Variables determining the response of invertebrate species to toxicants, A case study on the River Meuse.

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Chapter I

GENERAL INTRODUCTION

A century ago the large rivers in Europe were still characterized by a high faunal biodiversity. Especially benthic communities were rich in invertebrate species, but nowadays many populations have virtually collapsed in several rivers. Concomitantly, few ubiquitous species are blooming. Also the fish fauna has much declined, notably the migratory species have been eradicated in many river systems. Today, ecological recovery and rehabilitation of aquatic ecosystems have become major objectives of many governments. For some rivers, like the Rhine, international rehabilitation programmes have been established which aimed at restoring water quality so that formerly existing species may return, safeguarding river water as a source of drinking water, and reducing pollution of the river sediment (IKSR, 1987). Although a few typical riverine insects returned to the Rhine recovery proceeds slowly, while in other rivers, where no such rehabilitation schemes are in operation, recovery has not even commenced.

In order to restore the original biodiversity of disturbed rivers it is essential to know how species can benefit from improving environmental conditions and which environmental requirements are to be fulfilled. However, decisions on measures are hampered by a lack of knowledge on the autecology and the sensitivity to toxicants of many aquatic macrofauna species. This information is crucial, because most large rivers harbour habitats that are modified in many respects and at the same time many different water quality parameters indicate deteriorated conditions for aquatic life.

The lack of information on the specific requirements of macrofauna species to many environmental factors hampers the interpretation of data on the distribution of aquatic invertebrates severely. This scientific problem confronts also the water management with a dilemma: is there perspective for massive nature development programmes recreating interconnected habitats or is water quality to be further improved by reducing the input of toxicants and nutrients? Van Urk *et al.* (1993) observed that a decrease in concentrations of insecticides in the River Rhine coincided with the reappearance of the caddisfly *Hydropsyche contubernalis*, whereas Neumann (1990) suggested that the recolonization of this species was due to increasing oxygen levels. This example clearly demonstrates that the current ecological and ecotoxicological understanding of riverine invertebrates is insufficient to found river rehabilitation.

The aim of this study is to identify which key factors of water quality determine the response of invertebrate species to polluted river water. It is evaluated if toxic barriers exist for indigenous species to re-establish in rivers in the process of sanitation, and which variables determine the response of "pollution tolerant" species to deteriorated conditions. The River Meuse has hardly shown signs of ecological recovery in recent years and therefore this river was used in a case study.

River Meuse

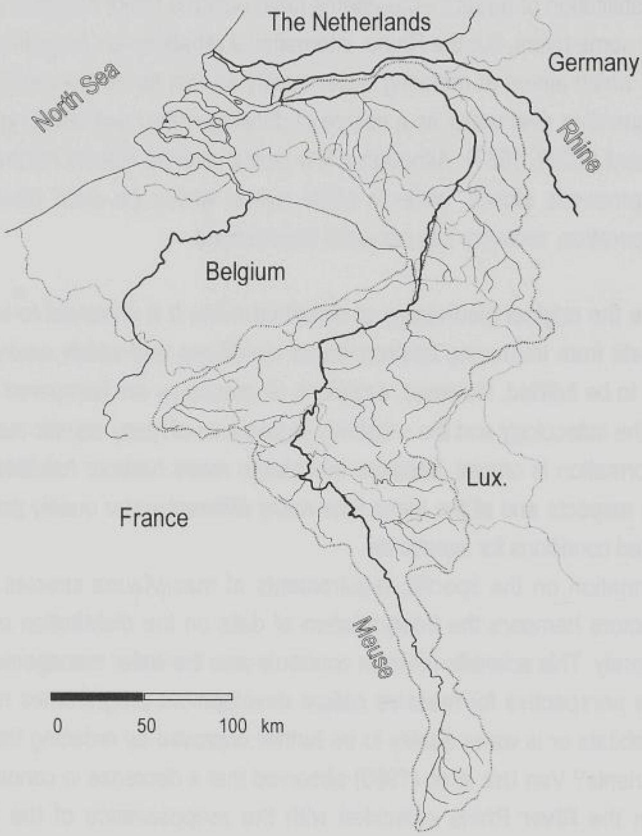

Fig. 1.1: Catchment of the River Meuse.

The Meuse is a rainfed river having a 35000 km^2 catchment area stretching from France through Belgium and The Netherlands (Fig. 1.1). The total length of the river is 850 km, of which the lower 250 km flows through The Netherlands (Breukel *et al.*, 1992). The Meuse serves many functions; its ecological value of certain reaches, e.g. the Grensmaas (part of the Dutch/Belgian Meuse which is not canalized), is of great importance. Meuse water is abstracted (as ground water and surface water) to provide drinking water for six million people in The Netherlands, Belgium and France (Descy and Empain, 1984; Van Urk, 1984). A large part of the water is abstracted for agricultural purposes; no less than one third of the Dutch part of the catchment consists of agricultural land (Van Urk, 1984). From the middle eighteenth century, the Meuse has become an important shipping route (Billen *et al.*, 1995). The river bed is used for the commercial extraction of gravel, and hydropower is used to generate energy. Finally, the Meuse serves recreational purposes (Breukel *et al.*, 1992).

The River Meuse has been exploited ever since the Middle Ages. Especially in the past decades, the morphology and chemistry of the Meuse was much altered. Habitat structures disappeared when dams, weirs and channels were constructed for shipping, and the water quality is greatly impoverished due to the input of industrial, agricultural and domestic waste (Admiraal *et al.*, 1993).

Focussing on water quality, it is apparent that the load of pollutants has changed during the past decades. In the seventies, metal levels were a major problem in the Meuse, but since the late seventies up to the mid nineties metal levels have dropped substantially (Baggelaar and Baggelaar, 1995). The load of organic toxicants like polycyclic aromatic hydrocarbons has decreased as well. However, oxygen levels have not improved, and nutrients are still as high as in the seventies. Bacteria (*E. coli* and fecal streptococci) even show a strong increase since then. The levels of cholinesterase inhibitors have also increased (Baggelaar and Baggelaar, 1995), indicating that pesticides are an increasing problem in the River Meuse.
Chemical measurements during recent years showed that the French part of the Meuse is relatively clean, and that the Meuse is most polluted downstream the industrial region of Liège (RIWA, 1996; RIWA, 1996; RIWA, 1997). The water quality improves to some extent further downstream, although the input of pollutants continues in the Dutch part of the Meuse (Fig 1.2 and Table 1.1 in appendix). Screening of an extensive data set on physical and chemical parameters (RIWA, 1996a; RIWA, 1996b; RIWA, 1997) shows that the Meuse carries the highest toxicant levels at the Dutch/Belgian border (Eijsden). Compared to other sites, pesticide concentrations are generally highest at Eijsden; the concentration of cholinesterase inhibitors (organophosphorous pesticides) even reached 6.4 μg/L in 1996

(Fig. 1.2). Although in general metal levels have dropped over the years, zinc levels are occasionally extremely high (990 µg/L in 1995 and 660 µg/L in 1996: Table 1.1) at this site. Aside from a high load of chemicals at the Belgian/Dutch border, the Meuse contains the lowest oxygen levels around Eijsden. While the minimum oxygen concentrations measured in other regions along the Meuse were at least 5.8 mg/L, they were as low as 2.4-2.8 mg/L during three consecutive years at Eijsden. One of the causes for these oxygen deficits is the input of sewage just upstream from Eijsden, around the city of Liège. The presence of sewer canals along this part of the Meuse is also apparent from the extremely high number of fecal bacteria measured at Eijsden.

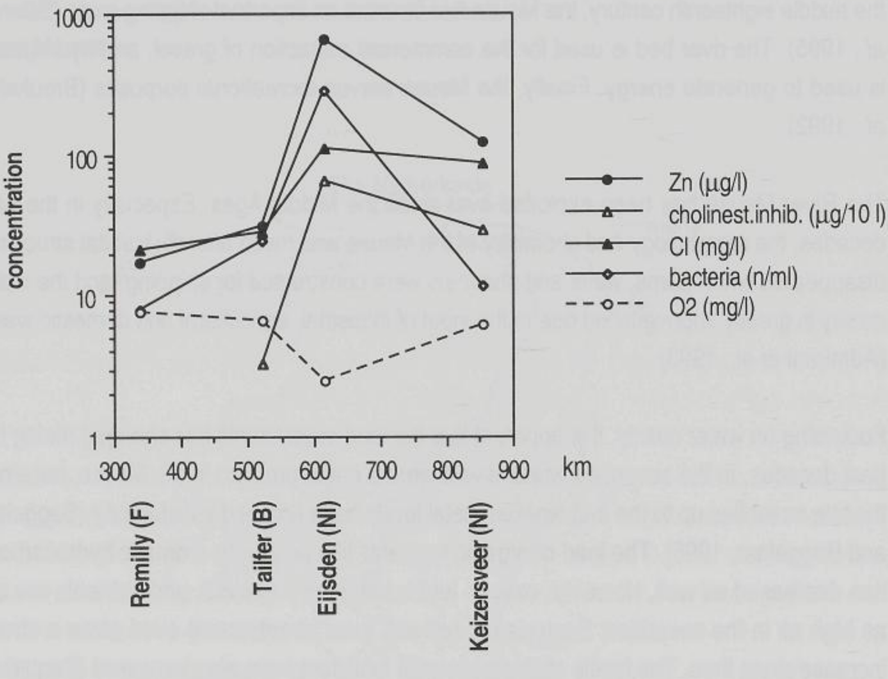

Fig. 1.2: Measured extremes at four sites along the River Meuse during 1996. Solid line: maximum levels, broken line: minimum oxygen levels.

Besides the regular sampling program performed by the Institute for Inland Water Management and Waste Water Treatment (RIZA) and RIWA, a chemical and biological warning system is installed at different stations along the Dutch Meuse (RIZA). As expected, the majority of reports made on incidental discharges came from the station situated at Eijsden. During the present study (1994 - 1996), there were more than 100 reports of incidental peak concentrations of toxicants (RIWA, 1996a; RIWA, 1996b; RIWA, 1997). Most incidents occurred in 1996, and the main problem during this year were pesticides

(25% of all cases; Fig. 1.3). Chlorinated hydrocarbons were also regularly measured (17% of all cases). There is a number of compounds that are repeatedly found in high concentrations over these (and previous) years: diuron, ammonium, diisopropylether and tributylphosphate. The latter two compounds are discharged during the industrial processing of phosphorous acid at Engis (Belgium) (RIWA, 1996). Diuron is a herbicide that commonly occurs at Eijsden, but it is also frequently discharged in the Dutch part of the Meuse. It is peculiar that not all measured extremes in the sampling program were detected as an incidental discharge; e.g., zinc was never reported by the chemical warning system, while data from the sampling program indicated that Meuse water occasionally contained up to 990 μg zinc/L in 1995. Many toxicants are routinely detected and identified and modern analyzing techniques have improved over the years. However, there are still many compounds that remain unidentifiable, demonstrating that chemical measurements fail to indicate a part of the potentially hazardous compounds in the Meuse.

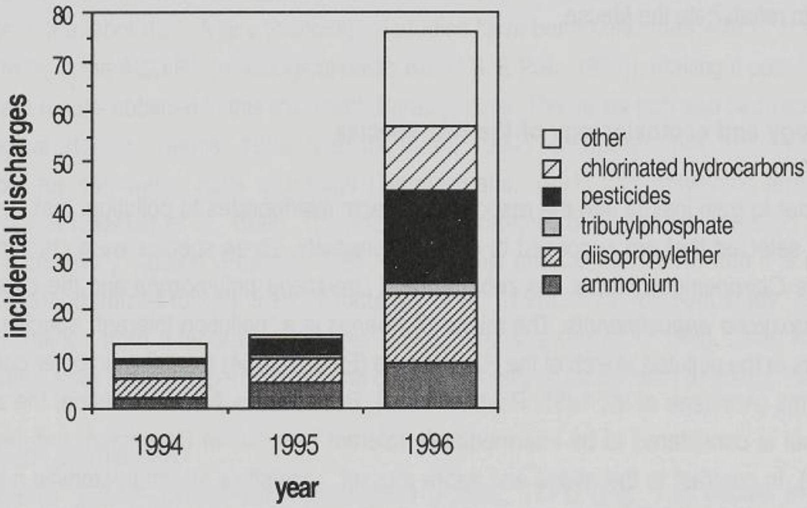

Fig. 1.3: Number of incidental discharges measured at Eijsden during 1994 - 1996 (adopted from RIWA, 1996a; RIWA, 1996b; RIWA, 1997).

Biological research on fauna in the River Meuse has mostly focussed on the distribution of species of fish (Vriese, 1992), macrofauna (Klink, 1985; Bij de Vaate, 1995; Ketelaars and Frantzen, 1995) and zooplankton (Marneffe *et al.*, 1996), while other studies concentrated on bioaccumulation of specific toxicants such as metals, PCB's, PAH's and pesticides (Kraak *et al.*, 1991; Van Hattum and Dirksen, 1992; Hendriks *et al.*, 1998). Most studies demonstrated that the environmental conditions are especially poor in the middle and lower region of the Meuse. Here the diversity and abundance of aquatic species are low and the bioaccumulated concentrations of toxicants are generally highest. These observations confirm the chemical state of the water in the upper, middle and lower reaches of the Meuse (Table 1.1).

Besides by a chemical warning system, the Meuse has been continuously monitored by a biological warning system at Eijsden since 1990, using fish (golden orf; *Leuciscus idus*) and daphnids (*Daphnia magna*). From 1992 until July 1998, there were no reports of alarms set off by the golden orf system. The *Daphnia* system, on the other hand, has elicited alarm on 3 to 5 occasions in 1995 and 1996 (there is some inconsistency on the number and also the causes of alarms (RIWA, 1996b and RIWA, 1997 vs RIZA, not published, resp.). The observed toxic effects on daphnids were related to pesticides (diazinon), mixture toxicity (ammonia, unidentified compounds, oxygen deficiency), (chlorinated) hydrocarbons or to unknown compounds. These reports show that the contamination of the Meuse is complex and variable and that it is therefore difficult to generalize on toxic effects. In contrast to the seventies, when metal levels were continuously high in the Meuse, nowadays there are no contaminants that dominate. This complex contamination obstructs the making of a detailed plan to rehabilitate the Meuse.

Ecology and ecotoxicology of the test species

In order to gain insight into the response of macroinvertebrates to pollution, test species were selected that are supposed to differ in sensitivity. Three species were chosen: the midge *Chironomus riparius*, the zebra mussel *Dreissena polymorpha* and the caddisfly *Hydropsyche angustipennis*. The midge *C. riparius* is a “pollution tolerant” species, and occurs in the polluted stretch of the River Meuse (Evrard, 1994) as well as in other polluted streams (Armitage *et al.*, 1995; Postma, 1995). Based on its field distribution, the zebra mussel is considered to be intermediately tolerant to pollution (Neumann and Jenner, 1992). In contrast to the midge and zebra mussel, caddisflies are characteristic riverine species (Ward, 1992), and are considered to be intolerant to pollution (Resh, 1993). Hydropsychid caddisflies are seldomly found in the polluted stretch of the Meuse (Ketelaars and Frantzen, 1995), in spite of having been abundant before the industrialization period (Klink, 1985).

Chironomus riparius

Larvae of the midge *Chironomus riparius* (Meigen, 1804) (Fig. 1.4) occur in both lenthic and lotic environments, throughout southern and western Europe (Ali, 1995; Postma, 1995) but also in North America (Beck, 1977; Rasmussen, 1985; Ali, 1995). Within these regions, the midge is mostly found in eutrophic waters (Beck, 1977). *C. riparius* is one of the characteristic taxa of which increasing density indicates a heavy loading of organic matter (Lindegaard, 1995), and is therefore often found in sewage canals (Pinder, 1995). The larvae feed on detritus (Rasmussen, 1985).

Fig. 1.4: Larva of the midge *Chironomus riparius*

C. riparius is a multivoltine species (Tokeshi, 1995). The development of midges is, however, highly dependant on temperature. At 20°C a life cycle of *C. riparius* can be completed in three weeks. At this temperature, larvae hatch from egg masses within three days. After 4 days as first instars, midges develop into second, third and fourth instar larvae over the following two weeks. After pupation, imagoes emerge from the water surface. After mating in swarms (Armitage, 1995), females attach their egg masses to plants or other solid substrates at the water surface.

C. riparius is often used as a test species, because it is widely distributed and it is easy to handle in the laboratory. Many toxicological studies have been performed with *C. riparius* (443 items in the AQUIRE toxicological database (U.S. E.P.A., 1997)), making it possible to compare results obtained in this study with literature data. The midge has also been used in ecological studies (Heinis, 1993; Van de Bund, 1994). *C. riparius* has proven to be suitable for estimating risks of metals (Timmermans, 1991) and polycyclic aromatic compounds (Bleeker *et al.*, 1998). The adaptation strategies of *C. riparius* to metals have been extensively studied (Postma, 1995; Groenendijk and Lücker, 1998) and it is often used in standardized toxicity tests (Grootelaar *et al.*, 1996). In the present study, larvae were obtained from a laboratory culture. This culture was started in 1986, using larvae originating from a small experimental pond of the University of Amsterdam (Postma, 1995).

Dreissena polymorpha

The zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Fig. 1.5) occurs in The Netherlands and other European countries (Kinzelbach, 1992), and has recently invaded North America (Riessen *et al.*, 1993). The mussel is abundant both in lotic and lenthic environments (Neumann and Jenner, 1992) and is usually most abundant in mesotrophic waters (Stanczykowska and Lewandowski, 1993). *D. polymorpha* is an important link in the aquatic food chain: it consumes large amounts of phytoplankton from the water by filtration (Stanczykowska, 1978). Zebra mussels are the main food source for diving ducks, coots (Suter, 1982) and benthivorous fish such as roach (Prejs *et al.*, 1990).

Fig. 1.5: The zebra mussel *Dreissena polymorpha*

D. polymorpha has a life span of 3-5 years. Reproduction occurs in late spring until late summer (Neumann and Jenner, 1992) and takes place by spawning of gametes, which leave the body through the exhalent siphon. Fertilization occurs in the water, resulting in free-swimming planktonic veliger larvae. After 3-5 weeks, the young bivalves settle on hard substrate using byssus threads (Neumann and Jenner, 1992). After attachment, the mussel starts to grow and is able to reproduce in the following summer.

D. polymorpha can be found throughout the year and it can be kept under laboratory conditions. As *C. riparius*, the zebra mussel has been extensively used in ecological and ecotoxicological studies (210 items in the AQUIRE toxicological database (U.S. E.P.A., 1997)). *D. polymorpha* is frequently used in the field as a "mussel monitor" (Borcherding and Jantz, 1997) or in bioaccumulation studies (Kraak *et al.*, 1991; Van Hattum and Dirksen, 1992), but is also suitable as a test organism in laboratory assays testing its filtration rate (Bleeker *et al.*, 1992; Kraak, 1992; Stuijfzand *et al.*, 1995) or re-attachment capacity (Mersch and Pihan, 1993).

Hydropsyche angustipennis

Hydropsyche angustipennis (Curtis, 1834) (Fig. 1.6) is a characteristic riverine species; it is widely distributed in small streams as well as in the lower reaches of large rivers (Edington and Hildrew, 1981), but is not found in stagnant waters. This caseless caddisfly spins nets from silk-like threads that are used to filter or capture food. Hydropsychids are important processors of organic matter in the stream ecosystem. The larvae eat algae, detritus and small invertebrates (Petersen, 1986).

Fig. 1.6: Larva of the caddisfly *Hydropsyche angustipennis*

H. angustipennis is a univoltine species, and the adult flight period occurs from April until early October (Hickin, 1967). After hatching of the egg mass, the larva develops via five stages. At the end of the fifth instar period, the larva constructs a stony pupal case which is attached to hard substrate. When the pupa is fully developed, it swims to the water surface and emerges. After mating, the female deposits an egg mass under a rock or another hard substrate in the stream.

Although *H. angustipennis* is considered to be more sensitive to pollution than *D. polymorpha* and *C. riparius*, it is not often used as a test species (23 items in the AQUIRE toxicological database (U.S. E.P.A., 1997)). This caddisfly is less easy to maintain in the laboratory, because it has more environmental requirements and is less well explored than the other two species. However, we argue that this is one of the main reasons why this characteristic riverine species should be used to identify barriers for ecological recovery of disturbed rivers. Therefore, following an initiative by Dr. Engels from the University of Cologne, efforts were made to initiate a laboratory culture of *H. angustipennis* at the University of Amsterdam. Since 1996, a successfull laboratory culture has been maintained, and it is possible to conduct standard experiments using first instar larvae (Greve *et al.*, 1998).

Outline of the thesis

Key factors of water quality in the River Meuse and toxic barriers for different indigenous species were analysed in an experimental study combining incubations in the field and biological tests on river water in the laboratory.

In *Chapter II*, it is evaluated whether the water of the Meuse directly affects macroinvertebrate species (1994 - 1995). To this purpose, the "intermediately tolerant" zebra mussel *D. polymorpha* and the "tolerant" midge *C. riparius* were kept in Meuse water under controlled conditions. Effects of Meuse water on filtration rates (mussel) and growth (midge) have been determined simultaneously. Water was sampled along different sites and in different seasons, as the pollution level is highly variable along the stream and throughout the year.

One of the questions raised at this stage of the study was whether pollution "tolerant" species are tolerant to toxicant levels, or whether they take advantage of the high nutrient levels in the river water. In many rivers, both toxicant input and organic enrichment are closely interrelated, making it difficult to distinguish between the effects of these separate factors. In *Chapter III*, an attempt was made to discern the effects of toxicants and the effects of organic enrichment on macrofauna in river water, using the midge *C. riparius* as a test species.
Following the results of *Chapter III*, it was hypothesized in *Chapter IV* that (in addition to species specific sensitivities to toxicants) the persistence of species in polluted rivers depends on species specific capacities to modify or compensate for negative effects of toxicants. The response to organic compounds present in site water, like humic acids, may

be essential in ranking pollution tolerant and pollution sensitive invertebrates. The responses of the zebra mussel *D. polymorpha* and the midge *C. riparius* to metals in the presence and absence of organic matter (humic acids) were compared in laboratory tests.

Following the results of *Chapter II*, it was determined in 1996 whether ecotoxicological barriers exist in the River Meuse for characteristic riverine insect species (*Chapter V*). To this purpose, caddisflies of the genus *Hydropsyche* were incubated in cages in the Meuse and their survival and development were monitored. A comparison was made with incubations in the River Rhine in which some caddisfly species populations have re-established.

According to the results in the preceeding chapters and the high incidence of chemical spills in recent years (Fig. 1.3), it is suggested that insecticides may be an important barrier for insect life in the Meuse. As the impact of insecticides may be strongly dependent on factors like the time of application and the residence time, the effects of a common insecticide on two insect species (*H. angustipennis* and *C. riparius*) were determined taking these variables into account.

In this study, invertebrate species were tested under (semi-)controlled conditions to differentiate the impact of the water quality from the impact of habitat loss. The findings of this study are discussed in *Chapter VII*, where the risk for aquatic biota in the River Meuse is assessed. Recommendations for water quality management are given there.

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Appendix

Table 1.1: Concentrations of physico-chemical parameters measured in the Meuse at Remilly (France, km 340), Tailfer (Belgium, km 522), Eijsden (The Netherlands, km 615) and Keizersveer (The Netherlands, km 855) during 1994 (RIWA, 1996a), 1995 (RIWA, 1996b), and 1996 (RIWA, 1997).

1994	Remilly (F)		Tailfer (B)		Eijsden (Nl)		Keizersveer (Nl)	
	average	minimum	average	minimum	average	minimum	average	minimum
oxygen (mg/L)	10.4	5.8	11.2	7.2	8.6	2.8	9.5	6.6
	average	maximum	average	maximum	average	maximum	average	maximum
suspended matter (mg/L)	15.3	65	17.5	106	12.9	60	10.2	40.2
conductivity (mS/m)	47	56	34	44	47	73	49	59
total hardness (mmol/L)	2.61	3.23	1.93	2.39	2.01	2.75		
chloride (mg/L)	15	19	15	19	40	94	48	75
ammonium (mg N/L)	0.07	0.14	0.08	0.31	0.37	1.01	0.19	0.59
total phosphate (mg P/L)	0.1	0.31	0.13	0.58	0.55	2.51	0.26	0.44
chlorophyll a (µg/L)	12	38	27	150	20	99	13	52
fecal streptococci (n/100 mL)	167	510	645	2090	2780	11000	354	1820
cadmium (µg/L)	<	0.4	<	0.1	0.21	1.28	0.25	2.82
copper (µg/L)	2	5	2	4	4	18	4	5
zinc (µg/L)	18	65	23	74	38	150	32	135
Σ6 PAH Borneff (µg/L)			0.03	0.09	0.12	0.71	0.07	0.31
diuron (µg/L)					0.11	0.4	<	0.02
parathion (µg/L)	<	0.25	<	<			0.29	0.89

1995	Remilly (F)		Tailfer (B)		Eijsden (Nl)		Keizersveer (Nl)	
	average	minimum	average	minimum	average	minimum	average	minimum
oxygen (mg/L)	10.4	7.2	10.7	6.2	9.1	2.4	9.5	7.6
	average	maximum	average	maximum	average	maximum	average	maximum
suspended matter (mg/L)	15.7	33.5	26.3	204	13.2	120	12.7	81
conductivity (mS/m)	46	54	36	44	44	64	47	61
total hardness (mmol/L)	2.53	3.29	1.96	2.36	2.04	2.52		
chloride (mg/L)	14	20	15	20	37	78	45	72
ammonium (mg N/L)	0.08	0.21	0.09	0.58	0.43	1.23	0.17	0.5
total phosphate (mg P/L)	0.06	0.11	0.1	0.4	0.32	0.75	0.23	0.34
chlorophyll a (µg/L)	30	161	36	282	15	65	11	37
fecal streptococci (n/100 mL)	257	574	339	1120	2810	14000	199	1250
cadmium (µg/L)	<	0.2	<	0.2	0.35	3.9	0.26	2.18
copper (µg/L)	2	8	3	15	4	10	5	9
zinc (µg/L)	16	37	19	52	70	990	38	158
Σ6 PAH Borneff (µg/L)			0.04	0.08			0.09	1.15
diuron (µg/L)			0.064	0.213	0.145	0.36	0.236	0.81
parathion (µg/L)					0.42	1.5	0.4	0.86

1996	Remilly (F)		Tailfer (B)		Eijsden (Nl)		Keizersveer (Nl)	
	average	minimum	average	minimum	average	minimum	average	minimum
oxygen (mg/L)	10.4	7.6	11.4	6.7	8.5	2.5	10.1	6.1
	average	maximum	average	maximum	average	maximum	average	maximum
suspended matter (mg/L)	13.8	30.6	16.8	54	12.4	150	14	64.4
conductivity (mS/m)	43	48	38	59	50	74	55	66
total hardness (mmol/L)	2.45	2.94	1.94	2.86	2.06	2.53	1.94	2.11
chloride (mg/L)	17	21	20	28	49	109	63	86
ammonium (mg N/L)	0.22	0.77	0.07	0.26	0.9	2.25	0.28	1.02
total phosphate (mg P/L)	0.06	0.18	0.09	0.24	0.47	1.2	0.28	0.63
chlorophyll a (µg/L)	22	62	27	158	12	72	12	62
fecal streptococci (n/100 mL)	136	800	445	2400	3940	28000	135	1170
cadmium (µg/L)	<	<	<	<	0.41	2.45	0.27	1.5
copper (µg/L)	1.2	3	1.7	5.3	5	15.9	4	6.8
zinc (µg/L)	10	17	22	31	73	660	34	122
Σ6 PAH Borneff (µg/L)			0.04	0.06			0.1	0.69
diuron (µg/L)			0.056	0.152	0.22	1	0.22	0.562
parathion (µg/L)			0.2	0.33	1.19	6.4	0.65	2.9