.

The recoveries of internal standard were generally low and exceptionally low in sample P9:1, in which the ethion recovery was only 10 %. In P9:1 the HBB recovery was 63 % which indicates that the ethion loss occurred before addition of HBB. Since HBB was added after GPC clean-up the losses of this substance represent losses from shaking with sulphuric acid and losses in the final GC determination.

6.1.3 Fish

In only three sites (B4, B9 and I4) both catfish species were caught and it is possible to compare the levels of pesticides between the species. When comparing the mean concentration of pesticides per fish and site (Table 8) it seems that the insectivorous-piscivorous bagre is more contaminated per fat weight than the herbivorous cascudo. At site I5 many of the highest pesticide levels for bagres were detected but no cascudos were caught at this site. Also, the bagres at site B4 (Furnas Stream) were among the most contaminated. For cascudos, the single pool from B4 (number 13) seems to be the most contaminated, but

this pool only contains samples from two specimens and the reliability is low. Pesticide levels in cascudos from I2 (Iporanga River) are also high.

The levels in samples from B9 and I4 are compared in figure 7 but no particularly great differences are found between the sites, but there are some differences between the species. According to the theory of bioconcentration there is a risk that predatory fish at a high trophic level accumulate pollutants to a greater extent than herbivorous fish at a lower trophic level. If the concentration is expressed per fat weight the differences in fat content of the species is important. The content of extractable fat is low in both species and the mean content is only 0.4 % in bagres while it is nearly 2 % in cascudo muscle tissue. The lower fat content in the bagres means that pesticides accumulated are concentrated in the small amount of fat and that the pesticide levels detected will be higher in the bagres than in the cascudos. This is illustrated in figure 7 a and b, with the exception that γ-HCH level in I4 is higher in the cascudos than the bagres. Some of the differences in pesticide level are explained by differences in fat content and when the pesticide content is expressed per muscle weight the situation is changed (figure 7 c and d). Then the differences are smaller and the pesticide levels in cascudos are in some cases even higher than in bagres. It is not possible to state reliably if the bagres have accumulated more pesticides than cascudos. Age and sex of the fish as well as the reproductive state have to be evaluated to determine how these factors are influencing the levels of pollutants in organisms. In this case, age of the fish is unknown and sex could not be determined in the smaller specimens. When plotting pesticide content against length or weight of the bagres and casdudos there is no indication that larger and probably older fish have a higher pesticide concentration than smaller fish.

When comparing the three watersheds in PETAR (figure 8) sites B4 and I5 seem to be among the most contaminated. The cascudo pool from B4 only contains samples from two fish and the results are somewhat less reliable for this species and sampling point. Samples from Pliões River and Córrego Preto Stream were expected to be more contaminated than other sites since there is much cultivation within their drainage area. No fish were caught at site P5 (Córrego Preto Stream) and the bagres caught at P9 (Pliões River) did not seem to be more contaminated than bagres from other sites. The graphs in figure 9 also illustrate these results.

Only persistent pesticides expected to accumulate in fat were included in the fish screening and ten out of fourteen different compounds were detected. The sulphuric acid clean-up treatment requires that the pesticides are rather stable so they can still be detected. Endosulfans were also included in the study but they are partly degraded during the clean-up step and can only be determined roughly. While endosulfan sulphate is readily degraded when treated with acid, α- and β- endosulfan can still be found at reduced quantities after the clean-up. Hence, concentrations are not given in cases where endosulfan was detected.

Heptachlor was detected in all the fish samples but its metabolite heptachlor epoxide was not. It is also degraded during the acid treatment and it is very much likely that the epoxide is present in the samples since it is the major metabolite of heptachlor, which is transformed after uptake by organisms. Heptachlor epoxide is, like heptachlor, easily accumulated in lipids and fatty tissues. The biotransformation of cyclodiene pesticides like heptachlor is an extremely slow process (*Klaassen et al., 1996*) and this might explain why heptachlor epoxide was not detected. If the fish have been exposed to heptachlor over a long period of time traces of its metabolite should be possible to detect. In this case the fish might have been recently exposed to heptachlor and the metabolite has not yet formed in detectable amounts. Heptachlor concentrations were determined from only one GC-column and there is some

uncertainty in the results. The heptachlor should be confirmed by running the samples on a GC with a mass spectrometer. The biotransformation of *p,p'*-DDT is also slow but extensive and the major metabolites are *p,p'*-DDE and *p,p'*-DDD (*Klaassen et al., 1996*). A mixture of DDT and its metabolites is often found, as in many of the fish samples in this study, when analysing body tissues. Usually, if *p,p'*-DDT is present in the environment this indicates a recent release of the pesticide since mainly its metabolites are found if a long time has passed after release. Binding of a pesticide to particles that settle on the bed of surface waters reduces its availability to organisms in the water while sediment-living or bottom-dwelling animals like the catfish species get more exposed.

The HBB recovery was quite low even though it was added to the extracted fat very late in the laboratory procedure. An internal standard should have been added to the muscle piece before extraction which would have revealed the losses during the extraction step. It is also desirable to run a spiked sample on the extracted fat and on the muscle before extraction to find out the recovery of analytes.

6.2 Concluding remarks

Pesticides were detected in samples from all the sites examined and the fish have also accumulated some of the more persistent pesticides and their metabolites. In the fish study, the small streams Furnas (B4) and Soarez (I5) seem to be more contaminated than the other sites. Samples from Iporanga River (I2 and I4) have among the lowest levels detected for fish. In the sediment study, the highest number of compounds found was at site B4. It is difficult to state anything about the levels in water but it should be borne in mind that there are many pesticides in use in the Apiaí area which for analytical reasons were not included in this study. These water samples indicate the situation during only a few seconds – the time it took to collect the samples. Aquatic biota in PETAR are most certainly exposed to pesticides dissolved in the water or bound to suspended particles or sediment. However, it is difficult to state anything about the risk to the exposed biota considering the fact that they are exposed to several different stressors. There is also a possibility of potentation of toxicity when organisms are exposed to a mixture of pesticides (*Walker et al., 1996*). This requires complex models for risk assessment are required. Furthermore, the results represent only a single sampling occasion that leaves no room for statistical evaluation. Finally, the present status clearly demonstrates that there is an environmental problem in this area, and more precise details can only be obtained through access to more time, money and fish!

7. Acknowledgement

I would like to thank Pedro Gerhard, Joel Homem de Melo Meyer and Joachim Sturve for the good work and all the good laughs in the field. The field trip was very successful! I would also like to thank the brilliant guides in PETAR, Assis and Jura, for all their help during the fieldwork and to encourage them to keep up their important work in PETAR. I also want to thank Jura and Hercilia for hospitality in their hotel. Eleonora Trajano at Instituto Biociências, USP kindly provided the fishing gear needed. PETAR administration gave us permission for research and lent us cars. I am very grateful to Luiz and Mara Luchini at LEA, IB in São Paulo, for the hospitality and all the support at the lab. I had a very pleasant stay there! Thank you Mara, Marcilio, Terezinha, Solange and the others at Instituto Biológico. I miss you all!

I have also enjoyed the stay at IMA, SLU in Uppsala and I want to thank my supervisor Henrik Kylin for advice on sampling, laboratory work, analysis, correcting the report and encouragement. Thanks also to Gunborg A., Åsa R., Märit P., Eva L., Anna H., Kerstin J. Anna A. and Karin W. for instructions and help with the practical lab work and answering all my questions.

I am very grateful to my Brazilian supervisor Rosana Moraes (Ph.D.-student at ESA, CTU, Gothenburg) for all the planning that made the field trip possible, for always being open for discussion, and for e-mail correspondence with quick answers to my questions. Thank you also for comments and ideas on the report. I also want to thank Sverker Molander (ESA, CTU) for support, discussions and help when writing the MFS application.

Finally, I want to thank Linda Andersson for being an excellent MFS colleague and travel company in Brazil and Andreas Broman for all the support.

May the fish be with you!

8. References

Almeida de, G.A., 1995. Resíduos de pesticidas organoclorados no complexo estuarino – Lagunar Iguape – Cananéia e Rio Ribeira de Iguape. Oceanographical institute, University of São Paulo. Master's Thesis in Portuguese.

Araújo, A.C.P., Telles, D.L., Gorni, R. & Lima, L.L.A., 1999. Endosulfane Residues in Brazilian Tomatoes and Their Impact on Public Health and the Environment. *Bulletin of Environmental Contamination and Toxicology*, 62, 671-676.

Arvidsson A., 1999. Determination of pesticides in an agricultural region in Sweden – sampling of water by solid-phase extraction. Department of Environmental Assessment, SLU, Uppsala 1999. Master's thesis.

Brown, C.D., Hodgkinson, R. A., Rose, D.A., Syers, J.K. & Wilcockson, S.J., 1995. Movement of Pesticides to Surface Waters from a Heavy Clay Soil. *Pesticide Science*, 43, 131-140.

Buck, S., in prep 2000. Alimentaçaõ e reproduçaõ em peixes Siluriformes (Pisces: Teleostei) em um rio da Mata Atlântíca, Alto Ribeira, São Paulo, Brasil. Instituto de Biociências, Universidade de São Paulo, São Paulo 2000. Doctoral Thesis in Portuguese.

Cullen, M.C. & Connell, D.W., 1992. Bioaccumulation of Chlorohydrocarbon Pesticides by Fish in the Natural Environment. *Chemosphere*, 25, 1579-1587.

Eke, K.R., Barnden, A.D. & Tester, D.J., 1996. Agricultural Chemicals and the Environment. Issues in Environmental Science and Technology, Ed. Hester and Harrison, Royal Society of Chemistry 1996.

EXTOXNET, 2000. http://ace.orst.edu/info/extoxnet/, 2000-08-17.

FAO (Food and Agriculture Organisation of the United Nations), 2000. Muhamed Barre via e-mail.

da Fonesca, G.A.B., 1985. The vanishing Brazilian Atlantic Forest. *Biological Conservation*, 34, 17-34.

Galetti, M. & Fernandez, J.C., 1998. Palm heart harvesting in the Brazilian Atlantic forest, changes in industry structure and illegal trade. *Journal of Applied Ecology*, 35, 294-301.

Gerhard, P., 1999. Ecologia de populações e comportamento de quatro espécies de bagres Heptapterinae (Teleostei: Siluriformes) en riachos do Alto Vale do rio Ribeira (Iporanga, São Paulo). Instituto de Biociências, Universidade de São Paulo: 128, São Paulo 1999. Master's Thesis in Portuguese.

Gevao, B., Semple, K.T. & Jones, K.C., 2000. Bound pesticide residues in soil: a review. *Environmental Pollution*, 108, 3-14.

Klaassen, C.D., 1996. Casarett and Doull's Toxicology The Basic Science of Poisons. 5th edition. McGraw-Hill, United States of America 1996.

Kreuger, J., 1998. Pesticides in stream water within an agricultural catchment in southern Sweden, 1990-1996. *The Science of the Total Environment*, 216, 227-251.

Kreuger, J., 1999. Pesticides in the Environment – Atmospheric Deposition and Transport to Surface Waters. Agraria 162, Department of Soil Sciences, Swedish University of Agricultural Sciences, Uppsala. Doctoral thesis.

Liljequist G.H., 1975. Jordens klimat. Generalstabens Litografiska Anstalt, Stockholm 1975.

Molander, S. & Moraes, R., 1998. Ecological Risk Assessment of Human Impacts on Ecosystems in Parque Estadual Turístico do Alto Ribeira (PETAR), State of São Paulo, Brazil – status report from an ERA project, August 1998. Report 1998:3, Department of Technical Environmental Planning, Chalmers University of Technology, Gothenburg, Sweden.

Moraes, R. & Molander, S., 1999. Ecological Risk Assessment in Parque Estadual Turístico do Alto Ribeira, Brazil, results from field trips and status report – August 1999. Report 1999:5, Department of Technical Environmental Planning, Chalmers University of Technology, Gothenburg, Sweden.

Olsson, M., Jensen, S. & Reutergård, L., 1978. Seasonal variation of PCB levels in Fish - An Important Factor in Planning Aquatic Monitoring Programs. *Ambio*, 7, 66-69.

Pardini, R., 1998. Feeding ecology of the neotropical river otter Lontra longicaudis in an Atlantic Forest stream, south-eastern Brazil. *Journal of Zoology*, London, 245, 385-391.

Polese, L., Minelli, E.V., Jardim, E.F.G. & Ribeiro, M.L., 1996. Small-scale method for the determination of selected organochlorine pesticides in soil. *Fresenius Journal of Analytical Chemistry*,354, 474-476.

Ranta, P., Blom, T., Niemelä, J., Joensuu, E. & Siitonen, M., 1998. The fragmented Atlantic rain forest of Brazil: size, shape and distribution of forest fragments. *Biodiversity and Conservation*, 7:3, 385-403.

Tomlin, C.D.S., 1997. The Pesiticide Manual. 11th edition. The British Crop Protection Council, United Kingdom 1997.

Torstensson, L., 1989. Bekämpningsmedel i den yttre miljön. Förekomst, spridning, effekter. Rapport 3536, Statens Naturvårdsverk (Swedish Environmental Protection Board), Stockholm, Sweden. (In Swedish but with English abstract.)

Traub-Eberhard, U., Henschel, K-P., Kördel, W. & Klein, W., 1995. Influence of Different Field Sites on Pesticide Movement into Subsurface Drains. *Pesticide Science*, 43, 121-129.

Walker, C.H., Hopkin, S.P., Sibly, R.M. & Peakall, D.B., 1996. Principles of Ecotoxicology. Taylor & Francis Ltd, United Kingdom 1996.

Appendix 1. Pesticides detected in 2 l water samples from rivers of Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil treated with ethylacetat:acetone, 1:1, methanol, NaCl (s) and internal standard (ethion). Pesticides were extracted with Solid Phase Extraction columns and eluted with dichloromethane. For multi analysis of pesticides (107 compounds) a gas chromatograph with EC-detector was used. The results represent the dissolved fraction of pesticides in the water. The sample sites are Betari river (B1, B7, B9, B10, B11), Furnas Stream (B4), Iporanga River (I2, I3, I4), Soarez Stream (I5), Corrégo Preto Stream (P5) and Pilões River (P6, P8, P9)

Site/ Sampling date	B1 990401	B10 990401	B11 990402	B4 990326	B7 990326	B9 990401	I2 990328	I3 990327	I4 990331	I5 990403	P5 990327	P8 990329	P9 990330
Pesticide (µg/L)													
HCB*			0.02	0.02	0.03	0.02	0.05	0.02	0.03	0.03	0.03	0.04	0.04
p,p'-DDE								traces					
Deltamethrin			traces						traces				0.04
Heptachlor		0.01				0.01			0.02		0.02	0.07	0.02
Carbofuran	traces												
α-Chlordane									traces				
γ-Chordane											0.01		traces
Chlorprofam										0.4		0.2	
Chlorpyrifos					0.1								
Metazachlor	2.5								1.1				2.2
Propachlor											0.3		
Tetradifon											0.02		

Site/ Sampling date	B1 981120	B7 981115	I2 981119	I3 981116	I4 981115	P5 981116	P6 981117
Pesticide (µg/L)							
HCB*	0.05	0.01		0.01	traces	0.02	0.02
Deltamethrin			0.5			traces	
Dichlorvos			0.1				
Chlorprofam	0.3						
Malathion		0.1		0.2	0.1		traces
Permethrin			1.9			0.1	

* Probable artefact (HCB was also found in blanks)

Chemicals

Appendix 2

Water extraction
Methanol, P.A. Chemitest® (PM 30.04)
Sodium chloride, P.A. Chemitest®
Ethylacetate, P.A. Chemitest®
Acetone, P.A. Chemitest®
Hydrochloric acid, P.A. Cinética® (PM 36.46)

Chemicals at IMA

Dichloromethane, pesticide grade MERCK
Acetone, pesticide grade MERCK
Cyclohexane, pesticide grade MERCK
Sodium sulphate, P.A. MERCK
Ethion
Carbofurane
Chlorothalonil

Fish extraction
Cyclohexane, pesticide grade Carlo Erba
Acetone, pesticide grade Carlo Erba
Sodium sulphate, pesticide grade Carlo Erba
Sodium chloride, P.A. -ACS ^{Reagentes}ECIBRA® (PM 58.44)
Phosphoric acid, P.A. MERCK

Chemicals at IMA

Cyclohexane, P.A. MERCK
Acetone, P.A. MERCK
Sodium sulphate, P.A. MERCK
Sulphuric acid, P.A. MERCK
HBB

Sediment extraction

Chemicals at IMA

Dichloromethane, pesticide grade MERCK
Acetone, pesticide grade MERCK
Cyclohexane, pesticide grade MERCK
Sodium sulphate, pesticide grade MERCK
Sulphuric acid, P.A. MERCK
HBB
Ethion

Appendix 3. Compounds used as standards in the analysis of pesticide residues in water samples from rivers of Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil January 2000. Standards used for the sediment samples are marked * and standards for the fish analysis are marked with #

Pesticide	Group	*Pesticide*	Group	*Pesticide*	Group
Acephate	*Org-Phosphorus*	Diflufenikan		Metalaxyl	*Phenylamide*
Aklonifen	*Diphenyl-ether*	Diclobenil	*Benzonitril*	Metamitron	*Triazinone*
Aldrin*#	*Org-Chlorine*	Dichlorvos	*Org-P*	Metazachlor	*Chloroacetanilide*
Atrazine	*Triazine*	Dimethoate	*Org-P*	Methiocarb	*Carbamate*
Atrazindesethyl		Diuron*	*Urea*	Methoxichlor	
Atrazinedesisopropyl		α-Endosulfan*#	*Cyclodiene org-Cl*	Metribuzin	*Triazinone*
Azinphos-methyl	*Org-P*	β-Endosulfan*#	*Cyclodiene org-Cl*	Mevinphos	*Org-P*
BAM		Endosulfan sulphate*	*Cyclodiene org-Cl*	Ethyl-Parathion- *	*Org-P*
Benazolinethylester		Endrin	*Org-Cl*	Methyl-Parathion	*Org-P*
Bitertanol	*Azole*	EPTC		Pendimethalin	*Dinitroaniline*
Captan*	*Trihalomethylthio*	Esfenvaletate	*Pyrethroid*	Penconazol	*Azole*
Carbaryl	*Carbamate*	Etrimphos		Pentachloranilin	
Carbofenothion		Fenfurfam	*Carboxamide*	Permethrin	*Pyrethroid*
Carbofuran	*Carbamate*	Fenitrothion	*Org-P*	Pirimicarb	*Carbamate*
Carbosulfane	*Carbamate*	Fenmedifam		Prochloraz	*Azole*
Carboxine	*Carboxamide*	Fensulfothion		Propachlor	*Chloroacetanilide*
α-Chlordane	*Org-Cl*	Fenvalerate	*Pyrethroid*	Propiconazol*	*Azole*
γ-Chlordane*#	*Org-Cl*	Flucynitrate		Propoxur	*Carbamate*
Chlorfenvinphos	*Org-P*	Fosfamidon		Propyzanid*	*Amide*
Chloridazon	*Pyridazone*	α-HCH*#	*Org-Cl*	Prosulfocarb	*Carbamate*
Chlorobensilate		β-HCH*#	*Org-Cl*	Quinalphos	*Org-P*
Chlorprofam	*Carbamate*	δ-HCH*#	*Org-Cl*	Quintozen	*Aromatic hydrocarbon derivate*
Chlorpyriphos	*Org-P*	γ-HCH*#	*Org-Cl*	Simazin	*Triazine*
Chlorothalonil		Heptachlor*#	*Org-Cl*	Sulfotep	*Org-P*
Cyanazine	*Triazine*	Heptachlorepoxid*#	*Org-Cl*	Terbutryn	*Triazine*
Cyfluthrin*	*Pyrethroid*	Hexachlorbenzene	*Org-Cl*	Terbutylazin	*Triazine*
α-cypermetrin	*Pyrethroid*	Hexazinone	*Triazinone*	Tetradifon*	
β-cypermethrin	*Pyrethroid*	Imazalil*		Thiabendazole	*Benzimidazole*
p,p'-DDD*#	*Org-Cl*	Ioxiniloctylesther		Toclofosmethyl	*Org-P*
p,p'-DDE*#	*Org-Cl*	Iprodion*		Tolylfluanid*	*N-trihalomethylthio*
p,p'-DDT*#	*Org-Cl*	Isofenphos		Triadimefon	*Azole*
p,p'-DDT*#	*Org-Cl*	Keto-endrin		Triadimenol	*Azole*
Deltamethrin	*Pyrethroid*	λ-cyhalothrine*	*Pyrethroid*	Trifluralin	*Dinitroaniline*
Desmedifam	*Carbamate*	Linuron	*Urea*	Vinclozolin*	*Dicarboximide*
Diazinone	*Org-P*	Malathion	*Org-P*		
Dieldrin		Methabenzthiazuron	*Urea*		

Appendix 4. Data on water samples (sampling date, extracted volume, extraction time, pH and ethion recovery) taken from rivers in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil, January 2000. The sampling sites are Furnas Stream (B4), Betari River (B9), Iporanga River (I2 and I4), Soarez Stream (I5), Córrego Preto Stream (P5) and Pilões River (P9)

Sample	Date	Extraction time (h and min)	Extracted volume (ml)	pH	Ethion recovery (%)	Comments
B4:1	000125	1.15	2000	6	41	Extraction flow fast in the beginning.
B4:2		1.35	1990	6	33	Problems with filter.
B9:1	000122	1.20	2000	6	15	Low recovery
B9:2		1.20	2000	6	19	Low recovery
I2:1	000121	2.35	2000	5.5	48	
I2:2		2.30	1920	5.5	32	Sample stored three days in refrigerator before extraction.
I4:1	000120	1.25	1670	6	13	Problems with leaking. Low recovery
I4:2		1.25	1970	6	13	Low recovery
I4:3	000128	2.15	2000	6	43	
I5:1	000124	1.35	2000	5	38	Problems with filter.
I5:2		1.10	2000	5	40	
P5:1	000121	1.30	2000	5.5	42	
P9:1	000128	2.15	1800	5	47	Problems with leaking.
P9:2		2.20	1995	5	42	Sample stored one day in refrigerator before extraction.
Std 1		1.10	2000	6	41	Distilled water+0.035 µg chlorothalonil and carbofuran+0.353 µg ethion. Low pH.
Std 2		1.15	2000	6	34	Distilled water+0.35 µg chlorothalonil and carbofuran+0.353 µg ethion. Low pH.
Int.std. 1		1.15	2000	6	48	Distilled water+0.353 µg ethion. Low pH.
Int.std. 2		1.15	2000	6	52	Distilled water+0.353 µg ethion. Low pH.
Blank 1						Columns were placed on extractor and then removed again.
Blank 2						

Appendix 5. Extracted amount of sediment and % of dry sediment in samples from streams in Parque Estadual Turístico do Alto Ribeira, State of São Paulo (Brazil), taken January 2000

Site	Sediment extracted (g wetw.)	Dry sediment (%)	Ethion recovery (%)	HBB recovery (%)
B4:1	6.01	69.0	90	96
B4:2	6.27	69.9	88	98
B9:1	6.36	77.0	85	90
B9:2	6.26	72.2	77	83
I2:1	6.09	78.0	72	86
I2:2	6.11	74.0	105	90
I4:1	5.93	78.1	87	105
I4:2	6.10	77.7	84	82
I5:1	6.03	70.2	84	92
I5:2	6.11	72.4	87	85
P5:1	6.30	76.7	90	57
P5:2	6.26	73.8	89	62
P9:1	6.01	80.5	10	63
P9:2	6.06	76.9	75	67

Appendix 6. Data (weights, length, recovery) for cascudos (*Isbrueckerichthys sp.*, Order Siluriformes, Family Loricariidae) caught at the sampling survey in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil, January 2000. The sites of sampling are Betari River (B), Iporanga River (I) and Pilões River (P). The cascudo is a bottom-dwelling grazer & scraper

Site	Date	Pool number	Sample number	Sex	Weight (g)	Muscle – maximum length (mm)	Muscle weight (g)	Pool muscle weight (g)	Fat weight (g)	Muscle fat (%)	HBB recovery (%)	Comments
I4	000120	1	1		8	69-86	2.4732	3.6571	0.0688	1.8	65	
			3		3.6	54-66	1.1768					
		2	2		6.9	67-81	1.8045	3.0327	0.0237	0.8	77	
			5		2.2	48-57	0.6461					
			6		2.5	48-58	0.5995					
		3	4		9.2	76-90	2.7208	3.3337	0.0325	1.0	79	
			10	F	2.6	45-54	0.6150					
		4	7		6.3	60-75	1.7636	3.1365	0.0580	1.8	71	
			8		4.8	54-69	1.3815					
			9		-	-	-					Fish number 9 missing.
I2	000121	5	11		1.9	43-52	0.5427	1.4503	0.0295	2.0	79	
			12		1.95	45-52	0.4758					
			13		1.75	43-51	0.4311					
		6	14		1.6	41-50	0.3332	1.524	0.0173	1.1	74	
			15		2.5	47-56	0.7273					
			21		2.0	44-52	0.4754					
		7	16		1.4	37-45	0.3786 (16+17)	0.8858	0.0137	1.5	72	
			17		0.95	34-41						
			18		1.4	32-41	0.5117 (18+19)					
			19		1.15	37-45						
		8	20	F	8.2	72-86	2.5398	2.5398	0.1573	6.2	59	
B9	000122	9	22		2.2	46-56	0.5891	4.6145	0.0929	2.0	68	
			23		9.6	72-?	2.5762					
			26		5.5	63-77	1.4475					
		10	24		9.0	71-85	2.2765	3.4409	0.1093	3.2	62	
			27	F	6.5	71-84	1.2125					
		11	25		7.9	65-82	2.0388	4.1771	0.0693	1.7	70	
			28	F	8.4	73-87	2.1426					Dropped muscle 28 on the floor.
		12	29		7.0	66-79	1.9594	5.1956	0.0413	0.8	66	
			30		5.7	67-82	1.1045					
			31		8.0	65-85	2.1466					
B4	000122	13	32		4.7	59-73	1.1214	1.8157	0.0078	0.4	75	
			33		3.4	53-66	0.6943					

Appendix 7. Data (weights, length, recovery) for over bagres (*Rhamdioglanis frenatus*, Order Siluriformes, Family Pimelodidae) caught at the sampling survey in Parque Estadual Turístico do Alto Ribeira, State of São Paulo, Brazil, January 2000. The sites of sampling are Betari River (B), Iporanga River (I) and Pilões River (P). The bagre is a carnivorous-insectivorous and piscivorous and bottom-dwelling fish

Site	Date	Sample number	Sex	Weight (g)	Muscle – max length (mm)	Muscle weight (g)	Fat weight (g)	Muscle fat (%)	HBB recovery (%)	Comments
I4	000120	101		3.9	70-81	1.4260	0.0067	0.5	66	
		102		4.9	87-95	1.5588	0.0044	0.3	76	
		(103)		2.7	71-79	0.8509	-			Not extracted.
		104		4.1	79-87	1.5875	0.0109	0.7	73	
		105	F?	14.5	125-142	6.0040	0.0365	0.6	66	
		106		8.0	98-107	3.2205	0.0097	0.3	69	
		107		3.1	76-83	1.2417	0.0040	0.3	70	
		(108)		2.4	68-84	-	-			Not extracted.
		109		33.0	158-176	14.018	0.2332	1.7	63	
		110	F?	20.0	141-164	7.8205	0.0525	0.7	69	
		111	M	16.0	129-144	6.3148	0.0163	0.3	68	
B9	000122	112	M	25.5	141-161	9.0455	0.0514	0.6	65	
		113	F	27.5	143-174	9.4860	0.0154	0.2	118	
		114	M	25.5	142-169	5.0835?	0.0330	0.6	53	
		115		15.5	121-143	?	0.0400		63	
		116	M	7.7	96-114	2.8678	0.0105	0.3	64	
		117	M	12.5	108-125	4.6540	0.0257	0.6	67	Lost some of the org. phase.
		118	M	8.5	97-117	3.2009	0.0136	0.4	66	
		119	F	18.0	128-151	7.0850	0.0355	0.5	67	
B4	000122	120		11.5	116-135	3.7919	0.0066	0.2	66	Wound at right gill.
		121	F	33.5	162-189	10.0342	0.0077	0.1	67	
		122	M	34.5	164-190	10.5500	0.0133	0.1	63	
		123	M?	28.0	152-176	8.9535	0.0163	0.2	67	
		124	M	11.0	109-124	4.0692	0.0129	0.3	67	
		125	M	4.5	77-92	1.6250	0.0043	0.3	70	
		126	M	11.0	97-116	3.5910	0.0171	0.5	60	
		127	F	53.5	195-?	13.7457	0.0168	0.1	53	Caught in net, not electro-shocked.
I5	000124	128		4.35	78-94	1.3677	0.0025	0.2	61	
		129	M	4.85	78-95	1.6329	0.0054	0.3	62	
		130	F	35.0	165-196	10.4923	0.0142	0.1	54	
		131	M	10.0	102-126	3.1060	0.0068	0.2	57	
		132	M	11.0	111-131	3.5915	0.0088	0.2	56	
		133	F	5.95	88-103	1.7244	0.0040	0.2	58	
		134		11.5	98-119	2.8077	0.0052	0.2	58	
P9	000128	142	M	43.0	169-198	10.4927	0.0670	0.6	51	
		143	M	21.0	132-161	5.7799	0.0281	0.5	47	
		144		8.2	96-118	2.5346	0.0073	0.3	55	
		145		7.8	92-111	2.1842	0.0050	0.3	55	
		146	F	26.0	152-181	7.2830	0.0119	0.2	54	
		147	M	9.5	99-113	2.9517	0.0076	0.3	56	
		148	F	40.5	170-202	12.8738	0.1007	0.8	45	
		149	F	25.0	157-198	8.4180	0.0084	0.1	53	
		150	F	26.0	155-191	7.9270	0.0124	0.1	47	
		151	F	23.0	140-180	7.0818	0.0082	0.1	51	

Appendix 8. Toxicological data and water solubility for pesticides detected in Parque Estadual Turístico do Alto Ribeira, Brazil (*Tomlin, 1997 and EXTOXNET 2000-08-17 at http://ace.orst.edu/info/extoxnet/*)

Pesticide	Class	Water solubility (mg/l)	LC50 (96 h) Fish (mg/l)	LC50 Daphnia sp. (mg/l)
Captan	Fungicide N-trihalomethylthio	3.3 (25 °C)	Bluegill sunfish 0.072 Brook trout 0.034 Harlequin fish 0.3	7-10 ppm (48 h)
γ-Chlordane	Insecticide Organochlorine	0.1 (25 °C)	Bluegill sunfish 0.07 Rainbow trout 0.09	0.59 (48 h)
Chlorfenvinfos	Insecticide Organophosphorus	145 (23 °C)	Guppies 0.3-1.6 Harlequin fish <0.32	0.0003 (48 h)
Chlorpyrifos	Insecticide Org-P	1.4 (25 °C)	Rainbow trout 0.003 Roach 0.25	0.0017 (48 h)
DDT	Insecticide Org-Cl	insoluble	*p,p'*-DDT: Channel catfish 0.00122 Northern pike 0.00027 Rainbow trout 0.00087 *p,p'*-DDE: Minnow trout 0.0032	*p,p'*-DDT: 0.00047 (48 h)
Dichlobenil	Hebicide Benzonitril	14.6 (20 °C)	Various species 5-13	6.2 (48 h)
Dieldrin	Insecticide Org-Cl			
Dimethoate	Insecticide Org-P	23000 (20 °C)	Bluegill sunfish 6 Mosquito fish 40-60 Rainbow trout 6.2	4.7 (24 h)
α-/β-Endosulfan	Insecticide Org-Cl	0.32(α)/0.33(β) (22 °C)	Golden orfe 0.002	0.075-0.750
Fenitrothion	Insecticide Org-P	21 (20 °C)	Carp 4.1 (48 h) Bluegill sunfish 3.8 Brook trout 1.7	
γ-HCH (lindane)	Insecticide Org-Cl	7.3 (25 °C)	Guppies 0.16-0.3	
Heptachlor	Insecticide Org-Cl	0.056 (25-29 °C)	Bluegill sunfish 0.026 Fathead minnow 0.078-0.130 Rainbow trout 0.007	
Hexazinone	Herbicide Triazinone	33000 (25 °C)	Bluegill sunfish 370-420 Fathead minnow 274 Rainbow trout 320-420	442 (48 h)
Malathion	Insecticide Org-P	145 (25 °C)	Bluegill sunfish 0.1 Largemouth bass 0.28	
Metamitron	Herbicide Triazinone	1700 (20 °C)	Golden orfe 443 Rainbow trout 326	101.7-206 (48 h)
Metribuzin	Herbicide Triazinone	1050 (20 °C)	Bluegill sunfish 80 ppm Catfish >10 ppm Goldfish >10 ppm Rainbow trout 76 ppm	4.5-35 (48 h)
Parathion	Insecticide Org-P	11 (20 °C)	Golden orfe 0.58 Rainbow trout 1.5	0.0025 (48 h)
Prochloraz	Fungicide Azole	34.4 (25 °C)	Bluegill sunfish 2.2 Rainbow trout 1.5	4.3 (48 h)
Quinalfos	Insecticide Org-P	17.8 (22-23 °C)	Carp 3.63 Rainbow trout 0.005	0.00066 (48 h)
Quintozen	Fungicide Ar. hydrocarbon derivate	0.1 (20 °C)	Bluegill sunfish 0.1 ppm Rainbow trout 0.55 ppm	0.77 (48 h)