



Glyphosate residues in surface water

A study of streams in Uppsala, summer 2000

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by

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Abstract

The substance glyphosate is widely used in herbicide preparations around the world. The fate of pesticide residues in water and their effect on non-target organisms are of considerable public concern. There is a fear about the potential long-term toxicity of herbicides to man and to aquatic organisms.

There are no formal limits for pesticide concentrations in surface water today. EU, who regulates drinking water in Europe, recommends that the level of 0.1 µg/l for individual pesticide shall not be exceeded, a regulation that has been adopted by the Swedish National Food Administration.

The herbicide Roundup with its active substance glyphosate is widely used by farmers in Sweden. Of the total usage of pesticides in Sweden, 30% are pesticides with glyphosate. A rapid annual increase of the glyphosate sale started in 1995. The causes of increased usage of glyphosate are complex.

The aim of this study was to find out if there were any traceable concentrations of glyphosate in surface water from the Uppsala area, in connection with rain events following application in the summer of 2000. This study will hopefully give a hint to the present situation regarding the occurrence of glyphosate residues in surface water in Uppsala.

The sampling of water and soil were made at intervals between the end of June and the end of August. Three different sites were chosen for the study. One at Storvad, River Fyris, the other site is at the Fyris River in Storvreta, at storm water pipe outflows. The third site is the brook Librobäck. Soil samples were also taken from a field beside Librobäck.

The limit of detection in this study was 0.1 µg/l for AMPA and varied from 0.03-0.05 µ/l for glyphosate. Sample concentration of glyphosate varied between traces of the substance up to 0.12 µg/l. No sample showed concentrations above the detection limit for AMPA.

The concentration of glyphosate and AMPA in soil samples varied between 0.01 µg/g and 1.22 µg/g for glyphosate, and 0.05 µg/g and 0.2 µg/g for AMPA.

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1. Aim

The aim of this study was to find out if there were any traceable concentrations of glyphosate in stream water from Librobäck, river Fyris (Uppsala) and in storm water effluents from the village of Störvreta, north of Uppsala, in connection with rain events following application in the summer of 2000.

2. Introduction

The substance glyphosate is widely used in herbicide preparations around the world. The fate of pesticide residues in water and their effects on non-target organisms are matters of great public concern. Most herbicides by their nature are less toxic than other pesticides to animals and zooplankton, but they may reach the water in substantial quantities during the management of aquatic weeds, a practice not allowed in Sweden, or by transport with surface water, or by leaching through the soil. There is a fear about the potential long-term toxicity of herbicides to man and to aquatic organisms. (Hutson & Roberts, 1987)

There are no formal limits for pesticide concentrations in surface water today. EU, who regulates drinking water in Europe, recommends that the level of 0.1 µg/l for individual pesticide shall not be exceeded, a regulation that has been adopted by the Swedish National Food Administration, (1993). Glyphosate has been used since the late 1970's. Despite that it has been used for nearly 30 years, little research about its behaviour and environmental effects has been carried out. One reason for this is that it is extremely difficult to measure glyphosate residues in environmental samples. This means that data are often lacking on residue levels in food and the environment, and that existing data may not be reliable.

Lately, more analyses of glyphosate and AMPA have been carried out. In a study carried out in Denmark, traces of glyphosate and AMPA were found in wells (MILJØ-styrelsen, 1997). Glyphosate and AMPA has also been found in stream water from Saxån-Braån, in the south of Sweden (Bengtsson, 2000).

This study will hopefully contribute to clarifying the present situation regarding the occurrence of glyphosate residues in surface water in vicinity of Uppsala.

2.1. Historical background

In the beginning of the 60's, researchers at Monsanto discovered a new method for the preparation of tertiary aminomethylphosphonic acids. Aminomethylphosphonic acids are of considerable interest for use as metal complexing agents, water softeners, detergents, in scale removal, and for many other applications. Beginning in 1961, aminomethylphosphonic acid type compounds were evaluated at the agricultural division at Monsanto for plant biological activity. Over a 9-year period, however, only a few products were found to exhibit interesting plant growth regulating and systemic herbicidal properties. The product glyphosine was eventually marketed as a plant growth regulator called Polaris. When sprayed on mature sugarcane plants glyphosine functions as a cane ripener and increases the yield of sucrose produced per cane. Although about 200 aminomethylphosphonic acid type compounds were prepared and tested as herbicides between 1960 and 1970, no new leads were discovered. Despite the discouraging test results, the research on phosphonic acid type compounds

continued. In mid-1970 one compound was showing herbicidal activity, the molecule glyphosate had been synthesised (Franz et al., 1997).

2.2. Chemical facts (Hutson, 1987)

Common name: Glyphosate

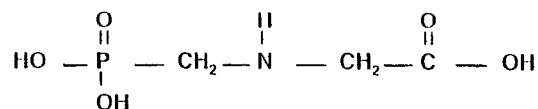
Chemical name (IUPAC): *N*-(phosphonomethyl)glycine

CAS nr: 38641-94-0 (isopropylamine salt)

Molecular formula: C₃H₈NO₅P

Molecular weight: 169.1

Chemical structure:



Water solubility 1.157% (by weight) in water at 25 °C

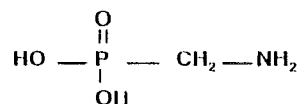
K_{oc} : 300-20,100

Vapour pressure: Negligible

Log K_{ow} : -4.1

pKa: pK₁ < 2.00; pK₂ 2.6, pK₃ 5.57; pK₄ 10.86

Chemical structure of AMPA (aminomethyl phosphonic acid, main metabolite)



2.2.1. Mode of action

Chemically, glyphosate is an organophosphate like many other pesticides but it does not affect the nervous system as other organophosphates do. It is a broad spectrum, non-selective herbicide which kills all plants, including grasses, broad leaf and woody plants. It is absorbed mainly through the leaves and is transported around the whole plant, killing all parts of it. It acts by inhibiting a biochemical pathway, the shikimic acid pathway (Grossbard & Atkinson, 1985). At low levels of application it acts as a growth regulator (Greenpeace, 1997). The low mobility of glyphosate in soil indicates a minimal potential for the contamination of groundwater. Glyphosate can, however, enter surface and subsurface waters by direct use near

aquatic environments or by runoff or leaching from terrestrial applications. This has been substantiated by reports that indicate the presence of glyphosate residues in water from direct overspray in forestry operations, from runoff, and from irrigation canal discharges.

Furthermore, the possibility of aquatic contamination from spray drift during agricultural or silvicultural applications also exists. Depending upon the suspended solids loading and the microbial activity of flowing water, glyphosate may be transported several kilometres downstream from the site of aquatic application. (WHO, 2001)

There are four formulations of glyphosate used as weed killers in Sweden (Swedish National Chemicals Inspectorate, 2001): 1. glyphosate-isopropylamine salt, 2. glyphosate-sodium salt, 3. glyphosate-trimethylsulfonium, and 4. glyphosate-ammonium salt. Common brand names are Avans, Kwick Down, Roundup, and Totex.

The rapid translocation of glyphosate from the foliage treated plants to the roots, rhizomes and apical meristems (figure 1) is one of its most important characteristics (Franz et al., 1997).

The unique biological properties of glyphosate include the following (Franz et al., 1997):

1. It is a broad-spectrum, non-selective, postemergence herbicide, with activity on essentially all annual and perennial plants.
2. Glyphosate is virtually non-toxic to mammals, birds and fish, insects and most bacteria. In addition, glyphosate does not bioaccumulate in the tissue of animals or agricultural crops.
3. Glyphosate is a systemic herbicide that is rapidly translocated from foliage to the roots, rhizomes, and apical tissues of treated plant.
4. Once inside the plant, glyphosate inhibits a key enzyme known as EPSP (5-enopyruvylshikimic acid-3-phosphate) synthase. The inhibition of this important enzyme prevents the plant from synthesizing certain aromatic amino acids that are essential for plant growth. EPSP synthase is present only in plants and some microorganisms. Other life forms do not possess this enzyme and have to obtain aromatic amino acids from their diet.
5. Because glyphosate is a broad-spectrum herbicide, it does not discriminate between crop and weed species. This limits its use in agricultural applications.

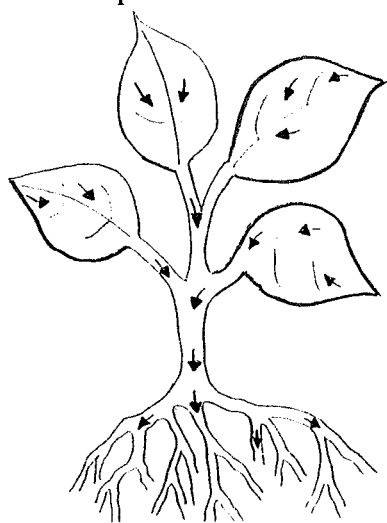


Figure 1. When glyphosate is sprayed onto plant foliage, it is absorbed and then moved, translocated, throughout the plant's tissues. Once inside the plant, glyphosate inhibits an enzyme, called EPSP synthase. This, in turn, prevents the plant from making certain aromatic amino acids essential for plant growth. (modified from Franz et al 1997)

2.2.2. Chemical degradation

Glyphosate is rather stable to hydrolytic and photolytic degradation (Roberts, 1998). Minimal hydrolysis occurs in various sterile pH buffer solutions (Franz et al., 1997). Most studies of the photolysis of glyphosate in either water or soil have indicated that glyphosate is not readily photodegraded. Under natural sunlight in sterile buffer at pH 5, 7 and 9, photodegradation was insignificant, with <1% degradation after about 30 days. Glyphosate underwent very little photodegradation in/on soil surface, under either natural or artificial sunlight. When Ca^+ was added in sterile deionised or natural water solution and irradiated with artificial sunlight (350-450 nm), observable degradation occurred with half-life values of 4 to >14 days. Glyphosate in deionised water was reported to be more stable in longer wavelength light (>400), but degraded faster under shorter wavelength UV light (254 nm). Natural sunlight was also reported to stimulate some of the degradation of glyphosate. AMPA (aminomethylphosphonic acid), formed via the N-dealkylation reaction, was the major degradation product. (Roberts, 1998)

2.2.3. Metabolism by plants

Glyphosate is metabolized very little by plants, the major metabolite being aminomethylphosphonic acid (AMPA). Glyphosate is rapidly and strongly bound to most soils and is not taken up by the roots of plants. (WHO, 2001, Franz et al., 1997)

2.2.4. Fate in soil

Because of different application methods and various environmental conditions, (rain, wind etc.), a significant amount of herbicide applied post emergence may eventually reach the soil. Herbicides that enter the soil are generally bound by adsorption to constituents such as clay minerals, organic material, metallic oxides, and humic substances. Since adsorption is often an equilibrium process, binding is usually reversible. (Franz et al., 1997)

Transport of herbicides in soil occurs in different ways. Since movement of a herbicide through the soil is generally mediated by water, water-solubility and adsorption characteristics are of great importance concerning the rate of the transport processes for any herbicide (Franz et al., 1997).

Although knowledge of the rates of herbicide dissipation from soil is important for both agronomic and environmental reasons it is difficult to predict the persistence or lifetime of a chemical in soil. Persistence is not a fixed property of a herbicide but is influenced by factors such as the chemical structure and physical properties, the microorganisms present in the soil and the cultivation method used (Franz et al., 1997).

The rate of metabolism of glyphosate can vary considerably between different soils, but usually can be correlated with the level of microbial activity, which is dependent on many soil factors. The half-life of glyphosate in soils is variable and commonly range from less than a week to several months. The half-life of glyphosate in two Hawaii sandy loam soils, was suggested to be several years, based on ^{14}C evolution. (Franz et al., 1997)

Metabolism by soil microflora is an important dissipation route of glyphosate in the environment. Degradation occurs under both aerobic and anaerobic conditions, in soils and in sediments. Soil degradation proceeds apparently without a lag phase, and can be considered a co-metabolic process. The primary soil degradation reaction of glyphosate yields AMPA and glyoxylic acid, which are both further degraded and mineralised to CO₂. (Roberts, 1998)

A second degradation pathway has been observed in isolated soil bacteria, which utilised glyphosate as a sole phosphorus source. In these bacterial culture incubations, initial cleavage of the P-C bond yielded inorganic phosphate and N-methylglycine, which was further degraded to glycine and ultimately to CO₂ and NH₄. This pathway could be inhibited by the presence of phosphate. Half-life values determined under either laboratory aerobic or anaerobic conditions varied and typically ranged from several days to several weeks. A field soil dissipation study generated a mean half-life of 14 days. Field dissipation studies conducted in forest systems in Oregon, Sweden and Canada generated half-life values in the range of 30-60 days. (Roberts, 1998)

A field study made by Torstensson and Stenstöm (1995), shows that the climate could effect the degradation rate. In an experiment conducted in northern Sweden, close to the arctic circle (about 66° N), and in the southern part of Sweden, (about 57° N), they found that glyphosate was initially degraded faster in the northern compared to the southern soil. It should be noticed that the northern soil had a higher basic respiration rate than the southern one, and that this higher activity probably resulted in a higher capacity to degrade glyphosate.

The extensive adsorption of glyphosate to soil clay and minerals together with its ready degradation in soil, are the principal deactivation and dissipation mechanisms in the soil environment. Several factors are involved in the adsorption mechanism. The extent of glyphosate binding is directly correlated to the inorganic phosphate sorption capacity in the soil and the presence of Fe²⁺, Fe³⁺ and Al³⁺ ions. Glyphosate has also been reported to bind to humic acid fractions. (Roberts, 1998)

2.2.5. Behaviour of glyphosate in water

Several studies have been reported in which glyphosate residues were measured following application of the herbicide Roundup to both static and flowing water. In static water, glyphosate was reported to be lost from the water body fairly rapidly. In flowing water, the rate of elimination from the point of application was dependent on the flow rate. In general, most studies indicate that glyphosate is strongly adsorbed by soil colloids, bottom silt, and suspended soil particles in the water. (Franz et al., 1997)

In New South Wales irrigation drainage systems, dilution by tributary inflow is used on a regional basis to control contamination. For example, daily applications of glyphosate are restricted to give concentrations no greater than 0.04 mg/l, assuming complete mixing in the combined discharge of the drainage basin. (Hutson & Roberts, 1987)

The uptake of glyphosate from flowing water by adsorption onto benthic sediments occurred slowly in several experiments in Australia and the USA. The load of glyphosate was attenuated by 13% per kilometre of travel in Australian conditions, while in one experiment in Washington, USA, 57% of the glyphosate entering the water travelled more than 14 km downstream. (Hutson & Roberts, 1987)

2.2.6. Metabolism in animals

In animals, glyphosate is excreted rapidly in the faeces and urine, and undergoes very little metabolism. Male and female Sprague-Dawley rats treated with a single oral dose of [¹⁴C]glyphosate rapidly eliminated >97% of the administered dose. About 30-36% of the dose was absorbed and eliminated in the urine, and the remaining radioactive dose was eliminated with the faeces. Other than unmetabolised glyphosate, the only detectable metabolites were AMPA and ¹⁴CO₂, which however, represented only about 0.2-0.3% of the administered radioactivity. Repeated dosing did not significantly change the metabolism, distribution, or excretion profile of glyphosate in rats. Studies conducted on laying hens and lactating goats showed similar metabolic fate. Rhesus monkeys treated intravenously with [¹⁴C]glyphosate rapidly excreted the administered dose as the parent compound, 80% in the urine within 24 hours and 94-99% within 7 days. (Roberts, 1998)

2.3. Usage

The herbicide Roundup with its active substance glyphosate is widely used by farmers in Sweden. It has been used since 1975 for weed control in agriculture, forestry and gardens. Glyphosate is also used by the Swedish National Rail Administration for weed control on railway embankments (Börjesson & Torstensson, 2000). Of the total usage of pesticides within agriculture, horticulture, and households in Sweden, 30% are pesticides with glyphosate (Swedish Board of Agriculture 1999).

In many other countries glyphosate, formulated as the isopopylamine salt and together with a surfactant, is very widely used as a broad-spectrum herbicide for the control of many ditch-bank, annual and perennial species and some floating weeds (Hutson & Roberts, 1987). It is not allowed to use glyphosate in any water systems in Sweden.

During 1985-1995 the usage of pesticides containing glyphosate corresponded to approximately 230 tons/year of active ingredient, with only small variations between years. A rapid annual increase of the glyphosate sale started in 1995. In 1997 and 1998, 500 and 470 tones of active ingredient, respectively, was sold on the market. Approximately 95% were used for agriculture. (Swedish Board of Agriculture, 1999) The pesticide is according to the Swedish National Chemicals Inspectorate (1998) less risky to health and environment than most other herbicides. The glyphosate containing herbicides approved in Sweden in the year 2001 are shown in table 1.

There are several reasons why the usage of glyphosate has increased over the last years. The price of glyphosate has decreased at the same time as the fuel price has increased. It is therefore cheaper to use glyphosate than to use mechanic weed control, e.g. when preparing fallow land for a new crop. The introduction of regulations for winter and autumn green fields has effected the need to use glyphosate. Environmental regulations with fallow fields (directed from the EU) have limited the possibilities to use mechanic cultivation. The patterns of increased usage of glyphosate are complex. (Swedish Board of Agriculture, 1999)

Table 1. Approved glyphosate-containing herbicides in Sweden, 2001. (Swedish National Chemical Inspectorate, 2001)

Name	Concentration
Avans	330g/l
Avans 440	440g/l
Clinic 360 SL	360g/l
ECO-plug	44 by weight %
Glyfonova 360 SL	360g/l
Glyphomax	360g/l
Jablo Glyfosat	360g/l
Keeper K	120g/l
Keeper Spray	7g/l
Kvick Down Bio	360g/l
Kvick Down 360	360g/l
Roundup Bio	360g/l
Roundup Dry	44 by weight %
Roundup G	120g/l
Roundup S	7g/l
Roundup	360g/l
RAMBO	103g/l
RAMBO SPRAY	7g/l
Totex (fluid)	34g/l
Totex (fluid spray)	3.4g/l
Verdys	360g/l

3. Material and method

3.1. The structure of this study

This study was focused on streams in or near Uppsala, Sweden. Water samples were collected for two months from the brook Librobäck (Appendix 1 sites 1-4), river Fyris (Appendix 3 site 1 and 3) and from storm water pipes in Storvreta (Appendix 2). Analyses were carried out to determine if the samples contained glyphosate and AMPA. The precipitation in the Uppsala area from June to August is given in Appendix 6.

Soil samples from a field beside Librobäck (Appendix 1 site 2), that was sprayed with glyphosate, were collected and analysed. The reason was to see if there were any connections between the concentration of glyphosate and AMPA in the water, in comparison to the concentrations in the soil.

Interviews were carried out with farmers in the Librobäck area to map the usage of glyphosate. The aim was to see if the brook water was affected by the usage.

3.2. Recovery study

A general recovery study was made independently from the recovery test made with each separate water sample from the Uppsala area. Two different water types were used in this study, one unspecified surface water and one unspecified spring water.

To sample 1 and 2, 10 µl of the standard mixture containing 1.0 µg/ml glyphosate and 1.8 µg/ml AMPA were added to 200 ml of surface water. The same water was used in sample 3

and 4. To these samples were 15 ml of the surface water added to 15 ml of ultra pure water to dilute the standard concentration.

To the samples with spring water (5 and 6), 30 µl of the same standard mixture were added to 200 ml of water. To sample 5, 10 ml of this water mixture were added to 20 ml of Merck ultra pure water. To sample 6, 5 ml of the water mixture were added to 25 ml of ultra pure water.

3.3. Sample sites

The sampling of water and soil were made at intervals between the end of June and the end of August. Three different locations were chosen for the study.

One location was the brook Librobäck. Four sampling sites along the brook were chosen; one at the beginning of the brook, two along fields that had been sprayed, and the last one close to the outflow of the brook into Fyris river. Soil samples were taken from a field beside Librobäck, just next to the water sample site 2. This field was sprayed with Roundup Bio on July 7.

The second location is at the Fyris River in Storvreta. Three different spots for sampling were chosen all at storm water pipe outflows.

The third location is at Storvad where surface water is taken from River Fyris for infiltration to produce drinking water.

3.3.1. Librobäck

Librobäck brook is approximately 13 km long. It is situated north-west of Uppsala, ending up in Fyris river (Appendix 1). The drainage area is 27.7 km², 46% is forest, 1% wetland, 50% fields and meadows and 3% remaining land. The area is surrounded by forest on till soil. A spring close to Ströby is open the hole year. It provides the brook with water when the water table is low. Librobäck is partly heavily meandering. It has created in some parts, a quite steep and broad ravine. The railroad Uppsala-Sala crosses the area. The western parts of Uppsala (Luthagen, Flogsta, Berthåga, Stenhagen) is situated in the lower, eastern parts of the area.

Interviews were made with five farmers in the area around Librobäck (table 2). The questions asked were:

What kind of herbicide containing glyphosate have you applied?

How much was it?

When did you apply it?

Table 2. Farmers application of herbicides containing glyphosate.

Farmer	date of application	used herbicide	dose (l/ha)	field location
1	2000-07-07	Roundup Bio	4	beside sample site 2
2	2000-07-03	Roundup Bio	3	between sample sites 3 and 4
3	2000-07-08	Roundup Bio	3	between sample sites 3 and 4
4	2000-07-02	Roundup Bio	3	between sample sites 1 and 2
5	2000-07-06	Roundup Bio	3	between sample sites 3 and 5

3.3.2. Storvreta

There are about 6 500 people living in Storvreta, mostly in houses with gardens. The sampling sites were outflows from storm water pipes that start within the housing area and ends up in river Fyris (Appendix 2). The samples were taken just before the pipes connect to the river.

3.3.3. Storvad

This site is just at the bathing place Storvad (Appendix 3). The sample was taken from one of the bathing bridges. A few 100 m upstream of this site the infiltration station for drinking water production in Uppsala is located.

3.4. Sampling method

Water samples were collected using a water sample collector connected to a telescope tube. The bottle (0.5 l) in the water sample collector as well as the sample bottle (1.0 l) was rinsed with sample water. The bottles are made of HDPE, high-density polyethylene plastic. Approximately 1 l was taken from each site. A smaller amount of the sample was poured into a 1 l plastic bag. All water samples were then frozen in the bottles and in the plastic bags, respectively. Soil samples were collected in plastic bags using a clean water rinsed tablespoon. Soil from six spots, approximately 10x10 cm, 5 cm deep, was mixed and then frozen at -18°C .

3.5. Sample treatment

The water samples were thawed and pH was measured at room temperature (21°C), using a PHM 210 Standard pH meter. If necessary the pH was adjusted to 7-8 by using 0.1 M HCl or 0.1 M NaOH. N-phosphonomethyl- β -alanin (30 μl of a 1.05 $\mu\text{g}/\text{ml}$ solution) was added as internal standard.

An ion exchange column (empty plastic column, Bond elute reservoir, with a 1 cm diameter, filled with <1 g of the ion exchange resin AG X-8) and an Isolate C18 column with 200 mg sorbent were put together (the C18 above the ion exchange column). A 70 ml reservoir, containing 40 ml of sample water, was put on the C18 column. The sample was allowed to drip through very slowly. The ion exchange column was then eluted with 8 ml of 0.5 M HCl into a pear shaped flask. The eluate was evaporated until 1-2 ml was left. The eluate was then mixed with \sim 1 ml of methanol/Merck ultra pure water, 80/20. The mixture was then moved in doses of \sim 0.5 ml at the time to a test tube that has been treated with saturated KOH in ethanol. The test tubes were blown to absolutely dryness by using air, before the derivatization reagents, 1.5 ml of TFAA (tetrafluoroaceticanhydrid) and 0.75 ml of TFE (tetrafluoroethanol), were added. Caps were put on as quickly as possible. The test tubes were heated to \sim 96 $^{\circ}\text{C}$ for 1 h. The test tubes were then cooled down to room temperature before they were blown dry by a gentle stream of air. When the sample was absolutely dry, 1.5-2 ml of ethylacetate was added and then dried again. This procedure was repeated one time. After this another 0.5 ml of ethylacetate were added.

The recovery test samples were treated the same way as the water samples. The recovery test samples were sample water from Storvad and Librobäck with different amounts of a standard mixture, containing 1.0 µg/ml glyphosate and 1.8 µg/ml AMPA, added.

The soil samples were analysed at the Department of Microbiology SLU, according to Börjesson & Torstensson (2000).

3.6. Gas chromatography and mass spectrometry conditions

GC-MS analyses were performed using a Hewlett-Packard 6890 system, equipped with a 30 m x 0.32 mm I.D. (0.25 µm film thickness) fused-silica capillary column (HP-5 for GC-MS), a UP5973 mass spectrometer, a split/splitless injector and Chemstation software, all from Hewlett Packard. Samples were injected 1 µl in the pulsed splitless mode at 250 °C, oven temperature 70 °C. After 1 minute, the oven temperature was raised to 250 °C at 15 °C min⁻¹. Helium (N47 grade, 99.997%) was used as the carrier gas and the flowrate was 0.8 ml min⁻¹. The mass spectrometer was operated in the electron ionisation (EI) mode; the auxiliary temperature was 260 °C and the MS source temperature was 230 °C. For the AMPA derivative *m/z* 302 was used for quantification and *m/z* 272 and 371 were used for identification. For glyphosate derivative *m/z* 411 was used for quantification and *m/z* 384 and 511 were used for identification.

4. Results

4.1. Recovery studies

The recovery of glyphosate from spring water was approximately 100% while it was higher, about 150%, from surface water (table 3). The recovery of AMPA was about 80%. The limit of determination was 0.05 µg/l for AMPA and 0.03 µg/l for glyphosate at a signal to noise ratio of 4:1.

Table 3. Recovery of glyphosate and AMPA from spring and surface water (µg/l).

Substance	Sample	type of water	Added concentration	Extracted concentration	Recovery (%)
Glyphosate	1	surface water	0.05	0.07	131
	2	surface water	0.05	0.1	204
	3	surface water*	0.025	0.03	137
	4	surface water*	0.025	0.03	137
	5	spring water*	0.05	0.05	100
	6	spring water*	0.025	0.024	96
AMPA	1	surface water	0.09	0.06	67
	2	surface water	0.09	0.11	121
	3	surface water*	0.045	0.04	84
	4	surface water*	0.045	0.04	81
	5	spring water*	0.09	0.07	81
	6	spring water*	0.025	0.03	76

* mixed with ultra pure water

4.2. Results of water samples

The limit of determination in the water samples from Librobäck and Fyris river was 0.1 µg/l for AMPA and varied from 0.03-0.05 µ/l for glyphosate (Table 4). The recovery and determination limit was determined using recovery samples with added concentrations for every individual analysis occasion. The sample concentrations were adjusted for the recovery in cases when it was not about 100% (Appendix 4 and 5). All samples where the concentration was under the determination limit, showed traces of glyphosate. (Appendix 4)

Glyphosate could be determined in samples from Librobäck 3 from the 21st and 25th of July as well as all samples from Librobäck 4, except for the sample from the 7th of August. Samples from Storvreta and Storvad from the 23^d of July and the sample from Storvreta the 13th of August also contained detectable concentrations of glyphosate. No sample showed concentrations above the detection limit for AMPA.

Concentrations for the different sample sites over time is shown in figure 2-8 (traces included).

4.3. Soil

Glyphosate and AMPA were detected in concentrations of 0.01-1.2 in soil samples (Table 5). The limit of determination was 0.01-0.2 µg/g with a margin of error of ± 20%.

In figure 9 the concentration of AMPA and glyphosate in the soil is plotted over time. The glyphosate concentration increases fast after the application, but decreases to its starting value within a month. The concentration of AMPA rises a little when glyphosate starts to decrease, but the concentrations of AMPA is also back to the start value within a month.

In calculations for the soil samples, the half-life of glyphosate in the soil was estimated to be approximately 13 days.

Table 4. AMPA and glyphosate concentration in water samples from Librobäck and River Fyris. The limit of determination was 0.1 µg/l for AMPA and varied from 0.03-0.05 µ/l for glyphosate. The bold values are above the determination limit, the remaining values should be regarded as traces. na= not analysed nd=not detected

Location	Date	Glyphosate concentration (µg/l)	AMPA concentration (µg/l)
Librobäck 1	2000-06-29	0.02	nd
	2000-07-12	0.02	na
	2000-07-21	0.03	nd
	2000-07-25	0.01	nd
	2000-08-07	0.02	nd
Librobäck 2	2000-06-29	0.01	nd
	2000-07-12	0.02	na
	2000-07-21	0.03	nd
	2000-07-25	0.02	nd
	2000-08-07	0.02	nd
	2000-08-23	0.03	nd
Librobäck 3	2000-06-29	0.02	nd
	2000-07-12	0.02	na
	2000-07-21	0.12	nd
	2000-07-25	0.04	nd
	2000-08-07	0.02	nd
Librobäck 4	2000-06-29	0.05	nd
	2000-07-12	0.05	na
	2000-07-21	0.06	na
	2000-07-25	0.04	nd
	2000-08-07	0.02	nd
	2000-08-23	0.07	nd
Storvad	2000-06-29	0.02	nd
	2000-07-06	0.01	nd
	2000-07-25	0.02	nd
	2000-08-23	0.1	nd
Storvreta 1	2000-07-03	0.1	nd
	2000-07-25	0.03	nd
	2000-08-23	0.03	nd
Storvreta 3	2000-07-03	0.03	nd
	2000-07-25	0.02	nd
	2000-08-23	0.1	nd

Table 5. Concentrations of glyphosate and AMPA in soil samples.

Date	Glyphosate (µg/g)	AMPA (µg/g)
2000-06-29	0.01	0.09
2000-07-12	1.2	0.14
2000-07-21	1.0	0.20
2000-08-02	0.02	0.05

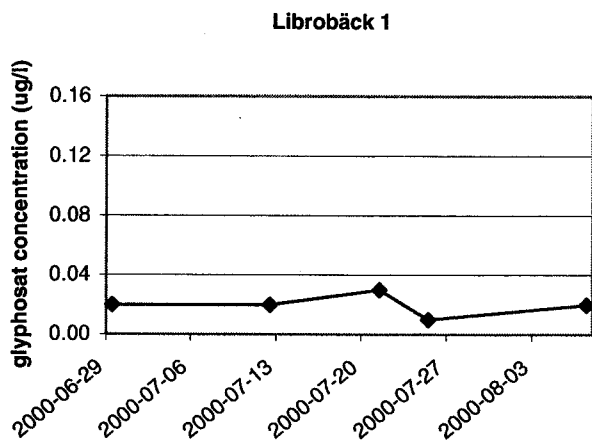


Figure 2. Concentration of glyphosate ($\mu\text{g/l}$) plotted over time from site Librobäck 1.

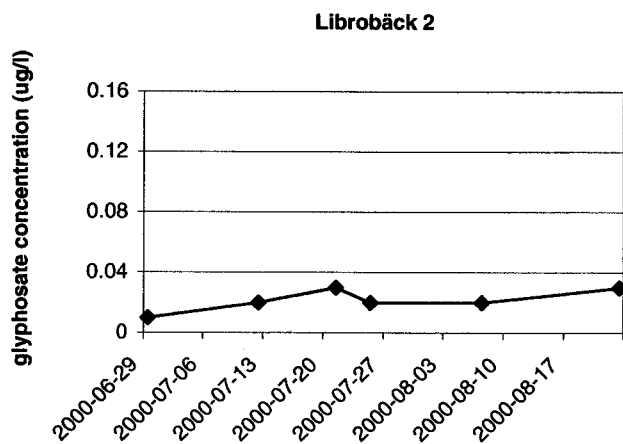


Figure 3. Concentration of glyphosate ($\mu\text{g/l}$) plotted over time from site Librobäck 2.

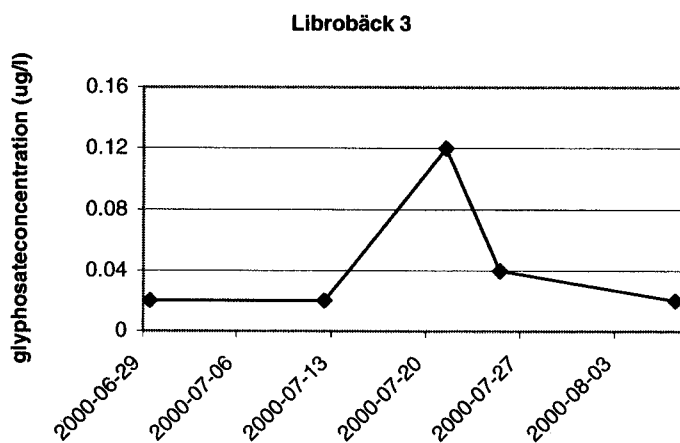


Figure 4. Concentration of glyphosate ($\mu\text{g/l}$) plotted over time from site Librobäck 3.

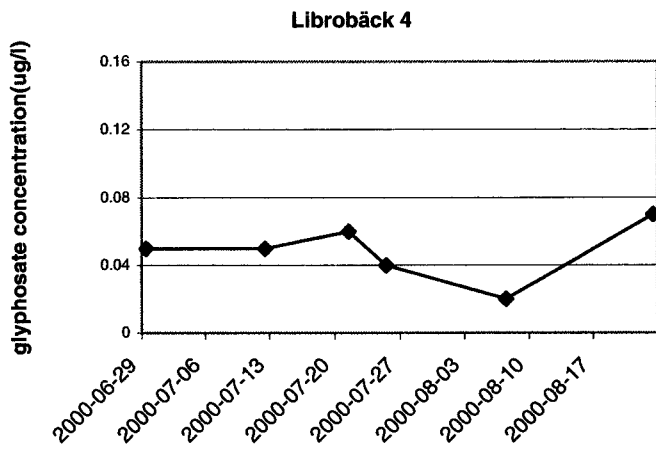


Figure 5. Concentration of glyphosate ($\mu\text{g/l}$) plotted over time from site Librobäck 4.

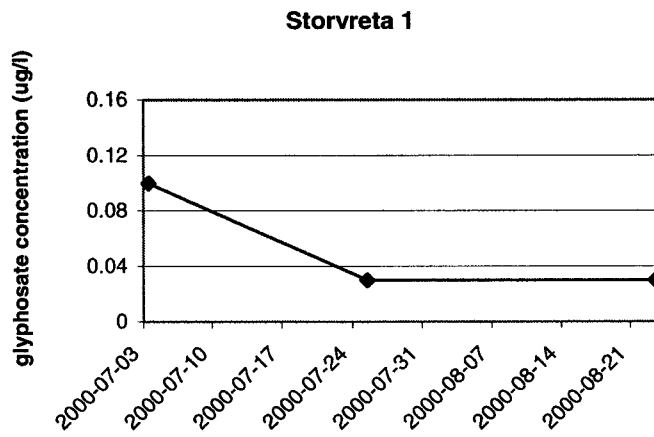


Figure 6. Concentration of glyphosate ($\mu\text{g/l}$) plotted over time from site Storvreta 1.

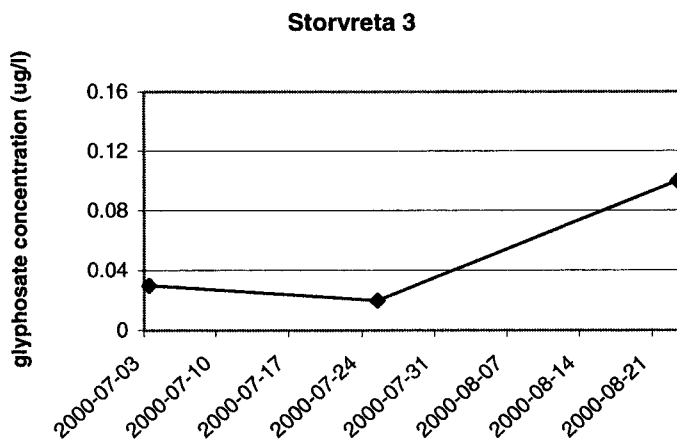


Figure 7. Concentration of glyphosate ($\mu\text{g/l}$) plotted over time from site Storvreta 3.

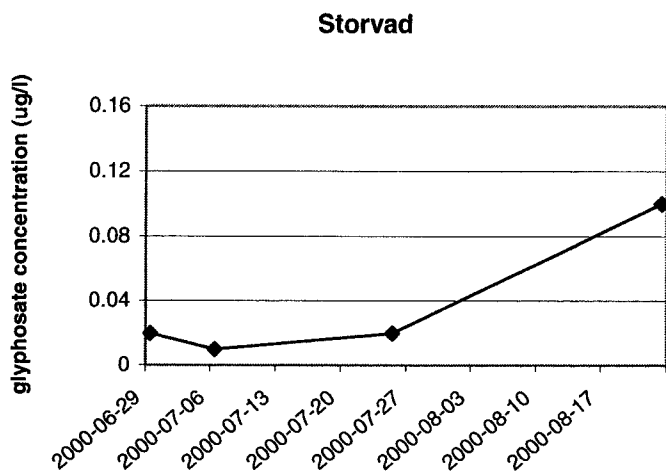


Figure 8. concentration of glyphosate $\mu\text{g/l}$ plotted over time for site Storvad.

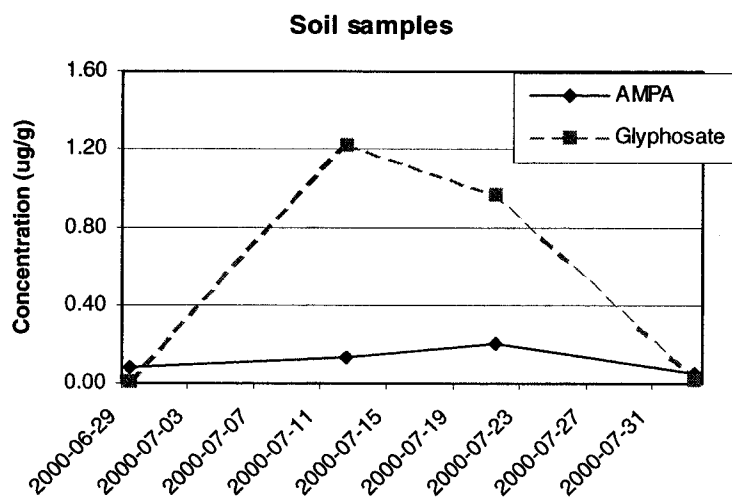


Figure 9. Concentration of glyphosate and AMPA ($\mu\text{g/g}$) plotted over time in the soil samples.

5. Discussion

5.1. Method

The method used for water analysis has been modified and changed during the time of this study. There are still questions on how to optimise the method. During the study we found out that the quantity of water analysed could affect the result. We found that 20-30 ml is the optimal volume. Since glyphosate has the ability to bind to surfaces, there are of course difficulties to be sure that you really measure all of the glyphosate in your sample. Although all glass material used for the analysis were washed with saturated KOH solution there could be a risk that some glyphosate was trapped on glass surfaces. When the glass material is washed with saturated KOH solution, the OH-groups binds to active sites on the glass surface. By doing this, the binding of glyphosate to the glass surface is reduced.

5.2. Recovery

The spring water gave better result than the surface water. The spring water is cleaner which means that it is less competition between ions when it comes to ion exchange in the columns and later in the derivatization. The recovery study also showed that the limit of detection can be lowered to 0.05 µg/l for AMPA and 0.03 µg/l for glyphosate.

5.3. Water samples

5.3.1. Librobäck, Site 1 and 2

In all samples from Librobäck 1 and 2, glyphosate concentrations were under the determination limit. The area around sample site Librobäck 1 was not treated with glyphosate, and the results from this site supports that so was the case. Quite far upstream sample site Librobäck 2 was an area treated with glyphosate as well as a field right next to this sample site. The reasons why the concentrations were under the detection limit could be that the glyphosate sprayed upstream this site were bound and eventually decomposed in the soil, and only a small amount actually entered the water. The glyphosate that went into the water could have been tightly bound to particles in the water, degraded, or diluted to under the detection limit. It is also possible that no glyphosate entered the water. The field beside sample site 2 had a protection zone with vegetation between the field and the brook (approximately 3 m wide). It is likely that this zone protected the brook from glyphosate contamination.

5.3.2. Librobäck, Site 3

Sample site Librobäck 3 shows another pattern. To start with there are very low concentrations of glyphosate. But, the sample from 2000-07-21 contains 0.12 µg/l. The reason for this could be a heavy rain that started a few days before this sample was collected (Appendix 6). It is possible that the rain washed out glyphosate from the fields that had been sprayed in the beginning of July, and therefore raised the concentration of glyphosate in the water. The water did also stay longer at this site because of a culvert that the water had to go through under a road. When the heavy rain came, the culvert was a bit too narrow to let all the water pass immediately. The sample taken a few days later shows a rapid decrease in glyphosate concentration. This could be due to degradation, or transport.

5.3.3. Librobäck, Site 4

Although almost all samples from this site were found to be above the limit of determination, the variation of concentrations is little. The concentration itself is also low. This site is situated just before the brook enters river Fyris. The water flow was generally higher at this site compared to the others. This could be an explanation to why there is not a high value at the same date as for site 3. The fields that were sprayed between sample site 3 and 4 were not situated right next to Librobäck. The time for the transportation of glyphosate could therefore be longer.

5.3.4. Storvreta

As only three samples from each site were analysed, is it difficult to say anything about patterns. The two sample sites do not show any sign of correlation in concentrations of glyphosate. Storvreta 1 had a higher concentration in the beginning of the summer where as Storvreta 3 had a higher concentration in the end of the summer. Since these sample sites are storm water pipes, the results are very dependent on the usage of glyphosate by the households.

In a proposal from the Swedish National Chemical Inspectorate (2000), it is suggested that the use of pesticides by private persons should be forbidden. This is a lead in the governments try to reach a non-toxic environments, which is one of the national environmental goals for the future (SOU 2000:52).

5.3.5. Storvad

The glyphosate concentrations found in samples from Storvad are generally very low except for one sample. Because the sample site is in Fyris River, a dilution effect of glyphosate is expected. Theoretically there would not be very high levels of glyphosate at this site. In one sample, however, the concentration was determined to be 0.1 µg/l. This could be an effect of a lower water table, which could be expected to lead to a concentration increase, as well as the effect of usage of glyphosate on stubble-fields in the neighbourhood.

5.4. Soil samples

The soil samples were taken in association with the water samples. The question was if you could see a connection between the concentration of glyphosate in water and the concentration of glyphosate in the soil. Since the concentrations from sample site Librobäck 2 were very low, and the samples are few, is it difficult to say anything of a pattern concerning this question.

Soil analyses often show that the concentration of AMPA increases when glyphosate starts to decrease (Börjesson, personal communication). This is a result of the degradation of glyphosate. In the soil samples from this study, no significant increase of AMPA was observed when the glyphosate concentration started to sink. This could mean that AMPA and maybe also glyphosate have moved down in the soil profile.

6. Conclusion

The analysis carried out on surface water from the Uppsala area, summer 2000, shows that glyphosate residues appear. The highest concentration of glyphosate was found after several intense rains. The rain is a likely cause for the transport of glyphosate from the fields into the surface water.

Because little analysis has been carried out of glyphosate in the environment, there is a great need of continuing and expanding the research. There is still a lot to find out about the behaviour in the environment of this complex molecule.

7. Acknowledgements

My stay at Miljökontoret, Uppsala kommun, and the Department of Environmental Assessment at SLU, Uppsala has been very pleasant. I would like to thank the staff at the Organic Environmental Chemistry Section, especially Gunborg Alex, for all their help. Gunborg Alex helped me with all the practical work and answered all my questions. My work would not have been this inspired and fun without Gunborg. I would also thank Jenny Kreuger and Peter Sundin for supervising.

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I am also very grateful to the farmers in the Librobäck area who provided me with information about their use of glyphosate.

Finally thanks to all of you that have answered my questions and helped me with this report, I am most grateful.

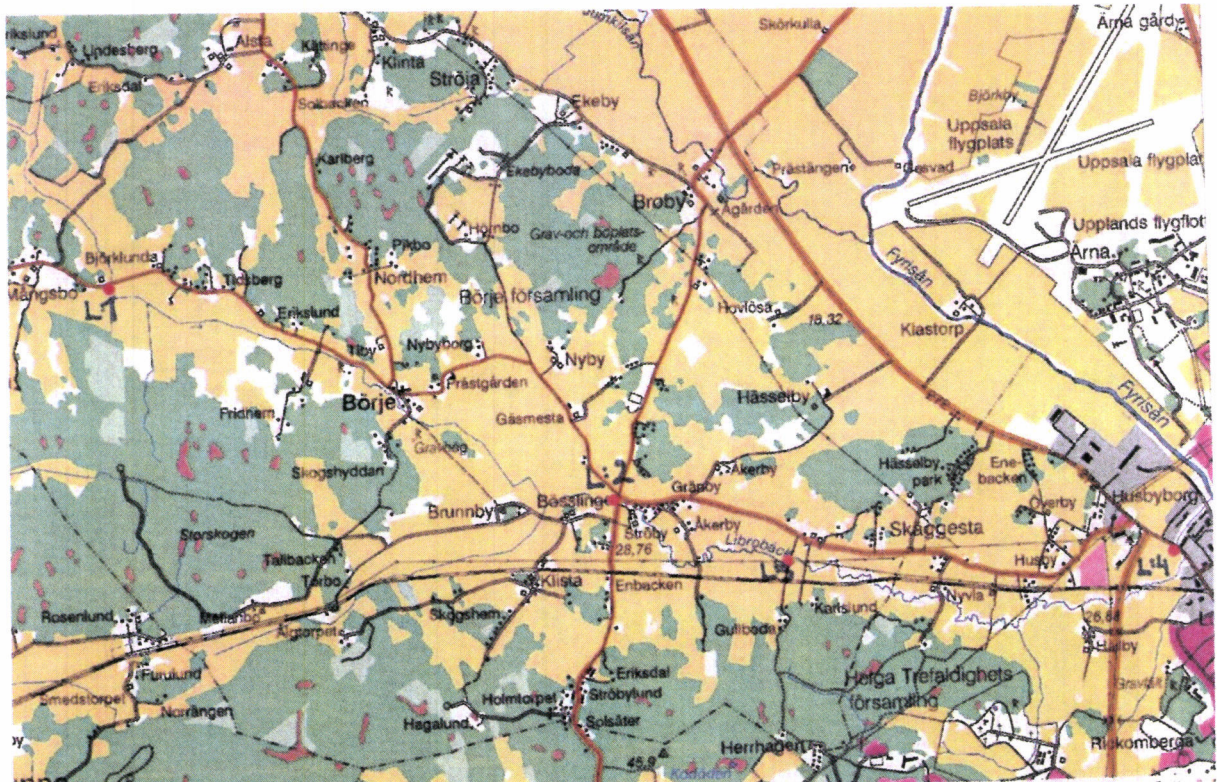
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Appendix 1

Map of sampling site Libroäck

The red spots denote the sampling sites. L1 is Libroäck 1, L2 libroäck 2, L3 Libroäck 3 and L4 Libroäck 4. The yellow area indicates cultivated land. Soil samples were taken on a field next to L2.

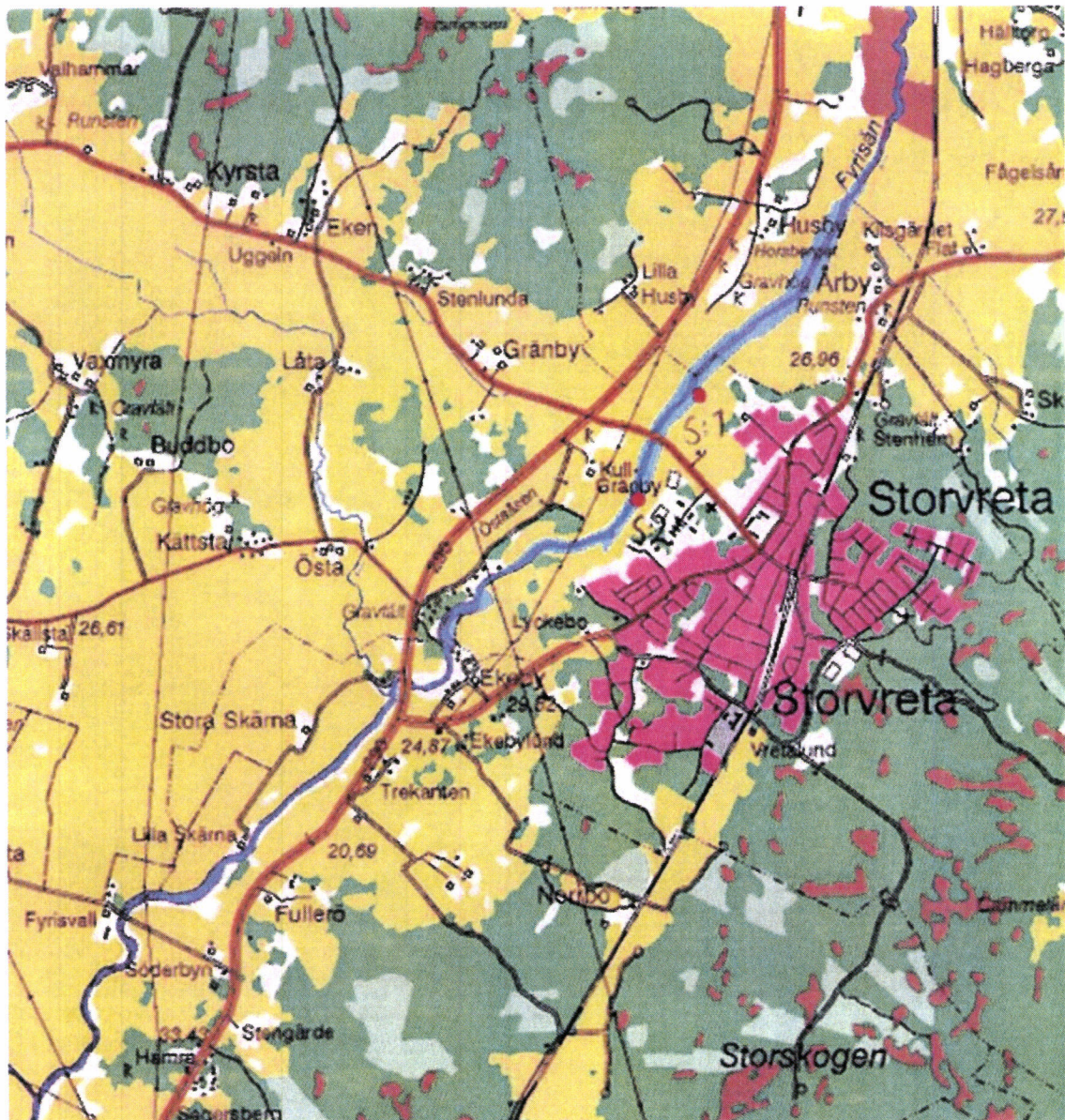


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Appendix 2

Map of sampling site Storvreta

The red spots denote the sampling sites. S1 is Storvreta 1, and S2 is Storvreta 3. The sampling sites are storm water pipe out flow before they enter the recipient, River Fyris.



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Appendix 4

Results of glyphosate determination and pH of the sample water

Concentrations and recovery of glyphosate. The pH is also shown for the different samples. The bold values show determined concentrations, the italic values are below the limit of determination and should be seen as traces of glyphosate, not as determined values.

nd=not detected

Location	Date	Measured glyphosate concentration (ug/l)	Recovery (%)	Glyphosate concentration adjusted for recovery (ug/l)	pH	Adjusted pH
Librobäck 1	2000-06-29	0.02	98	0.02	7.83	
	2000-07-12	0.04	220	0.02	7.7	
	2000-07-21	0.05	157	0.03	7.35	
	2000-07-25	0.01	116	0.01	9.48	7.7
	2000-08-07	0.02	96	0.02	9.16	7.47
Librobäck 2	2000-06-29	0.01	98	0.01	7.76	
	2000-07-12	0.03	220	0.02	7.74	
	2000-07-21	0.05	157	0.03	7.52	
	2000-07-25	0.02	116	0.02	9.37	7.83
	2000-08-07	0.02	96	0.02	8.97	7.3
	2000-08-23	0.03	98	0.03	8.81	7.71
Librobäck 3	2000-06-29	0.02	98	0.02	7.89	
	2000-07-12	0.03	220	0.02	7.61	
	2000-07-21	0.18	157	0.12	7.83	
	2000-07-25	0.04	116	0.04	9.39	7.58
	2000-08-07	0.02	96	0.02	8.97	7.31
Librobäck 4	2000-06-29	0.05	98	0.05	7.79	
	2000-07-12	0.1	220	0.05	7.75	
	2000-07-21	0.09	157	0.06	7.6	
	2000-07-25	0.04	116	0.04	9.35	7.43
	2000-08-07	0.02	96	0.02	8.88	7.65
	2000-08-23	0.07	98	0.07	7.71	
Storvad	2000-06-29	0.03	138	0.02	7.67	
	2000-07-06	0.01	138	0.01	8.69	7.18
	2000-07-25	0.04	170	0.02	9.17	7.69
	2000-08-23	0.2	170	0.1	9.3	7.74
Storvreta 1	2000-07-03	0.2	170	0.1	9.26	7.74
	2000-07-25	0.03	84	0.03	8.93	7.61
	2000-08-23	0.03	84	0.03	9.01	7.7
Storvreta 3	2000-07-03	0.05	170	0.03	8.29	7.31
	2000-07-25	0.02	84	0.02	9.52	7.83
	2000-08-23	0.1	84	0.1	9.5	7.34

Appendix 5

Results of AMPA and coordinates for the sampling sites

Concentrations and recoveries of AMPA. The co-ordinates for the sampling sites is also shown. The limit of detection was 0.1 µg/l.

na- not analysed

Location	Date	AMPA concentration (µg/l)	Recovery (%)	Co-ordinates X/Y
Librobäck 1	2000-06-29	<0.1	70	66 42 57/15 92 71
	2000-07-12	na	na	
	2000-07-21	<0.1	73	
	2000-07-25	<0.1	20	
	2000-08-07	<0.1	85	
Librobäck 2	2000-06-29	<0.1	70	66 40 85/15 96 46
	2000-07-12	na	na	
	2000-07-21	<0.1	73	
	2000-07-25	<0.1	20	
	2000-08-07	<0.1	85	
	2000-08-23	<0.1	85	
Librobäck 3	2000-06-29	<0.1	70	66 40 73/15 97 80
	2000-07-12	na	na	
	2000-07-21	<0.1	73	
	2000-07-25	<0.1	20	
	2000-08-07	<0.1	85	
Librobäck 4	2000-06-29	<0.1	70	66 40 73/16 00 70
	2000-07-12	na	na	
	2000-07-21	<0.1	73	
	2000-07-25	<0.1	20	
	2000-08-07	<0.1	85	
	2000-08-23	<0.1	85	
Storvad	2000-06-29	<0.1	70	66 44 30/16 01 70
	2000-07-06	<0.1	na	
	2000-07-25	<0.1	147	
	2000-08-23	<0.1	147	
Storvreta 1	2000-07-03	<0.1	147	66 51 10/16 05 60
	2000-07-25	<0.1	39	
	2000-08-23	<0.1	39	
Storvreta 3	2000-07-03	<0.1	147	66 50 80/16 05 40
	2000-07-25	<0.1	39	
	2000-08-23	<0.1	39	

Appendix 6

Precipitation at Ultuna meteorological observation station, June-August 2000.

