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Hexachlorocyclohexane
A well-known and highly explosive topic. Investigations on environmental levels of hexachlorocyclohexane in the Canton of Basel-Stadt.

Master thesis – Report

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Abstract

The hexachlorocyclohexane Lindane and its wastes have spread all over the world and are the most abundant organochlorine substances in Arctic air and fresh water. In the 1960s and 1970s, the pesticide Lindane has been produced in huge quantities by Ugine-Kuhlmann. The production lead to an estimated 120’000 tons of waste, consisting of different isomers of the hexachlorocyclohexane mixed with concrete. Open-air stock piling has led to distribution of the wastes throughout the city of Basel in the past. In this thesis, soils from various areas of Basel and vicinity have been analysed for hexachlorocyclohexanes, addressing several environmental questions.

Initially, three different extraction methods (accelerated solvent extraction, sonic extraction and hot Soxhlet extraction) have been evaluated. The hot Soxhlet extraction method fulfilled the requirements best and was hence fully validated. In addition, a sampling procedure was developed. The key parameters of this procedure have been determined and a robust sampling method established, in which samples can be taken at any chosen location.

Investigation of the pollution of the surrounding region of Basel and Basel-city has been carried out taking into account the main wind directions as an influencing factor. An increase in pollution was found in the prevailing wind direction for areas close to the former Lindane production site. Furthermore, the influence of the distance from source on the pollution in the main wind direction has been determined.

The origins of the pollution in the surroundings of Basel and Basel city have been investigated by soil profile analysis. These profiles have been sampled to study the migration of the pollutants into the soil. An equal distribution of the isomers has been found throughout the depth of 20 cm and beyond. A column test was used to simulate the migratory behaviour of hexachlorocyclohexane isomers in natural soil in a laboratory environment. Slight migration of the isomers could be shown to take place in the soil, whereas in sand the migration is significantly greater.

Samples mostly from park areas in the city of Basel show fortunately that the pollution is low. However, some sampling areas close to the former production site ARA STEIH, show significant contamination of the soil, albeit below the legal limit. With the passage of time, a shift in the ratio of the isomers has taken place, with the result that the beta- isomer is now the most abundant for these highly contaminated areas. The soils have further been analysed for other pollutants by the same extraction method and a degradation product of the formerly predominant alpha- isomer has been identified.

Zusammenfassung

Das Hexachlorocyclohexane Lindane und seine Abfälle haben sich weltweit verteilt und sind die am häufigsten vorkommende Organochlorverbindung in der arktischen Luft und Frischwasser. In den 1960er und 1970er wurde das Pestizid Lindane in grossen Mengen von der Firma Ugine-Kuhlmann produziert. Die Produktion führte zu enormen Mengen an Abfällen welche unter freiem Himmel offen gelagert wurden. Wind und Wetter haben die Abfälle in der Region Basel verteilt. Die Abfälle wurden später entsorgt, indem diese mit Beton vermischt und auf dem Areal vergraben wurden. Der kontaminierte Abfall wird auf 120‘000 Tonnen geschätzt.

In dieser Arbeit wurden Bodenproben aus Basel und der umliegenden Region auf Hexachlorocyclohexane untersucht um umwelttechnische Fragen zu beantworten.

Zu Beginn wurden drei verschiedene Extraktionsmethoden (Accelerated Solvent Extraction, Extraktion unterstützt durch Ultraschall und heisse Soxhlet Extraktion) auf ihre Fähigkeit Hexachlorocyclohexane zu extrahieren untersucht. Die heisse Soxhlet Extraktion erwies sich als die am besten geeignete Methode und wurde vollständig validiert. Zusätzlich wurde ein Probenahmeverfahren entwickelt und davon entscheidende Parameter untersucht. Dies führte zu einem robusten Verfahren welches überall eingesetzt werden kann.

Der Einfluss des Windes auf die Kontamination durch Hexachlorocyclohexane in Basel und deren Umgebung wurde untersucht. Einen Einfluss der Hauptwindrichtung, ausgehend von der ehemaligen Produktionsanlage, konnte ermittelt werden für nahe gelegene Gebiete. Des Weiteren wurde der Einfluss der Entfernung zur ehemaligen Produktionsanlage in der Hauptwindrichtung untersucht.

Der Ursprung der Verschmutzung in Basel und deren Umgebung wurde mittels Profilanalysen des Bodens untersucht. Insbesondere die Migration der Stoffe im Boden im Laufe der Zeit war im Focus. Die Profile wiesen eine gleichmässige Konzentrationsverteilung über die beprobten 20 cm auf. Einzelne Profile in grössere Tiefen wiesen ähnliche Resultate auf. Die Migration der Hexachlorocyclohexane wurde mittels einem Säulentest unter Laborbedingungen detaillierter untersucht. Geringe Migrationen der Stoffe in der Erde wurden festgestellt. Auf Sand wiesen die Hexachlorocyclohexane eine signifikant grössere Migration auf.

Bodenproben aus Basel-Stadt, zumeist aus Parkanlagen, wiesen glücklicherweise generell tiefe Hexachlorocyclohexan Konzentrationen auf. Gebiete nahe der ehemaligen Produktionsanlage, heute ARA STEIH, wiesen jedoch signifikant hohe Konzentrationen auf. Diese sind jedoch unterhalb des Grenzwertes. Die Bodenproben wurden zusätzlich auf weitere Organochlorverbindungen untersucht, wobei ein Abbauprodukt von alpha-hexachlorocyclohexane für die stark verschmutzen Gebiete in erhöhten Konzentrationen nachgewiesen werden konnte.

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1. Introduction

Lindane and its hexachlorocyclohexane isomers (HCH-isomers) are classified as organochlorine pesticides (OCPs). As persistent organic pollutants (POPs), they have attracted considerable global and environmental attention and have been banned by the Stockholm Convention since 2009. There has been broad concern for OCPs due to their high persistence, long-term toxicity and bioaccumulation. They are considered to pose a great threat to the ecosystems and may be detrimental to human health. Use of these chemicals has been banned almost all over the world, or allowed only under restricted conditions. These compounds have been used due to their low cost and versatility in action in agricultural, industrial and public health applications and are still in use in some countries such as India. Some 600,000 tons were produced worldwide between the 1940s and 1990s. Due to the extensive use in the past centuries HCHs are present all over the world in aquatic environments, the soil and even in air. Consequently, OCP residues might ultimately accumulate in mankind through the consumption of contaminated drinking water and food or even by inhalation. Improper disposal of HCH wastes have caused local major issues which have tried to be analysed and remedied. In the region of Basel, in Huningue, Lindane has been produced until 1974 by Ugine-Kuhlmann. The process of producing one tonne Lindane leads to eight to twelve times more HCH- isomers. These isomers are not active as pesticides and have, unlike the γ-isomer (Lindane), a malodour. Therefore, they were open air stock-piled, which lead to a major pollution of the area. Sandoz has constructed in 1978 on this area the waste water purification plant ARA STEIH which was shut down in 2012. Thankfully Novartis, who is now the owner of the ARA STEIH, has started voluntary efforts to remediate the area in collaboration with other parties. Unfortunately this lead to further release of the toxins which, due to the smell, was sensed by the citizens of Basel by its malodours initiating advanced investigation needs.

2. Assignment of Tasks

Soil samples taken in the city of Basel in September 2013 showed that there is still a high pollution with HCH in the soil remaining from the 1970s. This is now further complicated by new pollution due to the clean-up work has started early in 2013. It showed, that legal limits have been reached and that there are too few data. Due to this lack of information more extensive, detailed investigations are required.
Soil samples will be collected and analysed for all relevant HCH- isomers to provide in-depth information. The following points were addressed in this study:

1. Validation of an appropriate extraction method.
2. A Process to result in representative subsamples.
3. Sampling strategy resulting in representative samples and results.
4. Distribution of HCH due to the main wind direction.
5. Change of HCH- isomer abundance with distance to the ARA STEIH.
6. Origin of the pollution. Does the pollution originate from the remediation process or from the production time?

Sampling procedures will be discussed. Three different extraction methods will be compared, whereas one will be validated. Analytical techniques using GC-MS/MS are well established and do not need further improvement.

In the past, initial measurements of HCHs in soil near the source of the pollution have been made. Furthermore, detailed investigation of the pollution has been requested.
Several different methods of extraction of the main HCH- isomers from soil are to be investigated and validated.

A well-designed sampling procedure and the choice of sampling areas are important aspects of the project and have to be chosen with great care. In addition, several environmental and pollution questions need to be faced. The extent and geographical distribution pattern of the pollution in the region of Basel are to be investigated. Several sampling areas can be chosen to investigate the pollution near to the source and at further distance. Depth profiles of interesting areas may also lead to an in-depth knowledge of the HCH pollution. Depending on the results, further tasks can then be investigated, although it may not prove possible to answer all the questions that may arise.

3. Theoretical Part

3.1. Hexachlorocyclohexane

3.1.1. Introduction

Hexachlorocyclohexane (HCH) was one of the most widely used pesticides and is now classified as persistent organic pollutant. Its synthesis was discovered in 1825 by Michael Faraday.[1] However, the biological effect as a pesticide was only discovered in 1942. Imperial Chemical Industries Ltd. commercialised the photochemical chlorination of benzene using UV-light, which lead to the vast production of HCH in the past century. The photochemical reaction results in several isomers having different physical and chemical properties.[2–5]

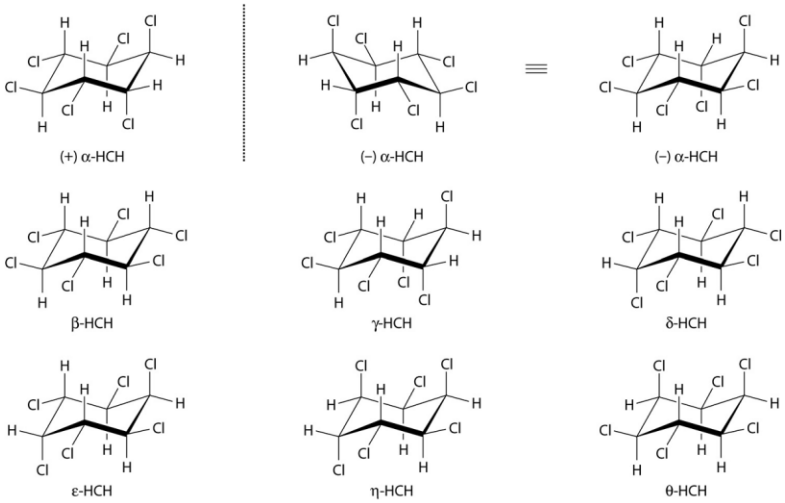


Figure 1. Hexachlorocyclohexane isomers.

Different conformations of all hexachlorocyclohexane isomers. [5]

The γ-isomer is known as Lindane and exhibits the desired insecticidal properties. However, often it was applied as technical HCH which mainly consist of the five isomers α-HCH (55 – 80 %), β-HCH (5 - 14 %), γ-HCH (Lindane, 8 - 15 %), δ-HCH (2 - 16 %) and ε-HCH (3 – 5 %). The other isomers and side products are produced very low concentrations.[3]

3.1.2. Lindane Properties

Lindane is an odourless white to brown crystalline powder. However, technical HCH which contains also the other enantiomers and side products exhibits a penetrating musty odour. All isomers are rather poorly water soluble and non-polar. Yet they do differ from each other. The chlorine atoms in the β-isomer are all oriented equatorial and thus lead to a thermodynamically more stable isomer which would explain some physical and biological characteristics. The physical properties of the most common isomers are listed in the Table 1.[6–8]

Table 1. Physical properties of the relevant HCH- isomers.

Property	α-HCH	β-HCH	γ-HCH	δ-HCH
Molecular weight	290.8 g/mol	290.8 g/mol	290.8 g/mol	290.8 g/mol
Specific gravity	1.87 kg/L	1.89 kg/L	1.85 kg/L	NA
Melting point	158 °C	309 °C	112.5 °C	141.5 °C
Boiling point	288 °C	60 °C at 0.5 mm Hg	323.4 °C	60 °C at 0.36 mm Hg
Log K_{OW}	3.8	3.78	3.72	4.14
Water solubility at 25 °C	0.002 g/L	0.0002 g/L [a]	0.0073 g/L	0.0314 g/L
Vapour pressure at 25 °C	4.5 x 10^{-5} mm Hg	3.6 x 10^{-7} mm Hg [a]	4.20 x 10^{-5} mm Hg [a]	3.5 x 10^{-5} mm Hg

Various physical properties of the relevant hexachlorocyclohexane isomers. [a] Measured at 20 °C. The conformation of the isomers leads to different physical properties.[7]

3.1.3. History of Lindane

Since its discovery in 1942 Lindane was often applied as technical HCH. The use of technical HCH was reduced already early since it left an unpleasant smell in food crops. In the 1970s the technical HCH was replaced by enriched HCH containing 40 % of the desired γ-isomer. However, already in 1979 enriched HCH was no longer produced in the EU and USA. The technical HCH was then purified to obtain Lindane (> 99 %). In developed countries purification of Lindane was conducted by recrystallization from methanol or acetic acid. The resulting waste was not used and dumped.[7–9] A boost in popularity occurred, when the ill-famed pesticide dichlorodiphenyltrichloroethane (DDT) was banned in the late 1970s. At the time, HCH was a low-cost, convenient and efficient alternative and was therefore used extensively, not only in agriculture, but also in other application fields. Lindane (and its isomers) were used in agriculture (for fruit and vegetables), forestry (Christmas trees), veterinary (pets) and even as an insecticide in pharmaceutical shampoos and lotions.[7,8,10]

The major drawback in the production is the amount of waste generated. However, there have been attempts to re-use the inactive isomers such as conversion to 2,4,5-trichlorophenoxyacetic acid, a herbicide and a major ingredient in “Agent Orange” used in the Vietnam war and 2,5-Dichloro-4-bromphenol-oxyphosphoric acid (insecticide Bromophos). When the Vietnam war ended there was no market for the 2,4,5-trichlorophenoxyacetic acid and the demand for Lindane declined and consequently Lindane production plants were closed one by one. In 2006 only the two countries, China and India, were certainly producing Lindane. Romania was, however, still selling small amounts.[11]

It is estimated, that globally over 600'000 tons of Lindane were produced between 1940s and 1990s. Experts estimated, that per ton Lindane 8 to 12 tons HCH- isomer waste was produced which corresponds to 4.8 to 7.2 million tons. From the compiled data of the countries, it can be estimated that more 2.9 to 5.6 million tons of HCH- waste is missing.[5]

3.1.4. Lindane in Basel

Near Basel, Huningue in France, the company Ugine Kuhlmann was producing Lindane. The waste which accumulated was stored in the open. Due to wind and weather the HCH- isomers became distributed around the region of Basel. This had the effect that in the 1970s HCH-isomers were found in milk from the region of Basel, which lead to the slaughter of the milk cows. More alarmingly, a recommendation not to breast feed was issued. In 1995 the documentary "Reizendes Gift" has been produced by Martin and Priska Forter, showing the extent of the environmental scandal. People, such as a customer's officer describe the white wind and its deposits.

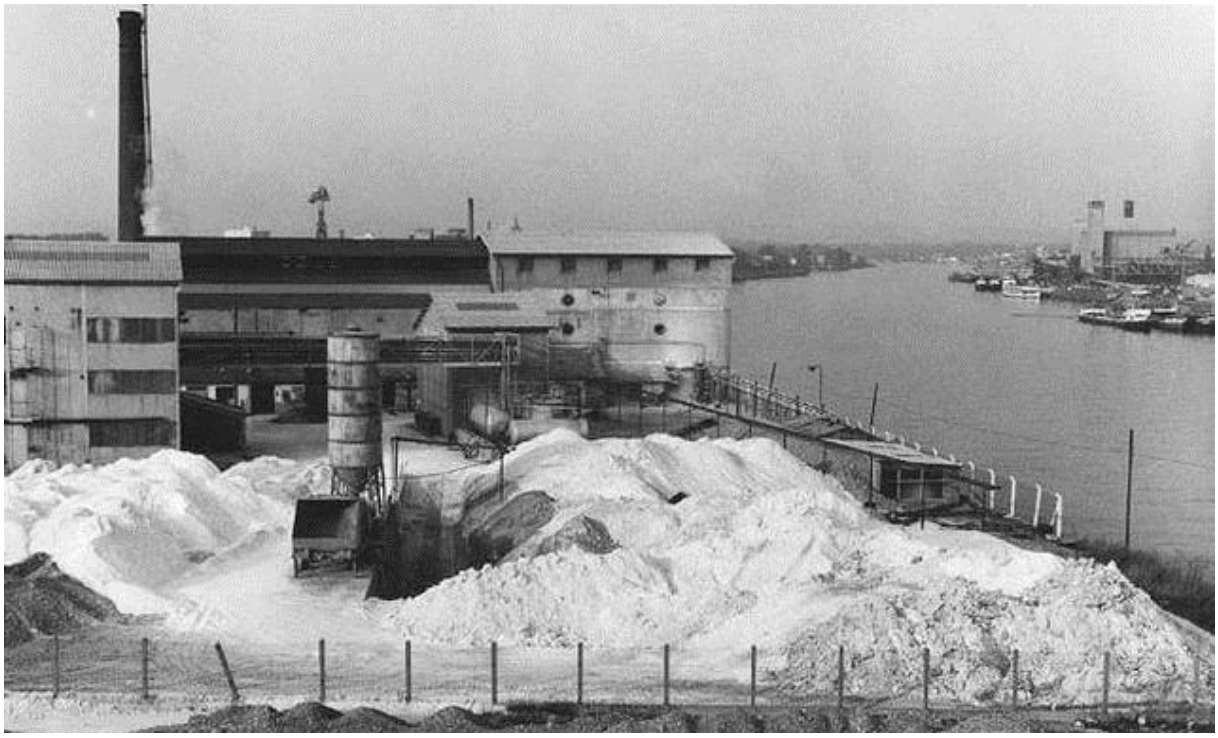


Figure 2. Open air stock-piling of Lindane wastes in Huningue.

Opan air stock piling of hexachlorocyclohexane production wastes of Ugine Kuhlmann in Huningue. Picture was taken on 02.11.1972.[12]

In 1974 Kussmaul and Borneff et al., 1983 measured HCH- isomer concentrations in the Rhein bellow Huningue. They found maxima of 2700 ng/l α-HCH, 300 ng/l β-HCH and 1100ng/l γ-HCH in the Rhein. Between 1976 and 1977 six measurements were conducted, whereas 1 - 9 ng/l α-HCH and 2 - 25 ng/l γ-HCH leading to the conclusion that the isomeric ratio between α- and γ-HCH have changed. Further the values have decreased dramatically.[6] Ugine

Kuhlmann has produced Lindane and its isomers until 1974. However, already in 1972, Ugine Kuhlmann was desperate to dispose of the waste. A huge pit was excavated disposing the gravel in Hagenthal-le-Bas and Hagenthal-le-Haut in France and the cavity filled with a mixture of HCH- waste and concrete. This site which was reconstructed and used as the industrial clarification plant Steih has been bought by Sandoz. This site is now being voluntarily decontaminated on the behalf of Novartis. The first decontamination project was a tremendous one involving roughly 100 million Euro. Soil which contained more than 20 g HCH per kg soil is burnt at 1000 °C. Soil below the contamination threshold is heated and the vapours are then burnt. Nevertheless, this constitutes an enormous project and involves many parties. The decontamination started in 2013. However, citizens of Basel complained that there was an aggravating smell, which could be traced back to the clean-up operation. Several measures were taken, including the construction of a tent over the site. Nevertheless, the first project had to be stopped. [12–15]

Figure 3. ARA STEIH currently.

Current situation of the ARA STEIH. Tents cover the construction site in order to reduce new contamination of the environment. Webcam image, taken on the 17th June, 2014, 13:42 o'clock.

3.1.5. Lindane Today

Today we are using more efficient processes to produce pesticides of lower environmental impact. Chemical disposal sites today are designed in accordance with more restrictive environmental protection legislation and a better understanding of the effects of disposal of wastes on nature.

Nevertheless, we are faced with the burden of an HCH- polluted environment. In general there are two different types of contamination sources. Very high local concentrations of HCHs can arise due to improper waste disposal, such as open-air stock-piling. Secondly, lower concentrations can be found due to the dispersion of open-air stock piles or application of the

insecticide.[5,8] The released HCH waste has reported to percolate both soil and ground water [16,17] and is the most abundant organochlorine substance in Arctic air, fresh water and the Arctic Ocean.[8]

The Lindane wastes are usually spread by the atmosphere and rivers and it is therefore not surprising that it is found in the oceans and higher concentrations near estuaries. HCHs have been also found in sediments and were absorbed by marine animals in which they accumulate.[6]

3.1.6. Toxicity of Hexachlorocyclohexane Isomers

HCH- isomers can be absorbed by plants via the gaseous phase or from the soil by the roots. In plants the HCH- isomers can be metabolised by dehydrochlorination, dehydrogenation, dechlorination and hydroxylation. The effects of HCH on animals and humans have been observed in several studies. It is known, that the HCHs are taken up and stored in fatty tissue. It is noteworthy, that due to its different physical characteristics, the β-isomer has slightly different biological/toxicological properties and the accumulation in fatty tissue is higher than for other HCH isomers.[6]

The absorption of HCH in humans occurs mainly via the uptake of food and less by respiration or by physical contact. The later depends on a variety of factors, such as the contamination of the environment, exposure time, concentration, etc.

The elimination half times of HCH- isomers from fatty tissue and brain tissue are isomer specific.

δ-HCH ~ γ-HCH > α-HCH >> β-HCH

Direct toxicological studies on humans have not been made. However, data from exposure reports and from experimental studies in animals are available.

It has been documented, that acute exposure to HCH- isomers results in accumulation in the nervous system leading to hyperexcitability, seizures and convulsions and eventually even to death.[6]

Symptoms of the ingestion of Lindane are vomiting and nausea. Studies on HCH causing cancer in humans have as yet been inconclusive.[9] However, in animal studies on rodents, there is evidence that HCHs cause liver tumors and hence it is reasonable to expect that HCHs are indeed human carcinogens and cause tumors of the lymphoreticular system. [7] It is also known, that HCHs act as endocrine-disrupting pollutants.[8,18,19]

Table 2. Toxicity of hexachlorocyclohexanes.

	α-HCH	β-HCH	γ-HCH	δ-HCH
Acute toxicity LD50 oral rat	177.0 mg/kg	6.000 mg/kg	88.0 mg/kg	1.000 mg/kg
Bioaccumulation factor (fish)	250	500	674	326
Acute toxicity (brain concentration)	80-100 µg/g	15-20 µg/g	6-7 µg/g	30-35 µg/g
Acute toxic effect	Generalised tremor	Generalised chronic convulsion	Ataxia	Locomotive excitation

Different toxicological of the major HCH- isomers. The toxicity is important for remediation decisions. [6,20–23]

3.1.7. Hexachlorocyclohexane in the Environment

The classification of HCHs as persistent organic pollutants already indicate, that HCH isomers break down only slowly in the soil and can migrate to some extent.

Several methods of decontamination of polluted areas are known. In case of the former production plant in Huningue the soil was removed to a disposal facility where the contaminated soil was heated and the vapours burned. Highly contaminated soil was incinerated separately. This is a very cost intensive and invasive method, which does apply to the current conditions. For large areas where this method cannot be applied, other methods have to be considered. Degradation of the HCH isomers does take place in the environment, but unfortunately the process is slow and isomer specific. Factors such as the weather, pH, soil composition and other environmental influences affect the rate of breakdown. In general the thermodynamically less stable isomers such as the α- and γ-isomer undergo higher degradation rates than the β- and δ- isomers. Remediation due to biotic procedures are possible. It has been reported, that contaminated soils have been treated with bacteria resulting in increased degradation of the isomers. However, the degradation rates depend on several factors. Nevertheless the β- and δ- isomer are usually transformed slower. The degradation may occur under both aerobic and anaerobic conditions. The isomeric pattern of contaminated soil changes over time to the favour of β- HCH.[20]

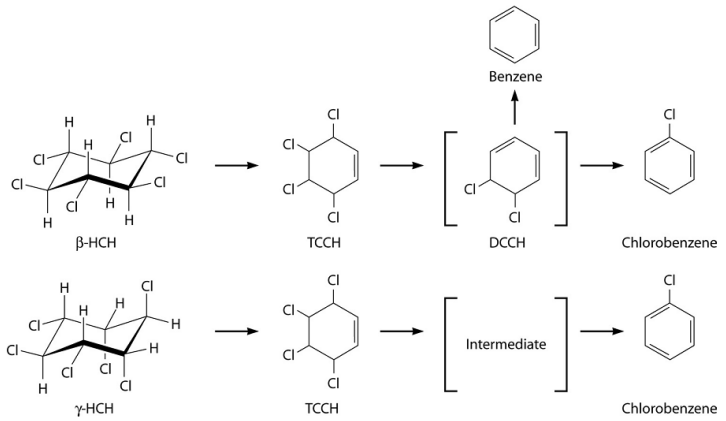


Figure 4. Anaerobic degradation of beta- and gamma- HCH.

β- and γ-HCH isomers feature the same anaerobic degradation pathway by eliminating hydrogen chloride to the final product Chlorobenzene.[5]

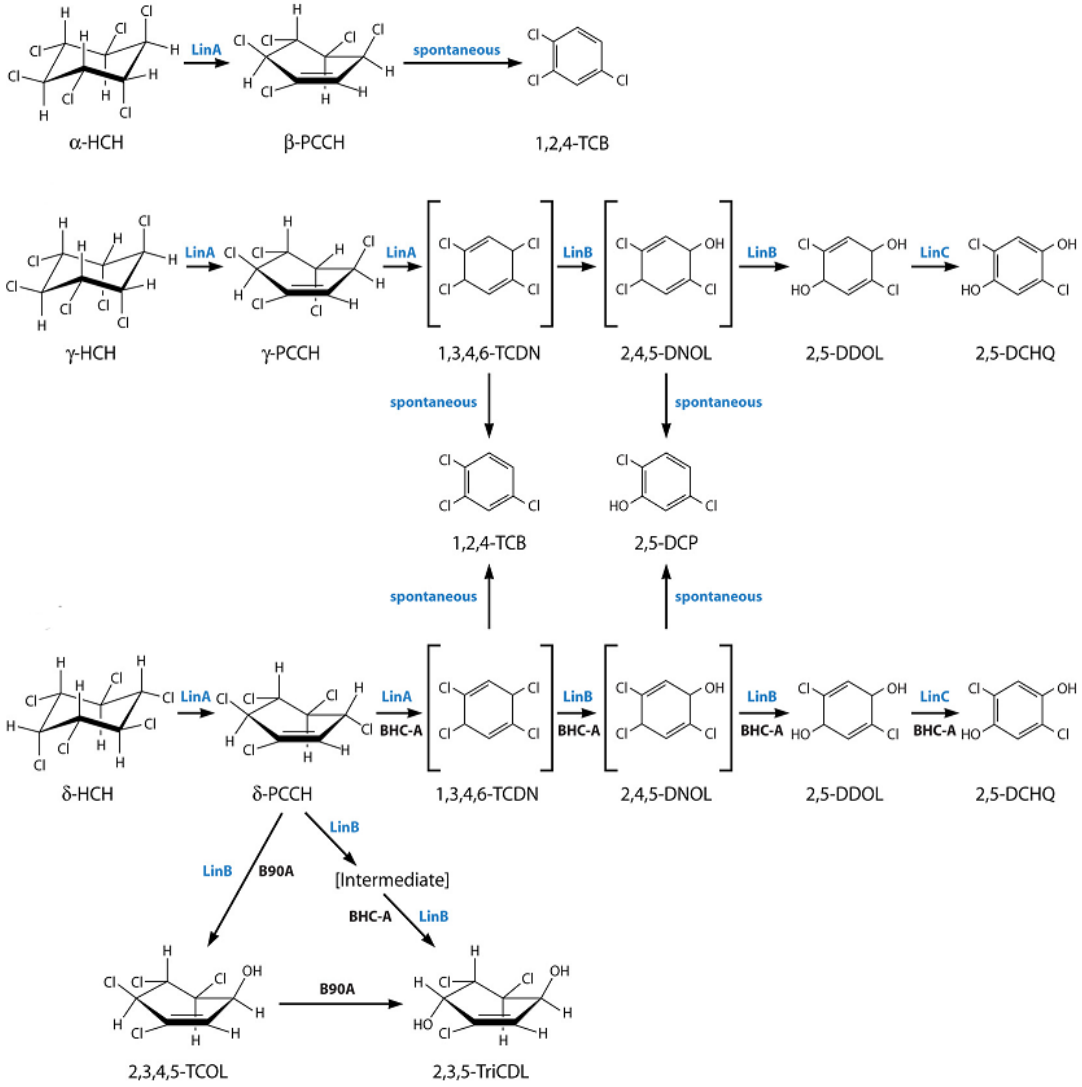


Figure 5. Biotic degradation of alpha-, beta- and gamma- HCH.

Upstream pathway for the aerobic degradation of α-, β-, and γ-HCH initiated by two successive dehydrochlorination reactions. LinA, LinB and LinC are enzymes believed to catalyse several reactions. The finally observed products were 1,2,4-trichlorobenzole 2,5-dichlorohydroquinone, 2,3,4,5-tetrachloro-5-cyclohexene-1-ol and 2,3,5-trichloro-5-cyclohexene-1,4-diol .[5]

The degradation of the HCH- isomers may also occur abiotically. However, the conditions might not be appropriate. Direct photolysis with UV irradiation (100-280 nm) does transform the isomers to 1,2,3,4,5-pentachlorocyclohexanes.[24] It has also been reported that indirect photolysis, assisted by hydrogen peroxide transforms the γ-isomer by using powerful OH radicals into further products.[5]

3.2. Soil

3.2.1. General Knowledge on Soil

The term soil is well known to all people, however, the functions of soils are more than just a medium on which plants grow. Moreover, it is a highly complex environment for a vast multitude of organisms. In general soils have a number of key roles. [25]

Medium for plant growth

Soils affect several aspects related to the growth of plants. For instance it is a physical support for the plants preventing uprooting due to environmental influences such as heavy wind. Furthermore, the soil pores retain rainwater. Plants can therefore rely on a fairly constant water supply which enables their growth and reproduction. Soil is also a good climate regulator preventing extreme changes in temperature necessary to life on the surface. The soil prevents plants from taking up toxins by ventilation, decomposing or absorbing organic toxins, or suppressing toxin producing organisms. The soils are also a supply for nutrients. Thus the properties of the soils often influence the vegetation and hence the number of animals due to the vegetation present. [25]

Regulator of water supplies

The water quality in rivers and lakes has become a more recognised topic of concern. The soil is an important factor affecting the water quality. Every drop of water in rivers and lakes has either travelled through the soil or has flooded over soil surface. The soaking ability of soil is thus an important aspect. Rainfall is stored in soil or is drained through soil preventing destructive floods.

The seeping of the water through soil over months or years purifies the water and kills potential disease organisms. This process is also used industrially for the production of drinking water.[25]

Recycling

The growth of animals and plants relies on the uptake of nutrients and conversion of small molecules into macromolecules for example in photosynthesis. However, when wastes are produced by organisms or when their life has been ended, all the materials are recycled. Thus

soils have the capacity to assimilate organic matter and form humus; converting mineral nutrients into a form that can be reused again and returning the carbon to the atmosphere as carbon dioxide. The accumulation of soil organic matter has also a major global influence in regulating the greenhouse effect.[25]

Habitat for soil organisms

Even though soil might look uniform, looking closer, the biodiversity is vast. Smaller and bigger pores can be filled with water, air or other gases and different pH and temperature environments exist, which promote the growth of organisms, each specialised to preferred conditions. In this ecosystem there exist prey, predators, producers, consumers and parasites resulting in a highly complex system, hidden from the world's eye and vulnerable to diverse influences and pollution.[25]

Influences on the atmosphere

Depending on the soil and its vegetation, there are several effects of the soil on the atmosphere. Wind can pick up soil particles from dry and barren soil which can be transported into the atmosphere. More moist and fertile soils can prevent such effects. Furthermore, the evaporation of moisture from the soils leads to an alteration of air temperature. Soils have further a major influence on the atmosphere. In an exchange of gases with the atmosphere, carbon dioxide and nitrous oxide is released by the soil and oxygen absorbed. This exchange has a major contribution on the atmospheric composition and global warming.[25]

3.2.2. Soil Classification

Initial classification of soils have been already been done in 1870s by the Russian scientist V.V. Dokuchaev and his associates. Soil classification of further soils in other countries by has lead to a large number of different classifications of soils. Nevertheless, many of the Russian names have been keept.

Soil taxonomy today is classified by six hierarchical categories: The twelve orders are divided in suborders, great groups, subgroups, families followed by series.

Each of the soil orders is largely based on the properties of the soils and the conditions that formed the soils. The classification includes a number of physical and chemical properties which are often analysed with great precision.[25]

Description of the soils in the field is often done according to colour, texture, consistence and other properties. For instance, the soil texture is defined as "the relative proportion of the various soil separates in soil material". Therefore the percentage of clay, slit and sand gives the twelve different textural classes as shown in Figure 6.[26]

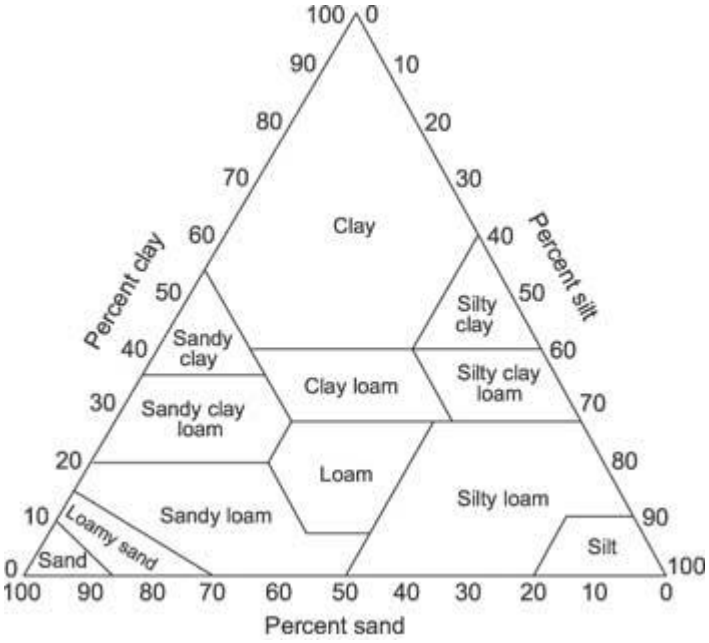


Figure 6. Twelve textural classes of soils.

According to the Soil Science Society of America 1996, the percentage of clay, sand and slit lead to twelve different soil texture classes.[26]

3.2.3. Bioturbation

Bioturbation means the biological reworking of soils and sediments. The first observations were recorded by Charles Darwin, who was very interested in the influence of earth worms on their environment. However, the initial results he presented were regarded as a speech on ‘worms’. At the time earth worms were regarded as garden creatures that needed eliminating. Nevertheless, Darwin continued with his research on this topic publishing a book he thought was of small importance. However, Darwin’s studies have since been shown to be of great importance and have become popular again. Disciplines such as ecology, pedology, hydrology, geomorphology and archaeology currently refer to his book.

Bioturbation is a result of a range of animal activities. Most obvious are the effects of larger subterranean mammals such as moles.

Figure 7. Bioturbation due to moles.

Subterranean mammals such as moles lead to obvious bioturbation. Hidden from the eye, small vertebrates and invertebrates have further significant influence on the soil construction. Image source: Meysman et al. [27]

However, there are further organisms contributing to bioturbation such as earth worms, nymphs and ants. The effects however, are of great importance for the ecosystem.

Burrowing organisms have been cited as examples for system engineers modifying the physical environment strongly. This engineering has major impacts on other organisms and further lead to heterogeneity of the pedological environment.

It is stated, that the effects of bioturbation also enabled important evolutionary phases such as the Cambrian explosion around 542 million years ago. Hence organisms developed biomineralised skeletons allowing some parts to use as digging tools. The new sediment environment was discovered to be not only a new food source but also a shelter from predators. The result of the turnaround of the sediments lead to an explosion of life. [27,28,29]

The importance of bioturbation on the distribution in soil of organochlorine pesticides has been shown by Zhang et al.: a principal downward distribution of HCHs and DDT by bioturbation was found. In layers beneath the plough pan the water solubility of the entities becomes more important.[30]

3.3. Sampling

3.3.1. Sampling Method

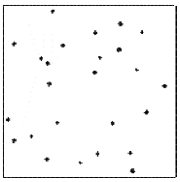
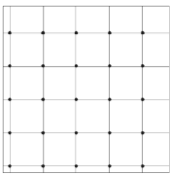
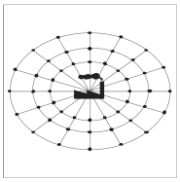
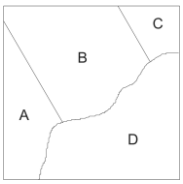
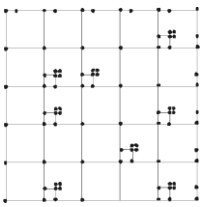
In order to draw conclusions from the results and improve procedures it is essential to acquire reliable data. The choice of sampling areas and the sampling procedure are therefore of great importance. Since the area of interest is rather large (Basel and environs), only rather random samples can be gathered. Hence errors due to inhomogeneity and sampling procedure must

be minimised as much as possible. It is therefore important to plan the sampling in detail, conduct the sampling professionally and to process the samples accordingly. The measures to be taken depend on the questions the analysis results must answer. In general, sampling areas differ significantly to laboratory conditions, whereas standardised sampling procedures are not feasible for all sampling locations due to environmental influences and other properties of the location. Documentation of different aspects at the sampling locations should lead to a reduction of errors and ultimately enable better conclusions to be drawn from the data.[31]

3.3.2. Sampling Plan

In a sampling plan several different aspects have to be considered. Thus, sampling of a specific location requires an adequate number of samples to represent the area of interest due to supposed inhomogeneity in deposition of the pollution. A sufficient number of probes must be chosen to answer the environmental and pollution questions kept as small as possible for reasons of efficiency. The sampling pattern is another important factor in deriving significant samples and is a major error source. There are different possibilities each with advantages and disadvantages listed in the Table 3.

Table 3. Different sampling patterns.

Patterns	Realisation	Advantages	Disadvantages
Random 	Distribution of the sampling positions by using random numbers with exclusion of any expert knowledge.	- Single objective procedure - Every sampling spot has the same likelihood - Low systematic error	- High number of samples required - Laborious realisation - Not proportional to the area
Systematic 	Distribution of the samples on a geometric grid: - Quadratic - Rectangular - Triangular.	- Low complexity - Lower sample numbers - Best coverage by triangular grid - Consistent distribution of the sampling spots - Area proportional	- Unsuitable grid size might lead to systematic errors - Triangular grid is complex
Directed 	Distribution of the sampling positions according to expert knowledge and plausibility considerations: - Point sources: target grid - Line sources: Line grid - Other sources depend on the hypothesis - Sampling positions are intensified near the source.	- Smallest number of samples - Consideration of the contamination hypothesis	- highest liability to systematic errors when false hypothesis is applied - Requires laborious pre-clarifications
Stratified 	Directed partitioning in homogeneous subareas and area proportional equal distribution of sampling spots inside the subareas.	Consideration of the contamination hypothesis	- Susceptible to systematic errors on false contamination hypothesis - Requires prior knowledge
Nested subroutine 	Systematic distribution of sampling locations with local condensed sampling with a given systematic.	- Heterogeneity is assessed at different spatial levels - Adequate for geostatic assessment.	- High number of samples necessary - extensive procedure

Different sampling strategies are discussed with drawbacks and positive aspects. Depending on the environmental issue a suited sampling pattern is chosen.[31]

3.3.3. Nature of the Sample

Samples can be retrieved as a single sample from a single borehole in the soil or can be pooled out of several individual boreholes to give an averaged sample.

The environmental and pollution question is one decision factor. However, for invasive sampling techniques it might be considered to use single samples in order to minimise negative effects on the environment.

Pooled samples have the advantage of levelling out the inhomogeneity of the sampling area. They are dependent on the sampling technique as well as the environmental and pollution question which is to be answered. The more samples that are pooled together, the more significant are the results for a given area.[31]

3.3.4. Sampling Depth

Sampling depth is a most important parameter in order to obtain representative samples. It is dependent on several factors such as the pollutant and its persistence in the soil, as well as the kind of immission. According to the VBBo, toxins should be examined to a depth of 20 cm for processed and unprocessed soils. However, sampling of profiles can be conducted even deeper than 20 cm. When measuring the sampling depth, the organic layer is also to be taken into account.[31]

3.3.5. Sample Quantity

The sample quantity must be carefully chosen in order to constitute a representative sample on the one hand. On the other hand the analysis method dictates a certain quantity. An aliquot may also be stored for later usage. When sampling at different areas, different soil compositions may also be found. Thus not all layers can be used for the analysis and will therefore influence the sample quantity.[31]

3.4. Extraction Methods

3.4.1. Accelerated Solvent Extraction

Accelerated solvent extraction (ASE), also known as pressurised liquid extraction (PLE), pressurised solvent extraction (PSE) or pressurised fluid extraction (PFE) is a rather recent extraction method which has been introduced by Dionex in 1995. Analytes are extracted from solids or semi-solids by using solvents at elevated temperatures up to 200 °C and 150 atm for a short period of time.

The desorption of the analyte is promoted by the elevated temperature of the solvent disrupting the hydrogen bonding, dipole interactions and van der Waals forces between the solute and

the matrix. The thermal energy assists in overcoming the cohesive (solute-solute) and the adhesive (solute-matrix) interactions.

The high pressure forces the solvent to remain in the liquid aggregate state above its atmospheric pressure boiling point. Moreover, the pressure increases the solvation power and speeds up the extraction kinetics of the solvent by forcing it into the pores of the matrix. Solutes which are normally trapped in the small pores are more easily extracted by the solvent.

The increased temperatures lead to a decrease of the dielectric constant thus making it suitable for the extraction of less polar compounds. Water has a dielectric constant of roughly 80 F/m at 25 °C. However, if it is heated to 250 °C, the dielectric constant of liquid water is reduced to 27 F/m, which is in the range of ethanol and methanol at 25 °C. Thus, at higher temperatures, it becomes more miscible with other organic solvents.

A further advantage of this extraction method is the fast time and the ease of automation which make it suitable to diverse applications, such as analysis of pesticides in food, etc. One limitation of the method is, however, the high temperature which may cause decomposition of analytes.[32]

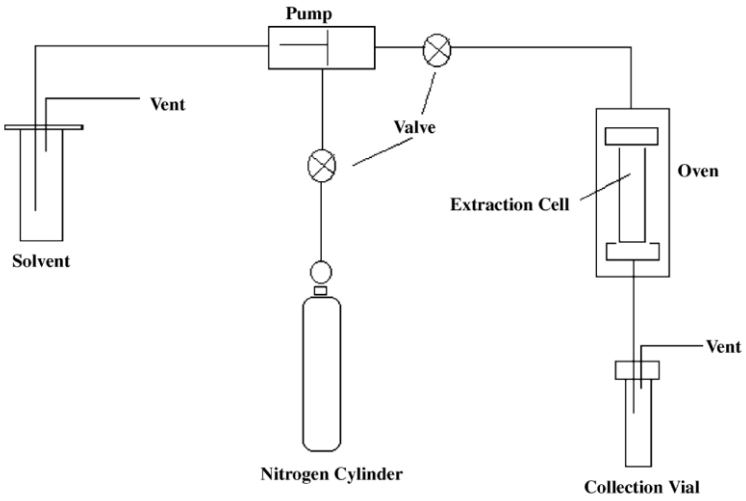


Figure 8. Schematic set-up of an ASE.

The solvent is transferred into the extraction cell and is set under pressure. The extraction cell is heated, whereas the solvent remains in the liquid aggregate state reducing the extraction time.[32]

3.4.2. Soxhlet

The Soxhlet extraction method has been described by Franz von Soxhlet and published in Dingler's Polytechnisches Journal in 1879 describing the gravimetric determination of milk fat. The principle has been adopted for numerous analytical problems and is a very powerful extraction method in which compounds of limited solubility are extracted requiring small volumes of solvent. In general, the solid is placed in a thimble made from filter paper and inserted into the Soxhlet chamber and a solvent below heated to reflux. The vapours then rise through the distillation arm and condense on the cooler placed above the Soxhlet chamber.

The condensed solvent continuously drips into the thimble flooding the chamber slowly. When the solvent level reaches the height of the siphon side-arm, the chamber is emptied into the distillation flask. In every cycle the solvent extracts a small fraction of analyte leading to an enrichment of the desired compound in the distillation flask. The solvent can then be removed resulting in the crude extract, which may be further purified.

The choice of the proper solvent is important since not only the desired analyte is extracted but also other substances. Furthermore the analyte must be thermally stable and non-volatile.[33]

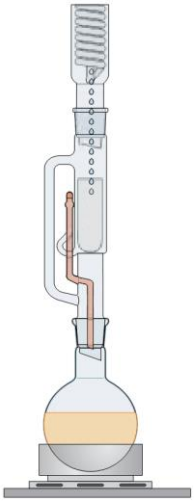


Figure 9. Soxhlet apparatus.

The vapours of the heated solvent rise until they are condensated on the cooler. Thus the extraction chamber is slowly flooded with solvent. When the solvent reaches the height of the siphon the chamber is emptied. This procedure is repeated several times.

3.4.3. Ultrasonic Treatment

Ultrasonic waves were discovered already in the 18th century. The application of ultrasonic waves is used for various applications. In nature, bats use ultrasonic waves to orient themselves. For medicinal applications ultrasound is used to examine unborn children. Ultrasonication is used for the disruption of cells in order to isolate the proteins produced by cells. For chemical applications ultrasound is often used to improve the dissolution of a substances in a solvent and also to degas solvents.

When ultrasound is passed into a liquid medium at high intensity, the waves cause high and low pressure cycles. During the low pressure phase small vacuum bubbles or voids are created. The bubbles grow until they cannot absorb any energy anymore. Thus the bubbles collapse by cavitation resulting in very high local heating and a pressure pulse. When ultrasound is used in liquids containing solids cavitation can occur at the probe surface. Then, due to the collapse of the cavities jets of liquid and associated shockwaves are driven into the surface. This results in substantial surface damage and exposure of fresh, highly-heated surface. Such conditions lead to the improved dissolution rates.[34]

3.5. Analysis Method

3.5.1. Gas Chromatography

Gas chromatography (GC) is a common method in analytical chemistry for analysing diverse compounds. The compounds in gaseous aggregate state or in solution are injected onto a column of various lengths and diameter. In general, a thin stationary phase inside the column is responsible for the properties of the column. Through the column a carrier gas is passed through with a constant flow. Furthermore, the column is placed in an oven whereas a temperature programme is applied. Thus the compounds are separated according to their volatility and secondly by their polarity. The separated analytes can then be detected by several different detectors. A well suited detector is the MS. Hyphenation with MS leads to a great gain of sensitivity and selectivity compared with other detectors.

Requirements for the separation of compounds by GC are thermal stability and volatility. Thus hexachlorocyclohexanes are well suited for the analysis by GC.[35]

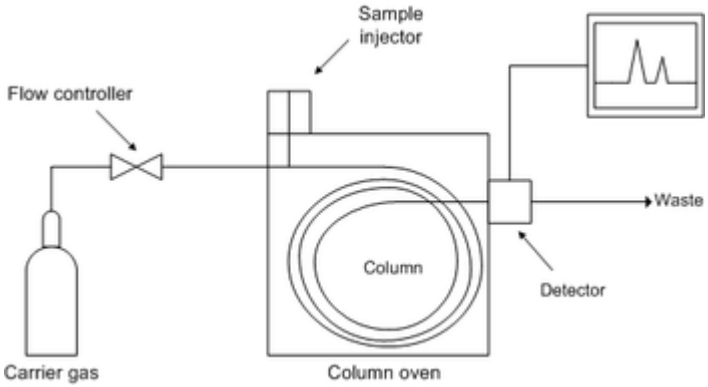


Figure 10. Schematic set-up of a GC.

In GC, the analyte is injected via injector onto the stationary phase. By passing through a flow of inert gas and a temperature program controlled by the oven, the analytes are separated on the column and detected.

3.5.2. Triple Quadrupole Mass Spectrometer

The triple quadrupole mass spectrometer, also known as QqQ, has become almost a standard analyser for mass spectroscopy. The QqQ consists of three quadrupoles in line, whereas several different analysis modes are possible.

Every quadrupole consist of pairs of opposite rods. The electric field generated by these rods, due to a voltage and a radio frequency voltage, is passed by the analyte, whereas only analytes with the adjacent m/z values have a stable trajectory and will reach the detector. By varying the electric field it can be determined which m/z ions can be detected.[36]

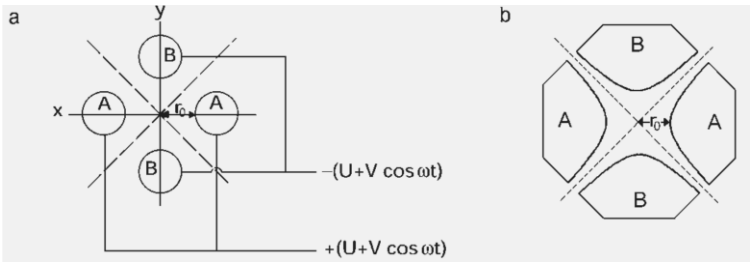


Figure 11. Scheme of the quadrupole poles.

By applying electrical fields between the rods, the passing of analytes with specific m/z values is regulated.[36]

The QqQ allows not only m/z determination, but can also conduct MS/MS. Therefore the middle quadrupole is used as collision cell leading to fragmentation of the analytes.

For the selected reaction monitoring (SRM) mode, various specific ions, selected by the first quadrupole, are fragmented in the collision cell looking for specific fragments in the third quadrupole. This method is well suited for quantification of compounds.[36]

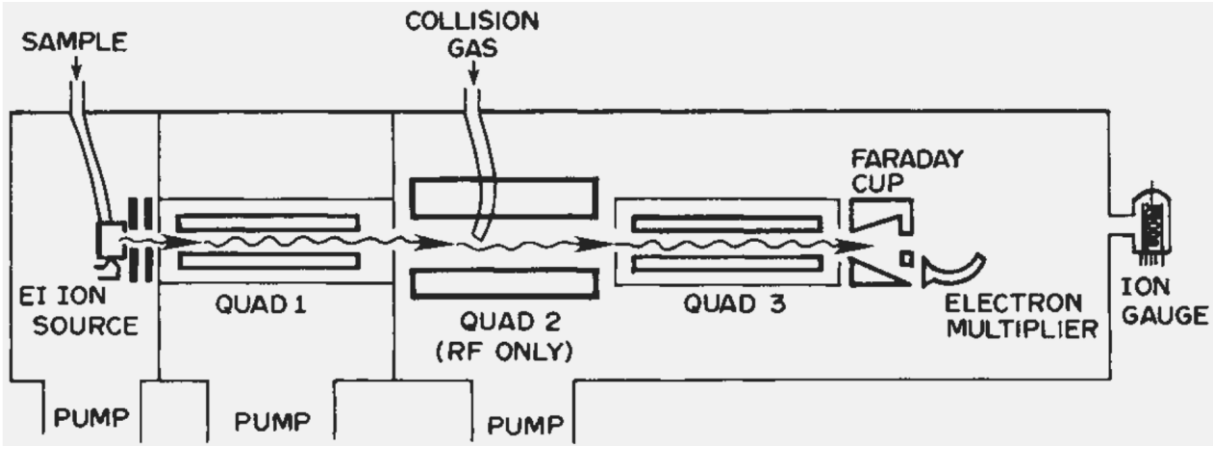


Figure 12. Principle of a QqQ.

The analytes have to pass the three quadrupoles in order to be detected. Further, the second quadruple may act as a collision cell in order to perform MS/MS experiments.[36]

4. Materials

4.1. Equipment

Drill sampler

Eijkelkamp, Edelman- Bohrer, Ton-Type Ø 7 cm

Core-sampler

Eijkelkamp, Split-Tube, Ø 5.3 cm

Infrared dryer

Satorius MA150

Used program: 4 to 6 g moist soil were drying at 105 °C until the loss is less than 5 mg/24 sec.

Ultrasound device

Hielscher UP100H

Specification: Sonotrode tip MS7 (max. amplitude 125 µm, sound density max 130 W/cm^2), 30 kHz, 100 Watt

ASE

Büchi Speed Extractor E-946

Extraction with Hexan: 1 min heating up, 8 min extraction at 130 °C and 103 bar, 1 min flushing with solvent and 2 min flushing with nitrogen. 3 cycles in total.

Extraction with Hexan/Aceton 1+1: 1 min heating up, 8 min extraction at 100 °C and 103 bar, 1 min flushing with solvent and 2 min flushing with nitrogen. 3 cycles in total.

Soxhlet

Büchi Extraction System B 811

Flushing with Aceton: 5 cycles, beaker heating level 11

Flushing with Hexan: 15 min, beaker heating level 10

Extraction with Hexan:

30 min beaker heating level 10 and top heating level 4

5 min beaker heating level 11, no top heating

Extraction with Hexan/Aceton 1+1

30 min beaker heating level 11 and top heating level 3

5 min beaker heating level 11, no top heating

Extraction hull

Macherey-Nagel, Extraktionshülsen MN645 33x94 mm, REF645022

Evaporation system

Büchi Syncore

Settings: 60 °C, min. pressure 200 mbar, 260 rpm

GC

Thermo Scientific, Trace GC Ultra

MS

Thermo Scientific, TSQ Quantum XLS

4.2. Chemicals

Hexan

Promochem, picograde, REF SO-1244-B025

Aceton

Merck, for analysis, REF 1.00014.2500

HCH

Sigma-Aldrich, REF 36756-250MG, Lot#SZBB045XV, alpha-HCH 24.6 %, beta-HCH 25.4 %, gamma-HCH 24.9 %, delta-HCH 24.7 %

Epsilon-HCH

Dr. Ehrenstorfer GmbH, REF LA1407500CY, Lot# 40308CY

Natural matrix certified reference material

PAHs, PCBs and Pesticides on Fresh Water Sediments, CRM CNS391- 50, Lot#011305, Resource Technology Corporation

Alpha-HCH D_6

Dr. Ehrenstorfer GmbH, REF XA1407140CY, Lot# 1124CY

Beta-HCH $^{13}C_6$

Cambridge Isotope Laboratories, Inc., REF CLM-3623-1.2, Lot#SDDF-012

Delta-HCH $^{13}C_6$

Cambridge Isotope Laboratories, Inc., REF CLM-3648-1.2, Lot#SDBG-005

2,2',4,5,5'-Pentachlorobiphenyl, PCB-101 $^{13}C_{12}$ 99%, EC-1405-1,2, Lot#10130-99-4, LGC Standards

5. Practical Procedure

In general a typical process of gathering data began by determining sampling areas according to the environmental pollution question. In a further step the samples were taken in sampling campaigns. These samples, usually several kilograms, were processed by removing stones, roots and visible organisms. The samples were sieved, homogenised and dried prior to extraction by the validated extraction method and analysis by GC-MS/MS.

5.1. Sampling Pattern

To assure the homogeneity of the samples five boreholes were combined to one sample. The pattern of the five boreholes was kept constant and has the same pattern as the five dots on a die – four at each corner and one in the middle.

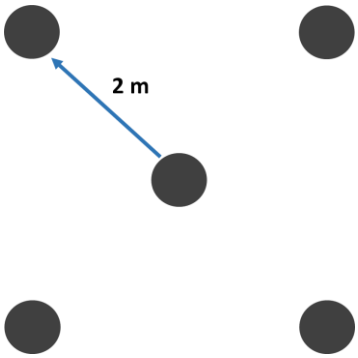


Figure 13. Sampling pattern.

Sampling of soils has occurred using a defined sampling pattern. The pattern corresponds to a five on die. Five boreholes with a defined distance to each other have been combined to one sample. Thus the heterogeneity of the soil has been averaged.

This pattern was applied to all areas except for the sampling area Uferstrasse, where the sampling area was not wide enough. Here samples were taken in a row each in two meters distance. The sampling of profiles has also occurred in the same manner.

5.2. Sampling Validation

5.2.1. Soil Sampling

The sampling of soils was carried out at previously carefully-chosen sampling locations dictated by the pedological considerations. In order to assure the homogeneity of the samples, five boreholes were combined to one sample. The borehole areas were marked and documented by taking pictures and GPS-data. Before sampling, the top grass was withdrawn without removing any soil. After sampling, the borehole was filled up with uncontaminated soil to reduce the impact of the sampling. In general the impact on the environment is very small

and hardly visible. Afterwards, the sampling equipment was cleaned using water and a brush followed by rinsing with isopropanol. Thus contamination of the following sampling areas was avoided. A first documentation of the soils was made at the sampling site: a more thorough documentation of the soil followed during the sieving process.

5.2.2. Sampling Precision

Multiple sampling of the same area was conducted in order to evaluate the sampling precision of the sampling areas and the total process. Therefore five boreholes, each 20 cm deep, were combined to one sample in the same manner as the described drill sampling. This procedure was carried out three times for each sampling area. Calculations of the standard error of means was done to compare different areas with each other. In total six sampling areas have been analysed in such manner. Further, for one sampling area (Neuerteich) a six-fold sampling was carried out, worked up and extracted each separately in order to obtain a relative standard deviation of the total process.

5.2.3. Sampling Robustness

Initially it was believed, that dust deposits could be washed down from trees resulting in local high pollution concentrations. Thus the influence of objects was investigated by sampling soil at a defined distance to the object in the direction of the source. Sampling locations were chosen in advance looking for large old trees beside an open field. In total five samples were extracted and analysed as shown in the Figure below.

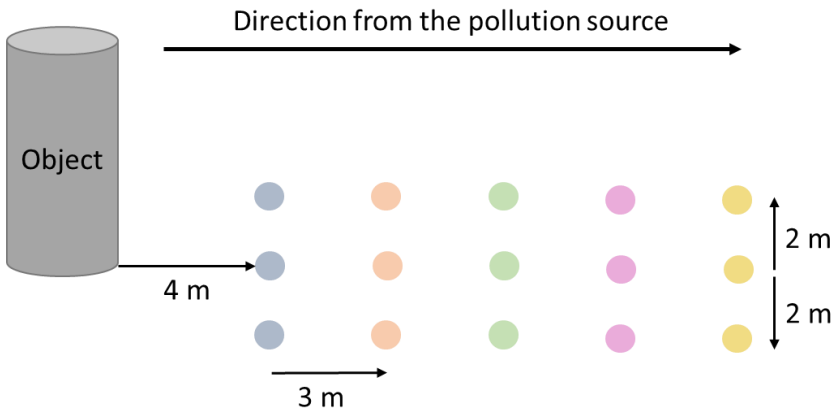


Figure 14. Sampling plan for the investigation of local inhomogeneities.

Sampling of soils close to objects have been taken in a defined procedure. Starting four meters from the object, every three meters samples have been taken to a total distance of 16 meters. Every sample is an average of three boreholes in two meters distance to each other and has occurred using a defined sampling pattern.

Sampling has started at 4 meters distance to the object (tree trunk). Every three meters a further sample has been extracted to a distance of 16 meters. Every sample was an average

of three borehole to a depth of 20 cm. A closer starting point would have been only partially feasible since large tress have large roots which could be damaged and result in inadequate sampling.

5.3. Determination of Sampling Areas

According to the environmental pollution question different sampling areas have been sampled. The influence of the main winds has been investigated as well as the origin of the pollution.

5.3.1. Influence of the Main Winds on the Pollution of Basel

It has been hypothesised, that the main winds have an influence on the pollution concentration in the city Basel and surroundings due to wind depositions. Samples in two rough circles around the ARA STEIH with different radius have been taken to assess the influence of the wind and the distance.

Sampling areas have been chosen in a rough circle with a large radius around the pollution source – the ARA STEIH. These areas of the surroundings of Basel are located in Germany, France and Switzerland (Canton Basel-Stadt and Basel-Landschaft). The main criterion on the choice of the sampling areas was that the soils were untouched since the 1960s. Trees, which make ploughing impossible since they are older than 50 years, and information from estate owners were good indicators.

In a second circle with smaller radius, predominantly the area of the city Basel, has been drawn. Perturbation of the soils in the city Basel could not be excluded for all the areas.

In general sampling has been carried out on state properties whereas in the city of Basel mostly park areas were affected.

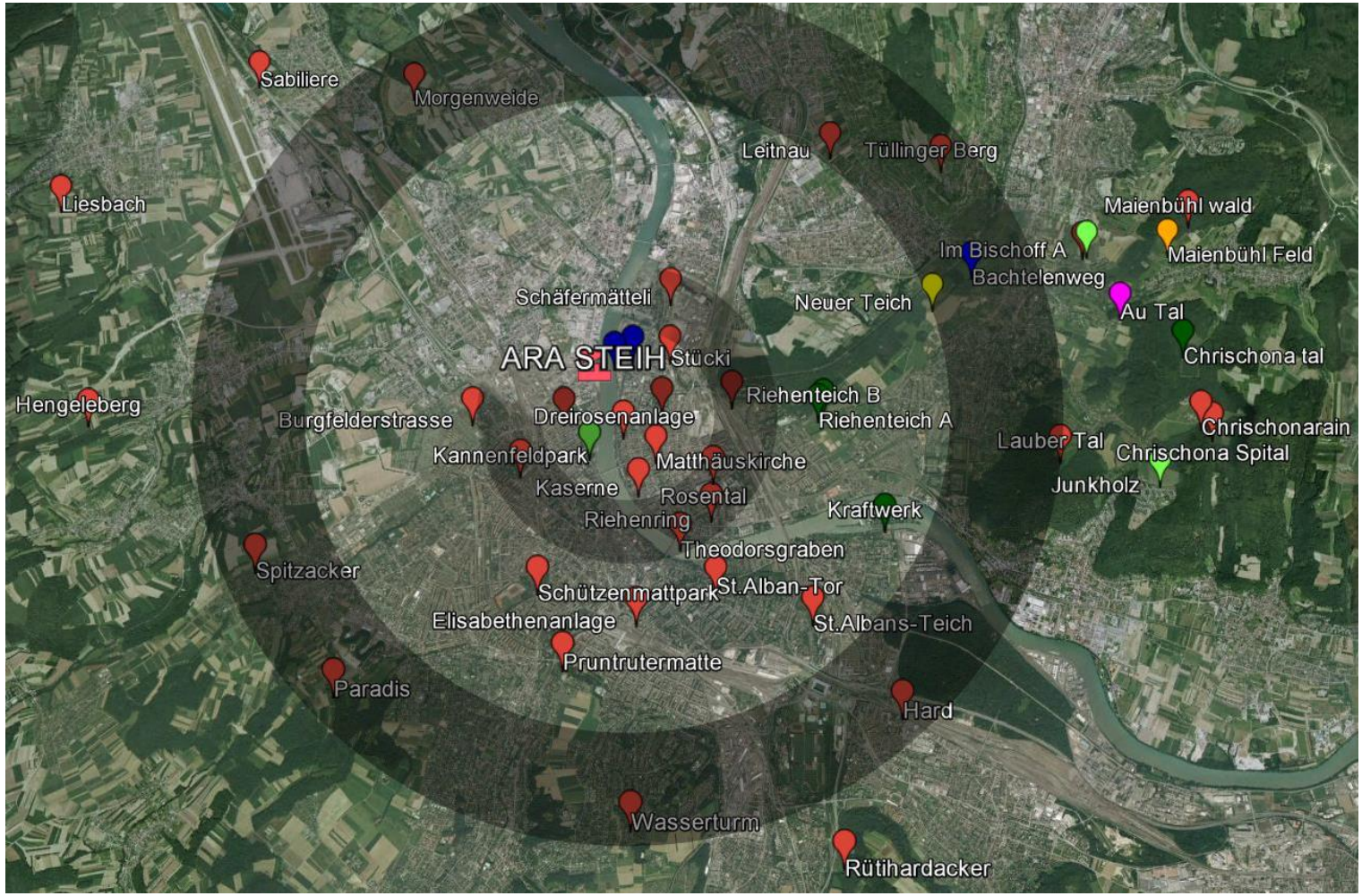


Figure 15. Sampling areas.

Samples have been taken in two rough circles. The circle with larger radius considers mostly the surroundings of Basel, whereas the smaller circle covers the city of Basel. The dark green coloured marked areas have been used for the evaluation of the robustness of the sampling. Bright green coloured areas mark the profiles which have been taken. The “Neuerteich (yellow) has been sampled six times for the validation of the sampling method. Blue Marked areas have been sampled three times investigating the homogeneity of the soils. The “Au Tal” (pink) has been sampled three times whereas one profile was taken. “Maienbühl Feld” (orange) has been sampled three times whereas one profile and also the investigation of the robustness has been carried out.

In addition, the prevailing wind directions were overlaid on the pollution source – the ARA STEIH. Thus probable sampling areas influenced by the wind could be identified.

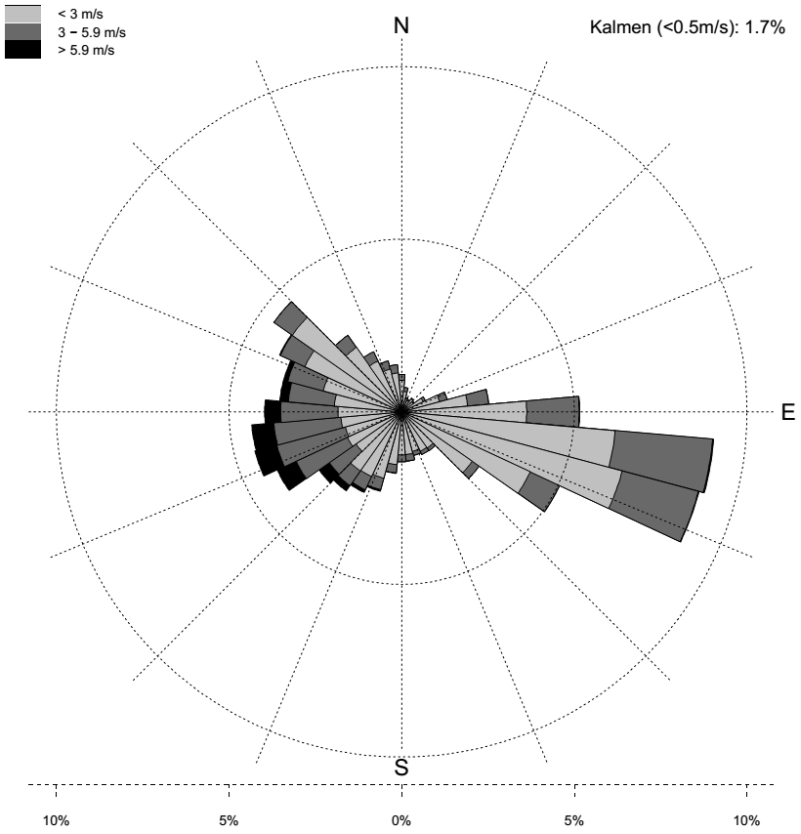


Figure 16. Wind rose of Basel - Binningen from 1981 to 2000.

The prevailing wind direction has been extracted from the wind rose of Basel – Binningen averaged from 1981 to 2000. The averaged wind direction has been further assumed for the total time period since the production of Lindane. The main wind directions have been used to assign sampling areas with respect to the wind influence.[37]

5.3.2. Profile Sampling

Sampling of profiles and also other data was used to determine the origins and the age of the pollution. Profiles were therefore used to investigate the pollution concentration of each isomer in 5 cm sections to a depth of 20 cm.

In total six profiles of unperturbed soils were sampled in the surroundings of Basel to a depth of 20 cm of which two were taken to a depth of 40 cm. The selection of areas for the profile sampling could not be planned in advance. Therefore, prior to the sampling of the profiles a test borehole was carried out using the drill sampler to investigate the soil properties. Three further profiles have been sampled in the city of Basel. However, perturbation of the soils in the city Basel could not be excluded.

5.4. Soil Sampling

Soils were sampled with two different samplers. In general a drill sampler has been used. However, for sampling of profiles a core sampler has been used.

5.4.1. Drill Sampling

Sampling with the drill sampler was the most used and preferred technique for the sampling of soils.

All soils were sampled to a depth of 20 cm, whereas the diameter of the borehole is roughly 8 cm. Soils containing large stones had an impact on the sample and the sample size. These stones lead to a widening of the borehole. When hitting a large stone during sampling, the object was remove using a small shovel. In general the sampling amount varies between 6 and 8 kg depending on the water and stone content. When sampling soils containing many stones less problems are encountered with the drill sampler than with a core sampler.

5.4.2. Profile Sampling

Sampling of profiles was conducted in the same manner as sampling with the drill except utilising a different sampler – a core sampler. Cores were taken by the pattern described in Section 5.1, at each corner of a four meter diagonal square and one in the middle boreholes were taken. However, profile sampling was difficult to achieve, since the soil composition is different for every sampling area. Thus usually a test sampling was carried out using the drill sampler. When the soil permitted such sampling, profiles were taken to a depth of 20 cm. In two specific cases it was possible to collect soil to a depth of 40 cm. The core was then divided into sections of 5 cm. This is an appropriate slice size, as cutting the slices is not always straight forward due to the presence of stones. The total slice was transferred to the storage glass, taking great care, not to contaminate the other slices with the previous slice residues. The equipment was washed with water using a brush, followed by rinsing with isopropanol after each borehole.

5.5. Sample Preparation

Soils from the sampling in the field have been processed to achieve a representative sample for the extraction. Therefore, stones and organic matter were removed from sampled soils followed by sieving through 5 and 2 mm sieves. The resulting soils were homogenised using a helical mixer for 3 min (1500 rpm). A subsample has been gathered by parting the evenly spread soil with an overlaid grid.

This subsample of soil was sieved through a 1 mm sieve and left to dry on an aluminium dish over night at ambient conditions. This resulted in a soil sample having a dry matter content of greater 95 %. The aluminium dishes were washed and heated over night at 400 °C before reusing.

A number of soils of different compositions were sieved: some featured a high loam content, others were rather sandy. The latter are simple to sieve, whereas loamy soils tend to be difficult. Loamy and very wet samples (> 80 % dry substance) were challenging. Nevertheless, all of them could be passed through the 5 mm sieve. However, some were hardly possible to pass

through the 2 mm sieve, and these were homogenised after the 5 mm sieve. A subsample was then passed through the 2 mm sieve and let to dry. The dry soils were then passed through the 1 mm sieve. Particles with greater diameter were crushed by using a mortar and pestle and then sieved.

Wet and loamy soils are difficult to homogenise. Since homogenisation using the drill was not possible, the soils were homogenised by hand mixing the soil for 7 min.

For the extraction of soils 10 g of dried soil (> 95 % dry substance) with particle sizes < 1 mm were required.

5.6. Evaluation of the Extraction Methods

Three different extraction methods have been evaluated for the purpose of the extraction of HCHs from soil.

5.6.1. General Remarks

Prior to this study, analysis of soil pollutants have already been reported by the Amt für Umwelt und Energie Basel-Stadt showing significant pollution. However, there was a need for more detailed investigations, resulting in this master thesis. Currently, hexachlorocyclohexanes are monitored by analysing air and dust deposition periodically. For this purpose, the analytical method has already been developed and optimised. Extraction methods for soils are yet to be evaluated.

In general, a number of different extraction methods have already been developed for the investigation of soils and sediments. Three commonly used methods are the accelerated solvent extraction, sonication and Soxhlet extraction. Further, these extraction techniques have been used for several different purposes with different solvents. For the evaluation of these three methods two different solvents have been used for the purposes of comparison: pure hexane and hexane/acetone (1:1).

5.6.2. Quality Control

It is to be noted, that the laboratory equipment used in this study has been carefully washed and baked overnight at 400 °C. Equipment which cannot be heated to such temperatures was washed carefully and rinsed with alcohol.

With every extraction campaign a recovery extraction was conducted using uncontaminated soil which had been doped with a known amount of HCH- isomers. In general a concentration of 10 µg/kg for alpha-, beta-, gamma-, and delta- HCH and 4 µg/kg for epsilon- HCH were applied for the hot Soxhlet extraction. Further, blank analysis have been carried out. The control of the analysis by GC-MS/MS has been done by the analysis of control standard solutions. The three different internal standards (ISTD) were added; before the Soxhlet extraction (ISTD alpha- HCH), before the concentration (ISTD beta- HCH) and before the analysis step (ISTD

delta- HCH). By comparing the ratios of the internal standards to each other errors in handling could be detected.

5.6.3. Accelerated Solvent Extraction

General procedure

Prior to the extraction it is necessary to flush the ASE with solvent, followed by carrying out a leak test. 10 g of dried soil (>95 % dry soil) were treated with a defined volume of hexachlorocyclohexane isomer mixture (alpha- HCH 24.6 %, beta- HCH 25.4 %, gamma- HCH 24.9 %, delta- HCH 24.7 %) and placed in 100 ml glass beakers. 25 µl of a 2 ng/µl solution of ISTD alpha- HCH in acetone were added. The solvent was left to evaporate. Diatomaceous earth was added to a volume of roughly 40 ml. The two solids were then mixed well. The sample was transferred into the ASE extraction flasks and was extracted by the corresponding programme. To the first extract, 25 µl of a 2 ng/µl solution of ISTD beta- HCH in acetone were added. The fractions were concentrated to a volume of 2 ml by vacuum distillation at 60 °C. The fractions were combined, filtered over glass wool and three times washed with solvent. The solvent was removed to a volume of 0.5 ml by blowing off using a constant nitrogen flow. The glass tube walls were washed with hexane and the solution concentrated down. The solution was then diluted to a volume of 1 ml and transferred to a vial. The samples were then analysed by GC-MS/MS.

For evaluation purposes the fractions were analysed separately doping each fraction with ISTD-beta- HCH.

Extraction programme

Hexane: 1 min heating up, 8 min extraction at 130 °C and 103 bar, 1 min flushing with solvent and 2 min flushing with nitrogen. This cycle was repeated three times in total.

Hexane/acetone (1:1): 1 min heating up, 8 min extraction at 100 °C and 103 bar, 1 min flushing with solvent and 2 min flushing with nitrogen. This cycle was repeated three times in total.

5.6.4. Sonication Extraction

General procedure

5 g of dried soil (95 % dry soil) were placed in a robust 40 ml glass flask and 25 µl of a 2 ng/µl solution of ISTD alpha-HCH in acetone were added. The solvent was left to evaporate. 25 ml solvent were added followed by sonication of the suspension for 40 seconds at (100 Watt). The suspension was centrifuged at 1500 rpm for 8 min. The supernatant was then collected. This cycle was repeated in total three times with the remaining soil. The fractions were combined, 25 µl of a 2 ng/µl solution of ISTD beta- HCH in acetone were added and concentrated to a volume of 2 ml by vacuum distillation at 60 °C. Filtration over sodium sulphate and washing three times with solvent followed. The solvent was removed to a volume of 0.5 ml by blowing

off using a constant nitrogen flow. The glass tube walls were washed with hexane and followed by concentration of the solution. The solution was diluted to a volume of 1 ml and was transferred to a vial. The samples were then analysed by GC-MS/MS.
For evaluation purposes number of cycles was increased to four in order to study the extraction progress. Furthermore, the fractions were analysed separately and thus each fraction was doped with ISTD beta- HCH.

5.6.5. Soxhlet Extraction

General procedure

10 g of dried soil (95 % dry soil) placed in a Soxhlet thimble. 5 µl of a 2 ng/µl solution of ISTD alpha- HCH in acetone were added. The solvent was left to evaporate. The extraction thimble was inserted into the Büchi apparatus and was extracted by the corresponding programme. To the resulting solution 5 µl of a 2 ng/µl solution of ISTD beta- HCH in acetone were added. The solution was concentrated to a volume of 2 ml by vacuum distillation at 60 °C, dried over sodium sulphate, followed by a filtration over glass wool and three times washing with hexane. The solvent was removed to a volume of 100 µl by blowing off using a constant nitrogen flow. The glass tube walls were washed with hexane. The solution first concentrated to 100 µl, 5 µl of a 2 ng/µl solution of ISTD delta- HCH in acetone were added followed by the dilution to a volume of 0.2 ml. The samples were then analysed by GC-MS/MS.
During the experimental phase the extraction time has been set to 2 h and the end volume was set to 1 ml (volume of internal standards was adjusted using 25 µl). Furthermore the ISTD delta- HCH was not available initially and was introduced later.
For evaluation purposes, the extraction time has been increased from 30 min to 1 h, 2 h and 4 h, with separate collection and analysis of the fractions.
In a later stage of the study the analysis of further pollutants in soils required 5 µl of a 1 ng/µl solution ISTD PCB-101 in acetone, which was applied simultaneously with the ISTD alpha-HCH.

Extraction programme using the Büchi extraction system (Soxhlet)

Hexane: 30 min extraction, beaker heating level 10, extraction chamber heating level 4 followed by 5 min flushing.
Hexane/acetone (1:1): 30 min extraction, beaker heating level 11, extraction chamber heating level 3 followed by 5 min flushing.

5.6.6. Evaluation Process of the Eextraction Methods

For the evaluation of the extraction methods described above, attention was paid to the following parameters.

Blank control of the extraction method

In a first evaluation process, the blank values were compared with each other. Therefore extraction of uncontaminated soil was carried out with the three above-mentioned methods and for each solvent system separately and in duplicate. For this purpose three cycles were combined to one sample for the extraction by Sonication and by ASE. For the evaluation by hot Soxhlet the extraction time was set to 2 h (which is equal to 25 depletions of the Soxhlet chamber).

Extraction efficiency

The uncontaminated soil was doped with 1000 µl of a 100 ng/µl HCH- solution in acetone (alpha- HCH 24.6 %, beta- HCH 25.4 %, gamma- HCH 24.9 %, delta- HCH 24.7 %). The solvent left to evaporate for 20 min followed by the addition of the internal standard alpha-HCH

This procedure was conducted in duplicate for each solvent and each extraction method.

5.7. Extraction Method Validation

The results of the evaluation of the extraction methods shows that the extraction by hot Soxhlet is the best suited for the purpose. The validation was made by using the hot Soxhlet extraction method as described in Section 5.6.4. For the validation purpose and further analysis, the extraction method parameters remained unchanged. Thus 10 g of dried soil were extracted for 30 min with hexane by hot Soxhlet, followed by 5 min flushing of the system.

5.7.1. Precision

The precision was obtained by extracting 5 µg/kg alpha-, beta-, gamma-, and delta- HCH and 10 µg/kg epsilon- HCH doped uncontaminated soil six times.

5.7.2. Recovery Rates

The recovery rates were determined by averaging the six-fold extraction described in section 5.7.1.

5.7.3. Robustness of Critical Steps

Critical steps of the extraction method and analysis have been explored.

Drying of the homogenised soils

The drying process of the wet soils was investigated by carrying out several experiments. First results were obtained from drying at 40 °C, as recommended in the literature, in an oven on glass and aluminium plates by doping uncontaminated soil and wetting this soil with 2 ml water (similar to the dry substance of the sampled soils). Based on the results, experiments were

made at ambient temperature in the same manner with soil samples which were dried in glass and aluminium dishes. All experiments were carried out at least twice. The final loss of contaminants by drying was averaged over six-fold drying.

Concentration of the solution by a nitrogen flow

The influence of the solvent removal using a constant nitrogen flow was investigated. Solutions containing the reference HCH- isomers and the three internal standards were concentrated using a constant nitrogen flow to defined volumes (0.2 ml, 0.1 ml and 0.05 ml). They were then analysed and compared with a reference solution not concentrated by a constant nitrogen flow.

Injection precision of the analysis

The injection precision of the GC-MS/MS analysis has been investigated by injecting an extraction sample with a 500 µg/kg solution of alpha-, beta- gamma- and delta- HCH and 40 µg/kg epsilon- HCH ten times. The calculation of the relative standard deviation for all of the isomers lead to the injection precision.

5.7.4. Accuracy

The accuracy of the extraction methods was investigated by extracting a reference material with a known content of hexachlorocyclohexanes. The reference material was extracted and analysed in duplicate. Furthermore, some samples were analysed externally by Heppeler GmbH and Bachema AG to prove the results and the accuracy.

5.7.5. Overall Process Linearity

For the investigation of the linearity, uncontaminated soil has been doped with a known concentration of HCH- isomers. Concentrations 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 50, 100, 500 and 1000 µg/kg were investigated. Except for epsilon- HCH concentrations up to 10 µg/kg were evaluated, since the expected contamination range has been estimated to be lower in natural soils. The concentrations 500 and 1000 µg/kg lie above the analytical linearity necessitating dilution of the samples.

5.8. Analysis

The quantitative analysis was carried out with a single GC-MS/MS method. All samples were prepared in the same manner as described earlier on. The identification of the analytes was done according to the retention times (± 1% of control standard) and their masses.
The quantifier ion was confirmed by several qualifier ions and their ratios (ratios originate from the control standard analysis).

5.8.1. Analysis of HCH

GC-conditions

Column: Phenomenex ZB-M µl tiresidue-2

Column length	30 m
Internal diameter	0.32 mm
Film thickness	0.25 µm
Max. temperature	340 °C
Injection volume	3.0 µl, split-less
Injector temperature	0.2 min 50 °C, then 8 °C/s to 310°C
Flow	1.3 ml/min helium

Table 4. GC oven temperature programme.

Time [min]	Temperature [°C]
0	110
4	110
6.5	160
16.5	260
18.2	310
20.2	310
20.2	310

The temperature programme of the GC-analysis has started at 110 °C increasing the temperature in several temperature ramps to 310 °C.

Ionisation

Ionisation	Electron ionisation
Electron energy	-70.0 eV, positive mode
Emission current	25 µA
Transfer to MS	280 °C
Source temperature	250 °C

MS

SRM mode	
Chrom. peak width	6.0 s
Collision gas	Argon
Collision gas pressure	1.5 mTorr
Collision energy	15 V
Cycle time	0.4 s

Table 5. SRM set-up for HCHs.

Parent [m/z]	Product [m/z]	CE	Start [min]	Stop [min]	Reference
185.010	150.080	20	8.50	16.50	ISTD alpha HCH D_6 [b]
222.050	148.040	20	8.50	16.50	ISTD alpha HCH D_6 [b]
224.040	150.010	20	8.50	16.50	ISTD alpha HCH D_6 [a]
186.910	151.010	25	10.00	18.00	ISTD beta HCH $^{13}C_6$ [b]
188.910	153.010	20	10.00	18.00	ISTD beta HCH $^{13}C_6$ [a]
222.930	151.020	20	10.00	18.00	ISTD beta HCH $^{13}C_6$ [b]
224.930	153.100	20	10.00	18.00	ISTD beta HCH $^{13}C_6$ [b]
186.910	151.010	25	10.00	18.00	ISTD delta HCH $^{13}C_6$ [b]
188.910	153.010	20	10.00	18.00	ISTD delta HCH $^{13}C_6$ [a]
222.930	151.020	20	10.00	18.00	ISTD delta HCH $^{13}C_6$ [b]
224.930	153.100	20	10.00	18.00	ISTD delta HCH $^{13}C_6$ [b]
180.970	144.970	15	9.50	16.50	HCH-isomers [a]
182.980	148.180	15	9.50	16.50	HCH-isomers [b]
219.040	145.960	15	9.50	16.50	HCH-isomers [b]

[a] Quantifier ions. [b] Qualifier ions.

The analysis of HCH has been carried out using the SRM mode. Thus a number of parent ions were disintegrated in the collision cell of the MS resulting in various product ions. Comparing the ion ratio of the product ions leads to very selective quantification of the analytes.

5.8.2. Analysis of Further Organochlorine Substances

Further organochlorine substances were semi-quantitatively determined by using the same vials and solutions as used for the analysis of the HCH- isomers. The identification of the analytes was done according to the retention times (± 1% of control standard) and their masses. The quantifier ion was confirmed by several qualifier ions and their ratios (ratios originate from the control standard analysis).

The following analytes were analysed:

1,3,5-Trichlorbenzol

1,2,4-Trichlorbenzol

1,2,3-Trichlorbenzol

Hexachlorobenzene

PCB-28

PCB-52

PCB-101

PCB-138

PCB-153

PCB-180

2,4'-DDT

4,4'-DDT

Aldrin

In a first extraction campaign investigating the influence of the main wind direction on the concentration of HCH- isomers in the soil, the samples have not been doped with the PCB internal standard. An estimation of the content of the various OCl was made by using the internal standard alpha- HCH for the quantification. For further analysis, 25 µg/ml internal standard PCB-101 was used. Thus the PCBs were then estimated using the PCB-101 internal standard. This was accomplished by doping the dry soils with 5 µl PCB-101 internal standard (1 ng/µl) simultaneously with the internal standard alpha- HCH.

GC-conditions

Column: Phenomenex ZB-M µl tiresidue-2

Column length	30 m
Internal diameter	0.32 mm
Film thickness	0.25 µm
Max. temperature	340 °C
Injection volume	3.0 µl, split-less
Injector temperature	0.2 min 50 °C, than 8 °C/s to 310°C
Flow	1.3 ml/min helium

Table 6. GC oven temperature programme of the HCH analysis.

Time [min]	Temperature [°C]
0	60
4	60
16.5	160
45.5	220
51.5	280
52.5	280
53.5	310
58.5	310

The temperature programme of the GC-analysis has started at 60 °C increasing the temperature in several temperature ramps to 310 °C.

Ionisation

Ionisation	Electron ionisation
Electron energy	-70.0 eV, positive mode
Emission current	50 µA
Transfer to MS	280 °C
Source temperature	250 °C

MS

SRM mode	
Chrom. peak width	6.0 s
Collision gas	Argon
Collision gas pressure	1.5 mTorr
Collision energy	15 V
Cycle time	0.4 s

Table 7. SRM set-up further OCl analysis.

Parent [m/z]	Product [m/z]	CE	Start [min]	Stop [min]	Reference
179.90	108.98	25	8.55	12.55	Trichlorobenzene [a]
179.90	145.00	25	8.55	12.55	Trichlorobenzene [b]
181.90	108.97	25	8.55	12.55	Trichlorobenzene [b]
181.90	146.97	25	8.55	12.55	Trichlorobenzene [b]
180.97	144.97	15	20.15	32.15	HCH [a]
182.98	148.18	15	20.15	32.15	HCH [b]
219.04	145.96	15	20.15	32.15	HCH [b]
283.94	249.09	25	20.25	24.25	Hexachlorobenzene [b]
285.93	213.98	25	20.25	24.25	Hexachlorobenzene [a]
285.93	251.08	25	20.25	24.25	Hexachlorobenzene [b]
185.01	150.08	20	20.46	24.46	ISTD alpha-HCH D_6 [b]
222.05	148.04	20	20.46	24.46	ISTD alpha-HCH D_6 [b]
224.04	150.01	20	20.46	24.46	ISTD alpha-HCH D_6 [a]
186.91	151.01	20	25.54	30.54	ISTD beta-HCH $^{13}C_6$ [b]
188.91	153.01	20	25.54	30.54	ISTD beta-HCH $^{13}C_6$ [a]
222.93	151.02	20	25.54	30.54	ISTD beta-HCH $^{13}C_6$ [b]
224.93	153.10	20	25.54	30.54	ISTD beta-HCH $^{13}C_6$ [b]
186.07	151.11	25	26.50	29.50	PCB-28 [b]
256.03	186.11	20	26.50	29.50	PCB-28 [a]
258.09	186.15	20	26.50	29.50	PCB-28 [b]
260.95	191.05	25	27.36	31.36	Aldrin [b]
262.93	193.09	25	27.36	31.36	Aldrin [b]
293.05	186.18	25	27.36	31.36	Aldrin [b]
220.10	185.10	20	28.13	32.13	PCB-52 [b]
255.03	220.20	20	28.13	32.13	PCB-52 [a]
292.06	222.12	20	28.13	32.13	PCB-52 [b]
254.05	184.07	20	34.80	38.80	PCB-101 [b]
326.01	256.17	20	34.80	38.80	PCB-101 [a]
326.01	291.05	15	34.80	38.80	PCB-101 [b]
266.09	196.15	25	35.78	37.78	ISTD PCB -101 $^{13}C_{12}$ [b]
338.05	266.21	25	35.78	37.78	ISTD PCB-101 $^{13}C_{12}$ [b]

338.05	268.21	25	35.78	37.78	ISTD PCB-101 $^{13}C_{12}$ [a]
164.79	115.09	20	42.00	50.00	DDT [b]
198.95	163.38	20	42.00	50.00	DDT [b]
235.15	165.15	20	42.00	50.00	DDT [a]
237.15	165.15	20	42.00	50.00	DDT [b]
237.15	199.13	20	42.00	50.00	DDT [b]
290.01	220.26	25	43.00	50.00	PCB-138\153 [b]
359.99	290.10	25	43.00	50.00	PCB-138\153 [a]
362.02	292.16	25	43.00	50.00	PCB-138\153 [b]
323.78	254.11	30	48.99	50.99	PCB-180 [b]
293.74	324.08	25	48.99	50.99	PCB-180 [a]
395.73	324.07	25	48.99	50.99	PCB-180 [b]
395.73	326.07	25	48.99	50.99	PCB-180 [b]

[a] Quantifier ions
[b] Qualifier ions

The analysis of many organochlorine pollutants has been carried out using the SRM mode. Thus a number of parent ions were disintegrated in the collision cell of the MS resulting in various product ions. Comparing the ion ratio of the product ions leads to very selective quantification of the analytes.

5.9. Mobilities of HCHs

Profile analysis show the distribution of the HCHs due to various natural influences in the natural environment. A laboratory experiment has been carried out to investigate the mobilities of the HCHs by simulated rain fall. Therefore a column test has been carried out similar to a lysimeter experiment.

5.9.1. Column Test Accomplishment

Essentially the column test is similar to a solid-phase, column chromatography. However, instead of using silica, soil is utilised. Furthermore, the flow of the mobile phase runs in the opposite direction, from the bottom of the column to the top. As mobile phase, deionised water with 10 % Evian was used. The stationary phase (soil) had been previously freed of stones, roots and had been sieved through a 2 mm sieve. The soil was used moist, with the same moisture content, as the original sampling (78.5 % dry substance).

Alternate layers of sand and soil were used to fill the column with a diameter of 10 cm. Since the column is rather long, the lower part of the column was filled with sand. Then a very thin layer of doped soil was applied followed by 5 cm earth. A 3 cm thick layer of sand was added between the following 5 cm earth. This was repeated until in total of 20 cm of earth had been added. The top layer was then covered with sand again.

All the masses and volumes were carefully noted for the calculations of the mass balance at the end.

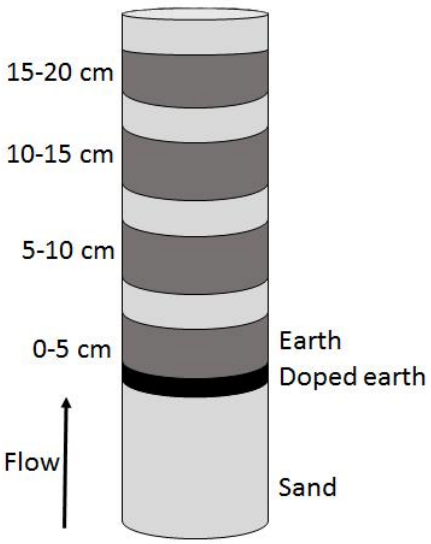


Figure 17. Schematic set up of the column test.

The bottom of the column has been filled with sand to reduce the dead volume. Alternating 5 cm earth and 3 cm sand have been inserted and compacted slightly. Between the sand and the first earth layer, a small amount of doped earth was introduced. The column experiment has been carried out by passing through the water from the bottom to the top.

Progressive filling of the column has occurred by compacting the soil or sand every 3 cm gently by mechanical impact and eluted with mobile phase with a flow of 2.2 ml/min before continuing with the next layer. After successfully loading the column, it was left to rest for 16 h. The elution of the column was carried out using a HPLC pump with a flow of 3.5 ml/min. The fractions were collected in 0.9 l fractions. Since the elution took several days, the flow was reduced overnight. The volume used to elute the column was calculated as noted in the experimental specification. The volume is six times the mass of the moist soil applied. For this experiment 12 l mobile phase were used. This is roughly the average yearly rainfall in Basel since 1960 on this area (12.8 l/year). After completion of the chromatographic process, the column was left to drain overnight. From top down, the sand and soil sections were carefully reclaimed. Due to the experimental set up, contamination of the soil sections could be avoided.

5.9.2. Mobile Phase Analysis

The mobile phase was collected in fractions of 0.9 l and extracted by liquid liquid extraction with hexane. 5 µl ISTD alpha- and beta- HCH (10 ng/µl) were added to the fractions from the column test. 10 ml hexane were added and extracted for 1 h by stirring vigorously. The organic layer was then removed, dried over dried sodium sulphate, washed twice with 2 ml hexane and concentrated under a constant nitrogen flow to a volume of 0.2 ml. The tube walls were washed with hexane before adding ISTD delta-HCH (10 ng/µl) and transferring the 1.0 ml solution to a GC vial. The analysis was carried out by the same method as the soil samples.
Furthermore, a blank and a recovery rate (10 µl 10 µg/µl alpha-, beta-, gamma-, and delta-HCH in acetone doped 900 ml blank mobile phase) extraction were made in the same manner in order to assure the accuracy.

5.9.3. Stationary Phase Analysis

The stationary phase was carefully reclaimed after completion of the experiment and the sections of soil and sand were kept separate. The sand fractions were vigorously homogenised and dried. The extraction of a 10 g subsample was made in the same manner as in the validated method. Homogenisation of the soil samples could not be achieved in the same manner, since they were too wet. Thus, the soil samples were homogenised by kneading. A subsample was sieved as well as possible through a 2 mm sieve, followed by drying overnight at ambient temperature, milling and sieving through a 1 mm sieve. 10 g soil was then extracted by the validated method and analysed.

5.10. Statistical Analysis

Statistical analysis of the data has been carried out using the “R-studio” software. For the analysis of the main wind direction influencing the pollution of the soils in the region of Basel and Basel-city, the sampling areas have been assigned to two categories: in the direction of the prevailing winds coming from the ARA STEIH and areas not in the main wind direction.
A second statistical analysis has been carried out, using a larger number of sampling areas potentially influenced by the wind. The latter analysis has the advantage of taking into account the fluctuations in wind direction and intensity.
Influences on the pollution concentration were assessed, not only for the sum of all isomers, but also for each isomer by itself and the ratios of the isomers relative to each other.
The data has been tested for normality followed by testing for equal variances. Thus the appropriate statistical calculations were applied to test whether there is a difference between the two groups. In Figure 18, the appropriate methods are listed for each statistical problem. However, the Brunner-Dette-Munk method did not have to be applied. All data and statistical operations and results have been enclosed on the data CD of this master thesis.

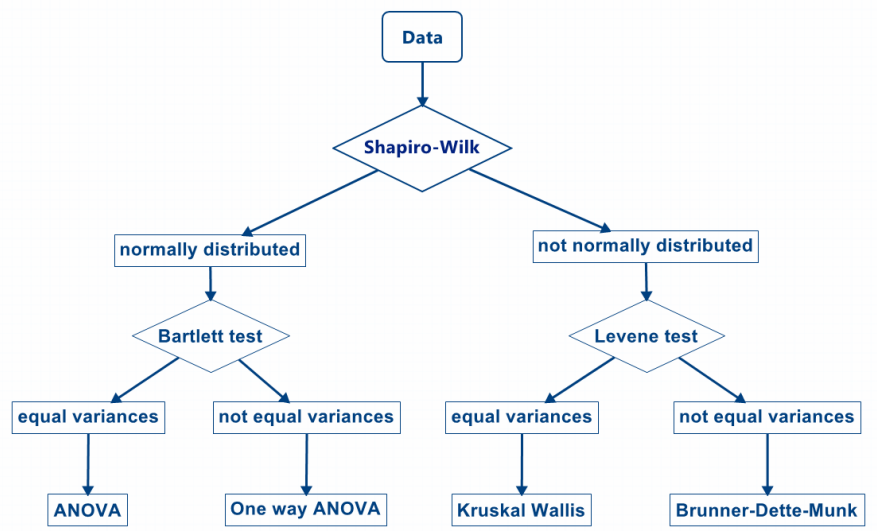


Figure 18. Statistical analytical methods.

The statistical analysis has been carried out using the software “R-studio”. Tests on normality and equal variances of the data were required prior to statistical analysis. Depending on the nature of the data the appropriate statistical tools were used. However, the Brunner-Dette-Munk analysis did not have to be applied.

Profiles were initially analysed using a regression analysis. Since results were not conclusive the slices were treated as groups. The assignment of the data to different categories was done on the basis of the sample depth. Differences between the categories were analysed in the same manner as the analysis of the prevailing wind direction.

The regression analysis was done using the Shapiro-Wilk test for the analysis for normality of the data, followed by the Breusch-Pagan test (ncv for R) for testing for equal variances.

The analysis of the data using “R-studio” was made using all the data, even though some data points were below the lower limit of quantification. The genuineness of the data points below the limit of quantification was confirmed by the confirming ions and the ratio of the ions to the analysed ion using GC-MS/MS.

6. Results and Discussion

6.1. Evaluation of the Extraction Methods

6.1.1. Blank Analysis

Extraction of an uncontaminated soil sample was made in duplicate for each method and each solvent in order to evaluate the extraction methods.

The analysis revealed that in none of these experiments HCH- isomers were present. Thus all materials and solvents used for the extractions were not contaminated and furthermore no interferences were observed. Graphically it was noticed that extraction using hexane/acetone (1:1) lead to more intense yellow colouring than when hexane is used on its own. The observation was confirmed by looking at the blank chromatograms, which showed more noise and disturbance.

6.1.2. Extraction Recovery

In the second part, the extraction efficiency was investigated by extracting a doped material in several consecutive extraction passes. The extraction cycles for ASE and sonication were collected separately. For the extraction by hot Soxhlet, the time was increased using the same extraction material. For each pass, fresh solvent was used.

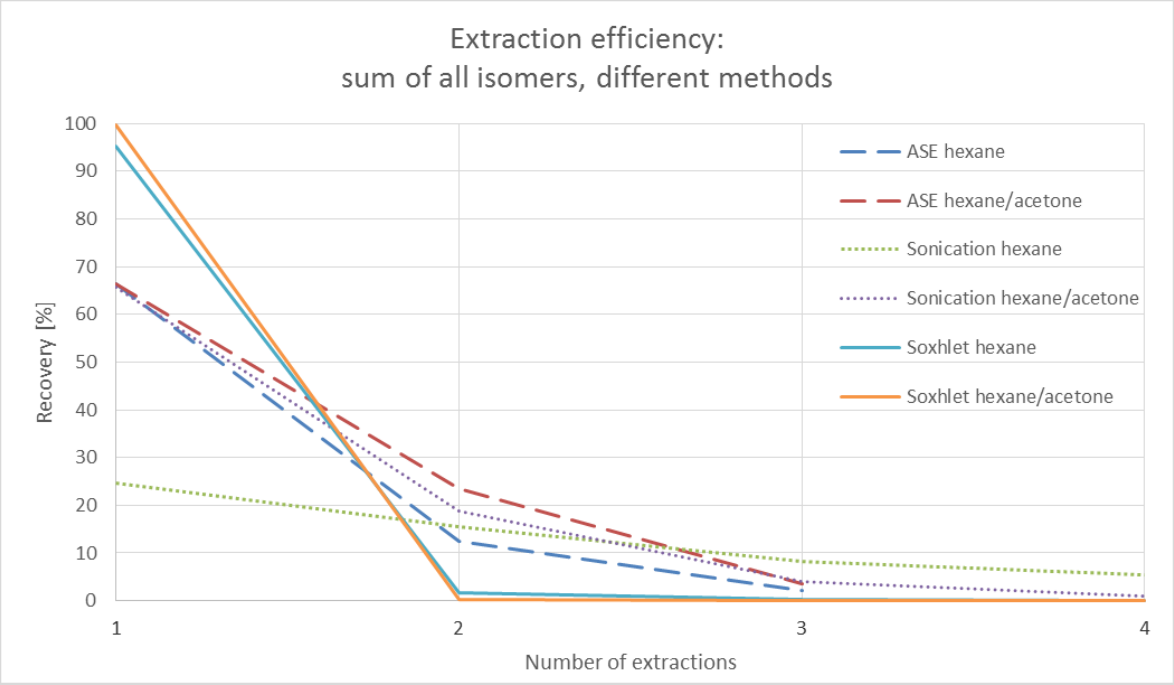


Figure 19. Extraction efficiency of the sum of all HCH by various methods.

Figure 19 illustrates the extraction efficiency of the three different methods: ASE, sonication and hot Soxhlet. The curves obtained for different solvents, such as hexane and hexane/acetone (1:1) are shown. The data are averaged from two extractions.

From Figure 19 it can be seen that the extraction recovery of the hot Soxhlet method, independent of the solvent used, is roughly 100 per cent after the first cycle. This corresponds to 30 min of extraction. Further extraction of the same sample leads to no additional extraction of HCH. Thus total extraction has accomplished after 30 min.

The efficiency of the ASE method is lower than the hot Soxhlet method, whereas after three cycles, most of the HCH- isomers have been extracted. However, evaluation of the three cycles leads to a total extraction efficiency of 81 % for hexane and 93 % for hexane/acetone (1:1). Using the latter solvent mixture, this extraction efficiency would be sufficient. If pure hexane were to be used, at least a further extraction cycle would be necessary. The efficiency might have been improved by other parameters than the cycle numbers. Since the pressure at the extraction temperatures used was already very high, the extraction cycle time might have been a parameter to increase in order to achieve better results.

Extraction assisted by sonication resulted in even greater differences between the solvents than for the ASE method. The extraction efficiency using hexane as solvent leads to poor results even after four cycles (54 %); whereas the solvent mixture hexane/acetone (1:1) leads to an efficiency of 89 %. The poor efficiency, high extraction cycle numbers and the tedious handling leads to the conclusion, that for this purpose the extraction method by sonication is less suitable. Although, the extraction time could have been prolonged, the method handling turned out to be not appropriate.

Figure 19 illustrates the total extraction efficiency of all isomers as a function of time. Figure 20 and Figure 21 illustrate the extraction efficiency of each isomer for the hot Soxhlet extraction.

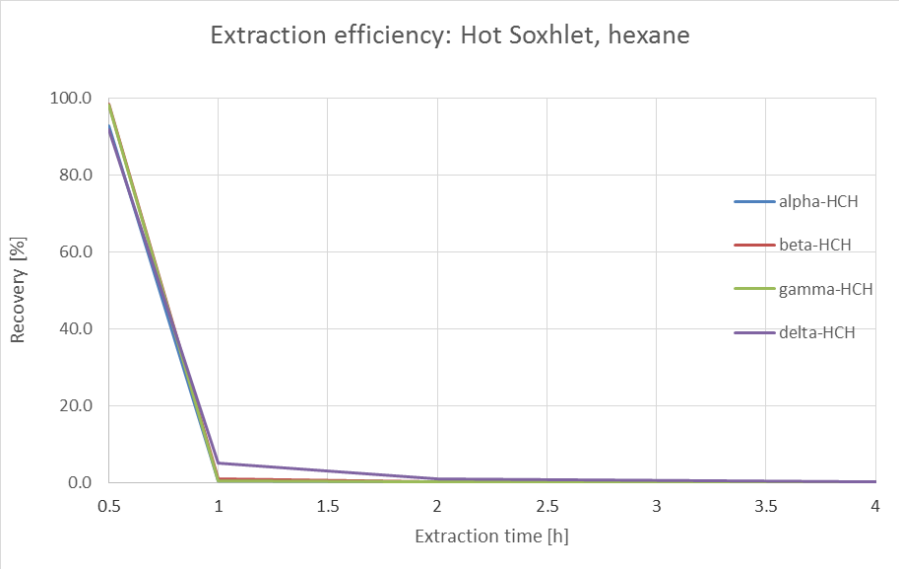


Figure 20. Hot Soxhlet extraction efficiency using hexane.

Extraction efficiency using the hot Soxhlet method for 30 min, 1 h, 2 h and 4 h in total using hexane as solvent resulted in total extraction after 30 min extraction. Additional extraction time did not lead to significant extraction of further HCH.

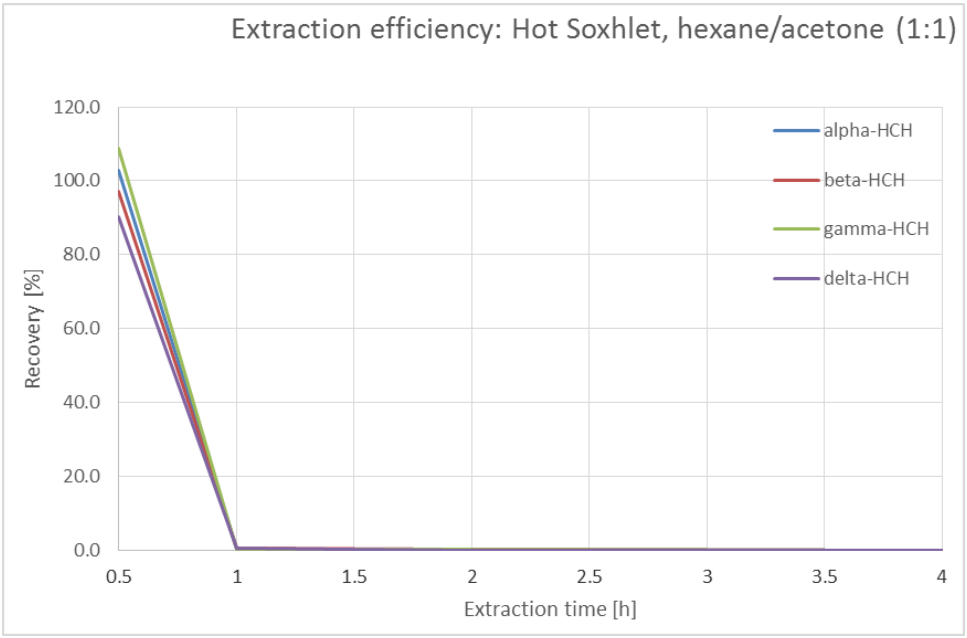


Figure 21. Hot Soxhlet extraction efficiency using hexane/acetone (1:1).

Extraction efficiency using the hot Soxhlet method for 30 min, 1 h, 2 h and 4 h in total using hexane/acetone (1:1) as solvent resulted in total extraction after 30 min extraction. Additional extraction time did not lead to significant extraction of further HCH.

Both Figure 20 and Figure 21 show that after 30 min total extraction of all the tested isomers has been achieved. The extraction using hexane leads to slightly lower alpha- and delta-HCH isomer concentrations than expected.

Table 8 shows similar results. However, the efficiency of extracting alpha-HCH is increased by only 0.5 % when the period of extraction is extended to 3.5 h. However, the amount of delta-HCH extracted is increased by this prolongation.

Table 8. Hot Soxhlet extraction efficiency using hexane.

extraction time	alpha-HCH [%]	beta-HCH [%]	gamma-HCH [%]	delta-HCH [%]	Sum of HCH [%]
0.5 h	92.9	98.5	98.1	91.7	95.3
1 h	0.4	1.0	0.5	5.1	1.7
2 h	0.1	0.1	0.1	0.9	0.3
4 h	0.1	0.1	0.1	0.2	0.1
sum	93.4	99.6	98.7	97.9	97.4

The extraction efficiency of the HCH- isomers in the soils has been investigated by prolonging the extraction up to 4 h. A total extraction has been reported after 30 min hot Soxhlet. Only a slight increase of delta HCH- was observed extracting additional 30 min.

Predefined attributes were rated in order to help decide which extraction method is to be validated. Thus the most important attribute is the extraction efficiency, which has been described previously. In conclusion, the efficiency and recovery rate of the hot Soxhlet method is remarkably good, closely followed by the ASE method. The sonication method efficiency was very

poor. It might have been improved by increasing even more handling steps, which was not desired.

The handling effort of the ASE and hot Soxhlet methods are roughly the same. Both are automated methods, requiring little work in preparing the extraction. The sonication method requires the most manual handling steps in the entire process. Numerous manual changes, cleaning and dead times make this process undesirable.

A further attribute is the extraction time. Here the methods are all roughly the same. However, the hot Soxhlet is able to extract four samples in 35 min; whereas the ASE takes an hour to extract six samples. The sonication method is rather difficult to rate. Every sonication extraction cycle requires ten minutes centrifugation. When applying four cycles, roughly eight to ten samples can be extracted in one hour. Therefore sonication is slightly faster than ASE and hot Soxhlet.

The solvent consumption is a further attribute to be considered. Here, the sonication method is the most economical. The ASE and the hot Soxhlet are again very similar. However, the flushing and leak check do require a significant amount of solvent. Thus the hot Soxhlet is slightly better in using less solvent.

The rating of these attributes has shown, that the hot Soxhlet extraction method is the most suitable method for the purpose. In Figure 20 and Figure 21, only marginal difference can be seen between the two solvents. Since the solutions made using hexane/acetone (1:1) are more intensely coloured than the solutions in pure hexane, which are slightly yellow. It can be concluded, that some further substances are extracted using the solvent mixture. This is in agreement with the blank analysis which shows more noise for the solvent mixture. The aim of the extraction is to extract as efficiently as possible the desired analytes, while ideally not extracting any further substances. Thus the hot Soxhlet extraction using pure hexane was chosen as the preferred extraction method.

6.2. Validation

Since the GC method has been established previously and has been extensively used for routine analysis of hexachlorocyclohexanes, the validation has been focused mainly on the extraction method and the sample preparation. The concentration of epsilon-HCH in soils is expected to be low and is not the main focus of the investigations. Also due to the limited availability of the epsilon- isomer some validation experiments were conducted without epsilon-HCH. Not only the extraction method was validated, but also the robustness of several processes were investigated as shown in Figure 22. Key steps of the sampling procedure have been chosen by scientific considerations and have been validated. The proof that the sampling procedure is valid has been carried out by an overall standard deviation. This deviation was discussed with collaborates of the AUE told to be good.

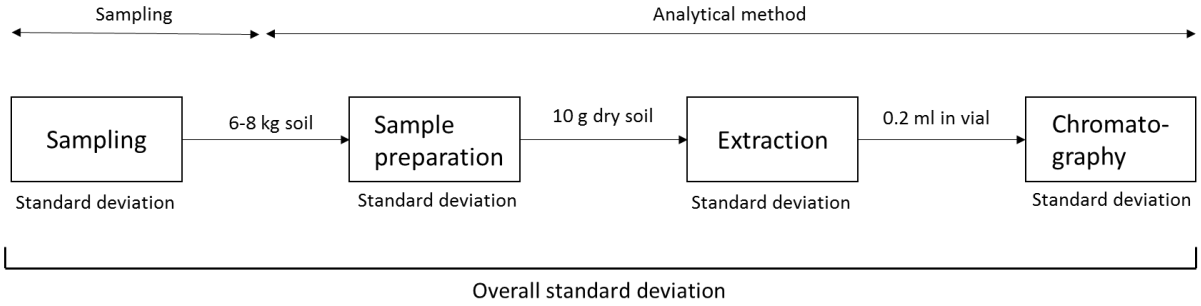


Figure 22. Overall process and their standard deviations.

Precision and standard deviations were investigated for a number of process steps. The overall standard deviation includes uncertainties of the soils, sampling, sampling preparation, extraction and even the chromatography deviations.

6.2.1. Validation of the Sampling

Several influences on the sampling have been explored. Analysis of the variation in HCH concentration in soil on a homogeneous area was done. Furthermore, the influence of large objects on the pollution concentration has been investigated.

Determination of the sampling uncertainty on a homogeneous area

Additional work has been done to evaluate how important the exact place of sampling is and to validate the sampling robustness and its procedure. Therefore, several sampling areas have been sampled three times and one even six fold. Thus the variation of the sampling and the variation of the soil were investigated.

For comparison reasons, the standard error of means are reported in relation to the mean value in Table 9

Table 9. Relative standard error of the means.

	alpha-HCH [%]	beta-HCH [%]	gamma-HCH [%]	delta-HCH [%]	epsilon-HCH [%]
Neuerteich, n=6	10	15	18	17	ND
Autal, n=3	37	35	15	ND	ND
Bachtelenweg, n=3	12	5	13	ND	ND
Maienbühl, n=3	31	30	6	ND	ND
Ackermätteli, n=3	22	16	69	42	21
Uferstrasse, n=3	29	33	26	33	32

ND = Not detected

The relative standard error of means has been calculated for the areas sampled three times. Six-fold sampling has been carried out for the area Neuerteich. Variation of the results has been observed due to local heterogeneities of the soils.

The results for Neuerteich are also shown in Figure 23 as GIS illustration.

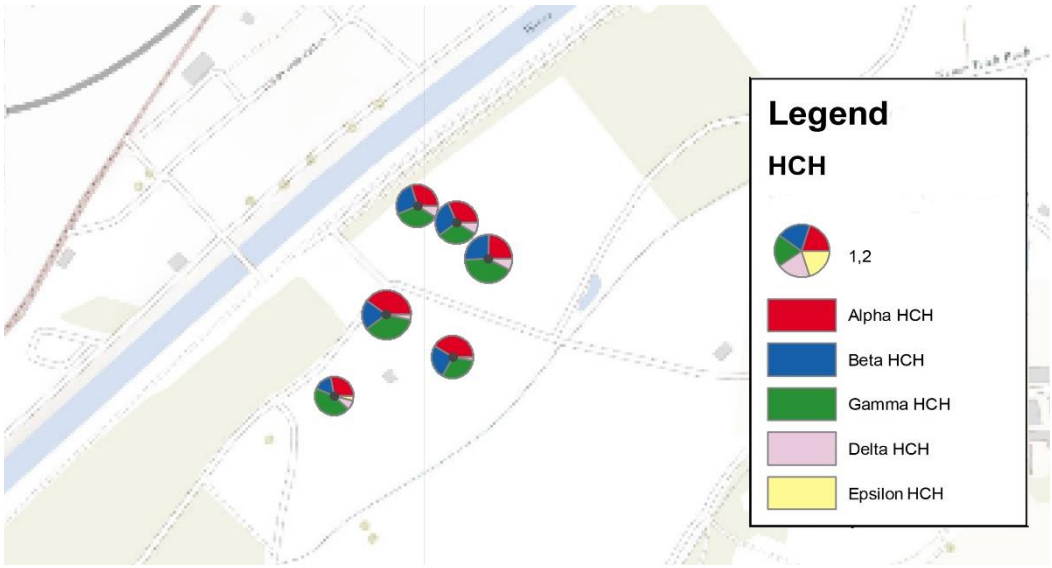


Figure 23. GIS of the six fold sampled Neuerteich.

Six-fold sampling of the area Neuerteich displayed as a GIS graph. For comparison reasons, all GIS graphs are normalised by the square route of the sum of all isomers (µg/kg). Sampled areas are up to 110 m from each other apart.

Except for the sampling areas Ackermätteli and Uferstrasse, the contamination concentration of the HCH- isomers were low which incurs a correspondingly greater error. It was possible to determine the relative SEM for the more concentrated sampling areas for all isomers. In general the relative SEMs for all sampling areas were roughly similar despite some outliers. The relative SEM for the sampling area Neuerteich is to be regarded as the most reliable result, since it has been calculated out of six independent values, whereas all others derive from three fold sampling. The SEM has also been used to calculate the possible range of the contamination of the more highly contaminated areas Ackermätteli and Uferstrasse. Thus a maximal contamination of the sum of isomers for Ackermätteli of 249 µg/kg and 713 µg/kg would be possible at a confidence interval of 95 %. A violation of the legal limit is not certain. However, these values have been calculated on the basis of a three-fold sampling. With increased sampling, the values could be determined more accurately. Furthermore, normality of the data has been assumed for the calculations.

Nevertheless, the results shown in Table 9 have been expected. During the sampling procedure, it has been noticed, that some layers of loam or small stones are thicker or are present in greater depth. Results of the column test do show, that the isomers adsorb more on loamy layers with humus then on sand. Thus such variation of the sampling is plausible. Furthermore it shows, that the standardised sampling procedure combining five boreholes to one sample is robust and delivers reliable results. Sampling locations in the area of Neuerteich were up to 110 m apart from each other, but nevertheless gave comparable results. It is concluded that the distance between the boreholes is well chosen and suitable for all sampling locations.

In Table 10 the relative standard deviations of the total procedure (sampling and analytical) are shown.

Table 10. Relative standard deviations of soil sampling processes.

	alpha-HCH	beta-HCH	gamma-HCH	delta-HCH	epsilon-HCH
Srel Injection precicion, n=10 [%]	6.6	5.6	6.1	5.8	5.1
Srel extraction precision, n=6 [%]	3.6	2.0	3.8	4.4	3.0
Srel sampling precision, n=6 [%]	25	37	44	ND	ND

Several variations have been analysed in this project. The relative standard deviations of the methods used are both very low. However, the sampling precision showed some significant influence of the soil heterogeneity. This result supports the observations made during the sampling.

The results of the injection precision and extraction precision have already been discussed in previous sections. The errors of the total soil sampling process include the extraction precision and injection precision. The results show, that the extraction method contributes only to a small part to the variation of the results and is thus well suited for the purpose. This has already been proven in the validation section of this report. Furthermore, the variation in soil has been reduced by combining five boreholes to a single sample. Nevertheless, a variation in the total process is present.

Influence of wind depositions behind large objects

Further investigations concerning the robustness of the sampling have been investigated by sampling at defined distances behind large objects, such as trees. It has been hypothesised, that winds deposit behind large objects. Furthermore, depositions on large trees could be washed down by the rain producing local a high pollution.

In total five different sampling areas have been analysed. Diagrams have been made to analyse the influence of the objects on the concentration of each isomer and their isomeric ratio.

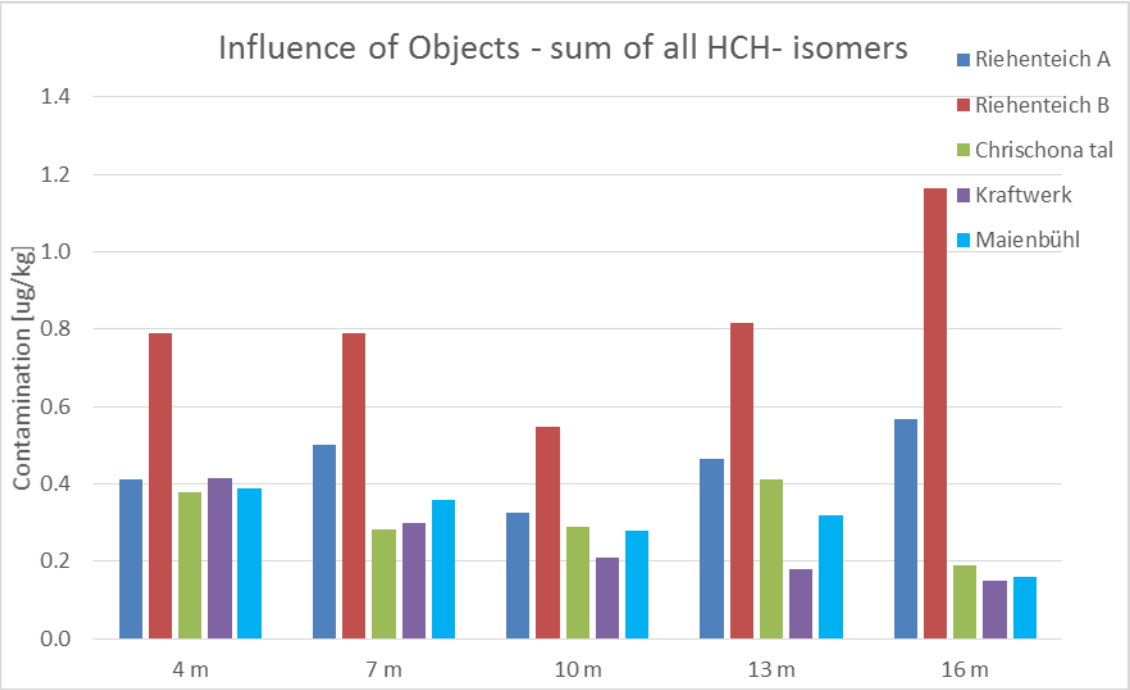


Figure 24. Influence of objects on the sum of all HCH- isomers.

Sampling of five different sampling areas close to a large object has been carried out to prove the robustness of the sampling procedure. Influence of the object on the sum of all isomers and each isomer has been analysed using statistics. An influence of the object on contaminations at defined intervals could not be proven. Thus the sampling procedure proved to be robust.

Figure 24 shows the influence of the sampling distance to large trees on the concentration of the sum of all HCH- isomers for the five different sampling areas for all HCH- isomers.

In general, results for delta- and epsilon- HCH were considered as informative, since the pollution concentrations were too low in some cases. It is concluded that there is no significant influence of the sampling distance to an object in the direction of the pollution source on the concentration of the sum of all HCH- isomers and each isomer. This has been confirmed by statistical analysis in the same manner as for the investigation of the soil profiles.

The p-value of 0.05 was used as significance threshold. Thus regression analysis was done exploring any correlation between the concentration and the distance as a numeric value. However, regression analysis with not normally distributed data resulted in no correlation. Transformation of the data did not result in any improvement. Thus, further analyses were made treating the distance as a category. Statistical analysis of these categories lead to the conclusion, that all the categories are the same, which supports the graphical impression of the data. Thus it could be proven, that big objects do not have an influence on the concentration of the sum of the HCH- isomers and on each isomer itself. Furthermore, this result enables sampling to be essentially carried out in any unperturbed area.

The validation of the sampling has shown, that samples can be taken anywhere. Decisive is the soil type.

6.2.2. Extraction Linearity

The linearity determination of the extraction method was determined by extracting the concentrations 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10, 50, 100, 500 and 1000 µg/kg of doped, uncontaminated soil.

Table 11. Extraction linearity of all targeted HCH- isomers.

Expected [µ/kg]	alpha-HCH [µ/kg]	beta-HCH [µ/kg]	gamma-HCH [µ/kg]	delta-HCH [µ/kg]	epsilon-HCH [µ/kg]
0.05		0.052			0.052
0.1		0.101		0.091	0.106
0.5	0.55	0.49	0.58	0.53	0.54
1	1.06	0.99	1.10	1.01	1.07
5	5.51	5.11	4.88	4.79	5.15
10	9.87	9.90	10.49	10.01	10.59
50	50.2	50.2	48.9	52.6	
100	102.4	95.4	98.0	103.2	
500	535.1	513.4	481.7		
1000	1015.6				
R^2	0.9994	0.9998	1.0000	0.9999	0.9998
Equation y =	1.0242x + 1.68	1.0261x - 0.94	0.9627x + 0.58	1.036x - 0.06	1.0546x - 0.01

The linearity of the extraction method has been proven for all the HCH- isomers. Low concentrations have been expected for delta-, and epsilon- HCH, whereas a linearity to high concentrations are not requested.

In Table 11, all values are listed which were used to determine the linearity of the extraction method. However, for none of the isomers was it possible to include the 0.01 µg/kg value into the linearity. Furthermore, the 1000 µg/kg value could only be used for the alpha-HCH isomer. The linearity for epsilon- HCH was deliberately only determined up to 10 µg/kg, since the expected contamination of the soils is very low. For all isomers a linear range was determined including at least six data points. Good values for the coefficients of determination for all isomers were obtained, indicative of excellent linearity.

Graphs are included in the attachment.

The limits of quantification (LOQs) and limits of detection (LODs) were determined using the same samples from the linearity study and are listed in

Table 12. The LOQs and LODs were determined by calculating the signal to noise ratio (S/N). Using S/N = 10 for the LOQ and S/N = 3 for the LOD.

Table 12. LOQs and LODs for all targeted HCH- isomers.

	alpha- HCH	beta- HCH	gamma- HCH	delta- HCH	epsilon- HCH
Concentration [µg/kg]	0.1	0.05	0.05	0.1	0.05
Signal	9.2	4.0	8.6	4.6	6.4
Noise	0.7	0.5	0.7	0.6	0.9
S/N	13.1	8.0	12.3	7.7	7.5
LOQ	0.074	0.061	0.040	0.127	0.065
LOD	0.022	0.018	0.012	0.038	0.019

The ratio of the signal to noise was used to determine the LOQs and LODs for all the isomers living up to the requirements which have been estimated.

The obtained LOQs were regarded as being sufficient for the purposes of this study. However, it must be noted, that the extraction of soils does result in rather high noise in the GC-MS/MS. The identification of the quantification peak is confirmed by its confirmation ions and their ratio to the quantification peak. Thus even very low concentrations can be analysed and interpreted with confidence. The condition of the GC and MS have an influence on the noise and the peaks. Thus a heavily-used liner is contaminated with deposits, which lead to undesired disturbance. Therefore, maintenance of the GC is of great importance. This includes changing of liner and septum as well as shortening the pre-column from time to time. Also the ion source is to be cleaned when necessary. The system is generally checked by before analysing samples. Furthermore, control standards are measured in every sequence to confirm the reliability of the analysis.

6.2.3. Precision

The extraction method precision was investigated by extracting doped, blank soil six times with a known concentration (5 µg/kg for alpha-, beta-, gamma-, beta- HCH and 10 µg/kg for epsilon-HCH).

Table 13. Extraction precision of the hot Soxhlet method.

	alpha-HCH	beta-HCH	gamma-HCH	delta-HCH	epsilon-HCH
Average [µg/kg]	5.5	5.1	4.8	4.8	10.6
Standard deviation [µg/kg]	0.2	0.1	0.2	0.2	0.3
Relative standard [%]	3.6	2.0	3.8	4.4	2.7
Recovery rate [%]	111	100	96	97	106

The precision has been determined by extracting a doped, uncontaminated soil six times. Results show outstanding precision for each isomer. Furthermore, recovery rates have been assumed as 100 % for all the isomers based on the data shown.

In Table 13 the resulting extraction precision is listed with further information. The relative standard deviation of < 5 % is an outstanding result, showing that the extraction method delivers reproducible results with small errors throughout the extraction procedure and analysis. The recovery rates for all the isomers show some differences. Thus alpha- HCH is slightly higher than the expected contamination. Since the alpha- HCH is applied as a mixture of alpha-, beta-, gamma-, and delta- HCH, an error in doping the samples can be excluded. With every analysis, a control standard sample is measured in order to assure the correctness of the analysis. Thus the alpha- HCH value was slightly higher than 100 % of the control standard, which explains the apparently anomalous result. The increased value for epsilon- HCH is still in an expected range. In conclusion, complete extraction of the HCH in soil samples was demonstrated resulting in precise and reliable results.

6.2.4. Accuracy

The accuracy has been proven by the analysis of a certified reference material. Soils were also extracted by a shaking method used prior to this study and compared. Furthermore, independent analysis of soil samples have been carried out by the external laboratories of Heppeler GmbH and Bachema AG.

Analysis of a certified reference material

The accuracy of the extraction method was assessed by extracting a reference material (PAHs, PCBs and Pesticides on Fresh Water Sediments, Resource Technology Corporation) in duplicate. Furthermore, the material that had already been once extracted, was extracted a second time and the solution collected separately. This assured the total extraction of the reference material and the validity of the method.

Table 14. Extraction method accuracy.

	alpha-HCH [µ/kg]	beta-HCH [µ/kg]	gamma-HCH [µ/kg]	delta-HCH [µ/kg]	epsilon-HCH [µ/kg]
Measured	43.5	26.8	9.2	31.6	0.2
Additional 30	0.18	0.15	0.04	0	0
Certified value min	22.8	12.30	6.39	NA	NA
Certified value max	51.4	30.00	12.60	NA	NA

NA refers to not available.
Extraction of a certified material has proven the accuracy of the extraction method.

In Table 14 the results are listed with the corresponding range from the certificate. The reference material tested includes only information on alpha-, beta- and gamma- HCH. However, the extraction of this reference material revealed, that delta- and epsilon- HCH are also present. The measured concentrations all lie within the certified range. Furthermore, the additional extraction of the soil did not lead to a significant increase of the content of HCH- isomers. Thus, the accuracy of the method is confirmed and must be considered valid for different soils. The accuracy for delta- and epsilon- HCH could not be tested in this experiment. In general, the extraction of doped material leads to successful recovery of the HCH for all isomers, also for delta-, and epsilon- HCH.

Method comparison

Prior to this project, soil samples had been sampled and analysed in September 2013 using a shaking extraction method. Therefore a sample from previous sampling dated back to fall 2013 has been reanalysed by the established hot Soxhlet method. Furthermore, the samples Ackermättli and Uferstrasse have been analysed by shaking extraction. In addition, samples have been analysed externally by Bachema AG using an ASE method and by Heppeler GmbH.

Table 15. Method comparison – results.

Sample Name	Extraction method	alpha-HCH [µ/kg]	beta-HCH [µ/kg]	gamma-HCH [µ/kg]	delta-HCH [µ/kg]	epsilon-HCH [µ/kg]
Riehenteich	hot Soxhlet	0.20	0.12	0.23	0.01	0.01
Riehenteich	ASE (Bachema)	<0.5	0.8	<0.5	<0.5	<0.5
Riehenteich	ASE (Bachema)	<0.5	1.4	<0.5	<0.5	<0.5
Uferstrasse 1	Hot Soxhlet	28	391	3.1	4.5	2.7
Uferstrasse 1	Shaking	12	189	1.2	1.3	1.8
Uferstrasse 2	Hot Soxhlet	31	456	3.2	4.0	2.2
Uferstrasse 2	Shaking	16	206	1.3	1.2	1.1
Ackermätteli 2	Hot Soxhlet	15	166	5.7	2.8	0.6
Ackermätteli 2	Shaking	4.7	57	0.3	0.4	0.4
Ackermätteli 2	Heppeler	4	30	< 4	< 4	ND
Ackermätteli 2	Heppeler	4	40	< 4	< 4	ND
Ackermätteli 1	Hot Soxhlet	6.4	100	0.6	0.7	0.3
Ackermätteli 1	Shaking	2.8	35	0.3	0.3	0.2
Ackermätteli 1	ASE (Bachema)	11.1	105	1.1	0.6	<0.5
Ackermätteli 1	ASE (Bachema)	11.3	97.9	0.7	0.6	<0.5
Ackermätteli 2013	Hot Soxhlet	9.4	74	0.7	0.6	0.3
Ackermätteli 2013	Shaking from 2013	14.2	52	0.6	0.5	0.3
Ackermätteli 2013	Shaking	5.1	47	0.4	0.2	0.2
Ackermätteli 2013	ASE (Bachema)	16.4	114	1.0	0.7	<0.5

ND refers to not determined.
All analysis have been done in 2014 except one analysis of the Ackermätteli sampled in 2013 has also been analysed in September 2013.
The hot Soxhlet extraction method has been compared with several other extraction methods. In general, results obtained by shaking extraction have always shown to be lower than the results obtained by other methods. External analysis of the soils using ASE leads to comparable results. Thus the accuracy of the method has been proven again. Heppeler GmbH has extracted the soils by a shaking method combined with treatment in an ultrasonic water bath.

Table 15 shows all the results of three different extraction methods. A first conclusion is that all results are in the same concentration range and thus are comparable. However, there are differences. In general, the values derived from the shaking extraction method lead to lower concentrations of each isomer. Nevertheless, re-assessed analysis of the same soil (stored at 4 °C) showed similar, slightly lower values than the analysis dated back to 2013. Thus the shaking method was carried out properly. Soils have also been analysed externally by Bachema AG. Despite some minor differences, the results are comparable. Unfortunately there is no information available on the variance of the method. However, the data has been analysed as blind double with some variation.

In conclusion, all three methods deliver results in a similar range. However, the shaking method gives results which are consistently lower than the other methods. This method was used in September 2013 due to lack of human resources and time pressure. The analysis carried out by Heppeler GmbH resulted in even lower concentrations of HCH than extraction by the shaking and hot Soxhlet method. The analysis have been done in duplicate, whereas a significant difference of the concentration of beta- HCH (33 %) in the same sample was observed. Since the exact extraction procedure of Heppeler GmbH is unknown, no definite conclusions can be made. Further analysis by the external laboratory using ASE showed comparable results. This is a further proof of the accuracy of the method.

6.2.5. Validation of Critical Steps

Potentially critical steps have been identified and investigated. Thus the drying process as well as the solution concentration by a nitrogen flow were examined thoroughly.

Drying of soils

Hot Soxhlet extraction with hexane requires dry soils. Therefore, the soils have to be dried. According to the VbbO the drying should occur at 40 °C. This drying process has been investigated by several experiments.

A triple workup on glass and separately on aluminium dishes showed some loss due to drying. Therefore, the condition has been changed to 30 °C and to ambient temperatures (six-fold workup). In Table 16 the results of the experiments are displayed. Since the extraction is based on dry soil (>95 % dry substance) the drying process is a vital process not covered by any internal analytical standards. The first experiments were unsatisfactory, in yielding great losses for all isomers. The experiments at 30 °C showed some improvement. However, six-fold drying at ambient conditions yielded acceptable results. The major difference between the experiments at ambient conditions was, that there was no air flow due to an oven fan. Thus the HCH-isomers were not lost due to evaporation. The data reported in this study, were obtained using the drying procedure optimised for minimal loss.

Table 16 shows the results of an investigation of the drying process of soil samples.

Table 16. Recovery after drying.

	alpha-HCH	beta-HCH	gamma-HCH	delta-HCH	epsilon-HCH
Residual, aluminum dish 40 °C [%]	53	49	51	50	65
Residual, glass dish 40 °C [%]	55	64	56	51	60
Residual, aluminum dish 30 °C [%]	89	71	81	51	108
Residual, glass dish 30 °C [%] [a]	88	68	81	49	92
Residual, aluminum ambient temp. [%] [b]	88	74	90	62	96

[a] experiment was carried out as duplicate.
[b] experiment was carried out six times.
All experiments, except the marked, have been done by three-fold experiments.
The loss of HCH- isomers due to the drying process is significant. Best results were obtained on drying at ambient condition.

Concentration of solutions by a nitrogen flow

For the analysis by GC, the dried solutions are concentrated to result in an end volume of 0.2 ml. This procedure has been explored by concentrating solutions to several volumes and diluting them again to the end volume.

In duplicate, the volume was reduced to 0.2 ml, 0.1 ml and 0.05 ml before adjusting the volume to 0.2 ml.

Table 17. Influence of the reduction by a constant nitrogen flow.

	alpha-HCH	beta-HCH	gamma-HCH	delta-HCH	epsilon-HCH	ISTD alpha-HCH	ISTD beta-HCH	ISTD delta-HCH
Reference [%]	100	100	100	100	100	100	100	100
0.2 ml [%]	71	73	77	93	75	71	75	103
0.1 ml [%]	54	58	59	72	57	55	58	79
0.05 ml [%]	54	61	60	78	62	55	58	81

The concentration process using a constant nitrogen flow has been investigated by blowing of solvents to defined volumes. The ratios to a reference showed significant loss. However, the precision of the method indicates no influence on the concentrations.

In Table 17 the signal intensity of all isomers and internal standards are compared. When reducing the volume of the samples, a loss of signal intensity is found. However, the intensity differences between the samples, reduced to a volume of 0.1 ml and 0.05 ml, is almost the same in comparison to the reduction to 0.2 ml. The intensity of the internal standards behave in the same manner. Since there are differences between the volumes reduced to, it is important to keep this parameter constant. For the validation and for all other samples, the volume was reduced to 0.1 ml and then diluted to 0.2 ml. The influence of this process step is,

however, not of vital importance, since the precision and the linearity of the method were outstanding. Furthermore, it concerns the signal intensity and any losses are compensated by the internal standard.

Injection precision

Error sources have been identified throughout the entire process. Therefore, the injection precision has been explored by a tenfold injection of the same sample. The standard deviation of this process has then been determined. The relative standard deviation for all the isomers are 6.6 % for alpha- HCH, 5.6 % for beta- HCH, 6.1 % for gamma- HCH, 5.8 % for delta- HCH and 5.1 % for epsilon- HCH. Clearly the relative standard deviations for all isomers lie close together. However, when comparing with the relative standard deviation from the extraction process (analysis included) with the injection precision, the latter are higher. An explanation for this phenomenon is the condition of the analysis instrument. Hence the injection precision was assessed after a long series of analysis. The precision of the extraction, including the analysis, is sufficient for the analysis of soil samples because soils naturally vary in composition.

In conclusion, for the purpose of extracting soil samples, a well-suited method has been developed and validated. The method exhibits a linear range covering the expected contamination range and additionally has very good precision. Analysis of an independent sample and doped samples have shown, that the accuracy of the method is fully adequate to give reliable results. These results have been confirmed by the method comparison with the shaking extraction method and with the analysis by external laboratories. Investigation of several robustness parameters have shown where precise working is required and that the method is suited for the purpose.

6.3. Influence of the Main Wind Direction

It has been hypothesised, that the main winds have an influence on the pollution concentration in the city Basel and surroundings due to wind depositions. Samples in two rough circles around the ARA STEIH with different radius have been taken to assess the influence of the wind and the distance.

6.3.1. Influence of Wind on the Surroundings of Basel.

Sampling areas with a larder radius to the ARA STEIH – surroundings of Basel have been assigned to areas in the main wind directions and others. In total 26 different sampling areas have been analysed and are shown in Figure 25.

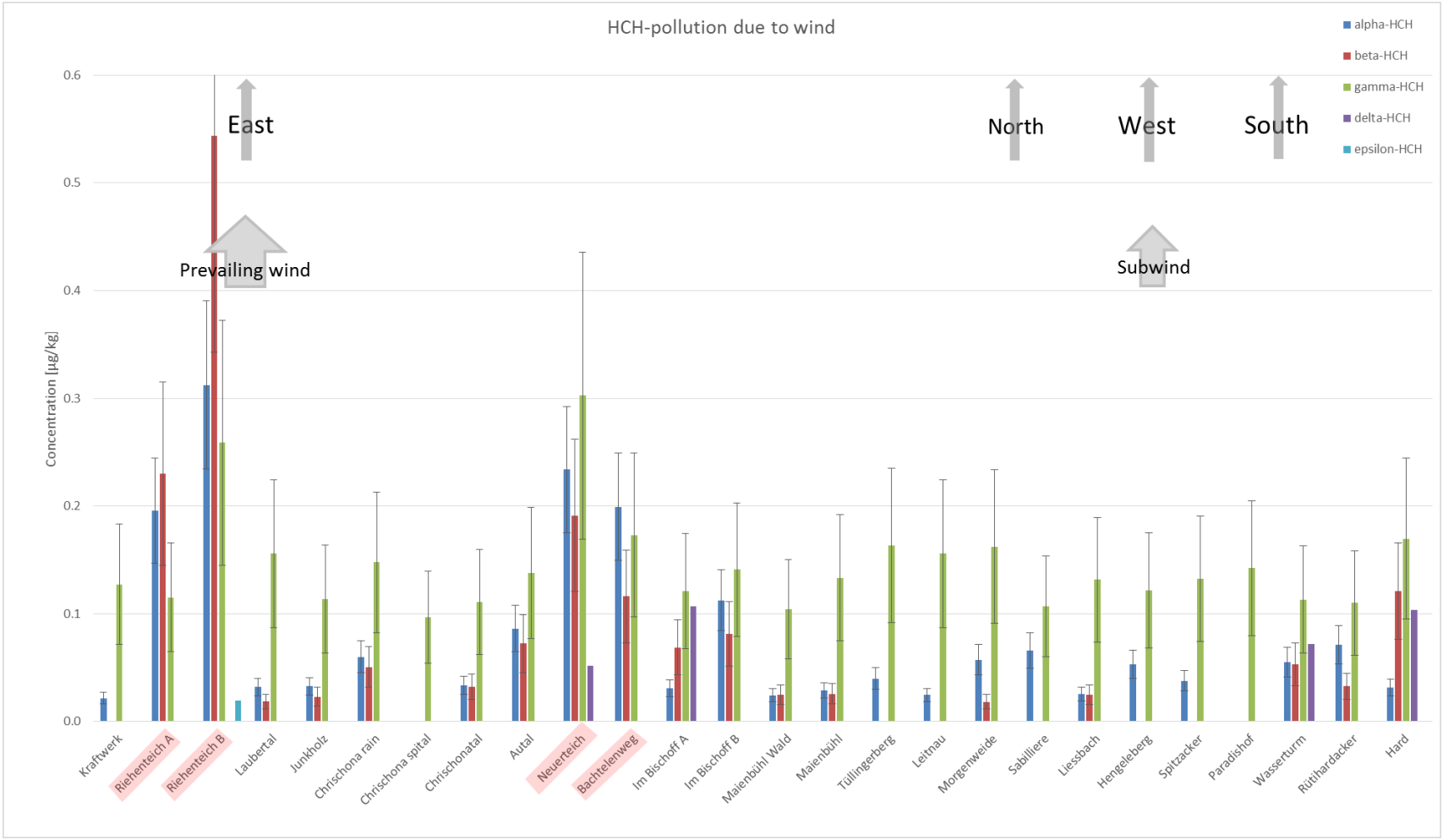


Figure 25. HCH- pollution due to wind influence on the surroundings of Basel.

Pollution of the surroundings of Basel shows low concentrations of HCH. The predominant isomer is generally gamma- HCH. Areas closer to the pollution source (marked red) show greater pollution concentrations, mostly beta- and alpha- HCH. No apparent influence of the wind direction was found on the concentration of HCH in the further region of Basel.

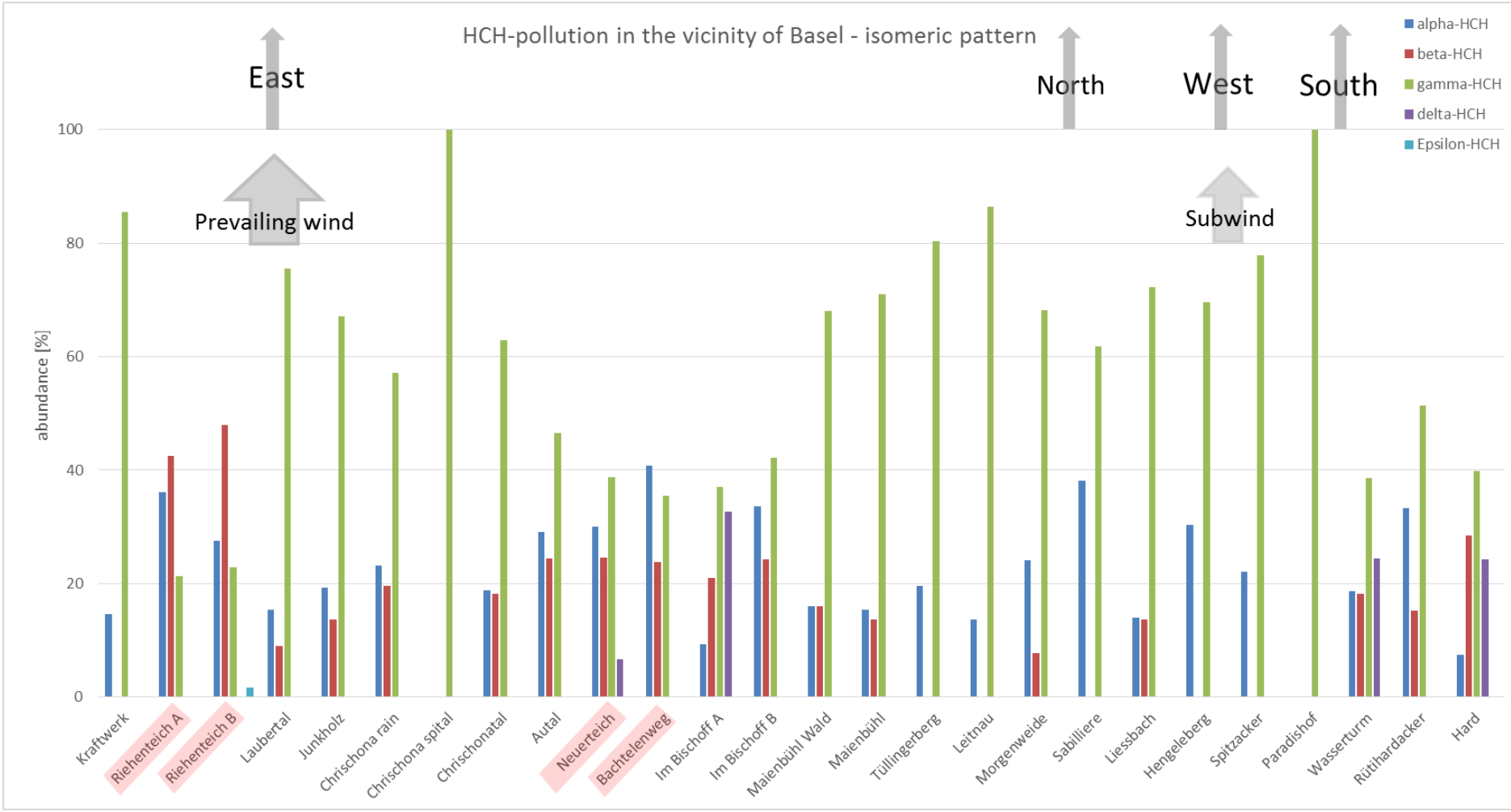


Figure 26. Isomeric pattern of the pollution in the further region of Basel.

The predominant isomer of the wider surroundings of Basel is gamma- HCH, followed by alpha- and beta- HCH. The pollution has most probably occurred from usage of the pesticide in the past and not from any one specific source. Areas closer to the pollution source marked red.

In conclusion, no obvious influence of the wind was observed, at least within the radius used. Furthermore, the presence of gamma- HCH in every sample in roughly the same concentration probably derive from historical use of the pesticide in the past.
The greater concentrations of alpha- and beta- HCH compared with delta- and epsilon- HCH is a typical fingerprint of the pollution due to open-air stock piling. However, the concentrations are too low (lower than a background pollution of Lindane) to give a conclusive answer, yet they probably derive from the pollution source. The higher contamination values of alpha- and beta- HCH from areas closer to the pollution source indicate that the influence of the main wind direction is not as important as the distance from the ARA STEIH.

In Figure 25, all data is displayed in a single graph. The sampling areas have been listed counter clockwise with regards to the rough circle in which the samples have been taken.
Some data points are averaged by several sampling measurements. Furthermore, two different samplers (core sampler and drill sampler) have been used, whereas the average from the profiles to a depth of 20 cm were taken for this data. Interpretation of the graph leads to several conclusions. In general the pollution concentrations are low. Some values such as Riehenteich A and B as well as Neuerteich exhibit slightly higher concentrations of alpha- and beta- HCH probably because these sampling areas are closer to the ARA STEIH. Analysis of soils near to the pollution source delivered high values for beta- and increased values for alpha- HCH. Thus this hypothesis is supported by results from the sampling in the city of Basel. In general the main isomer present in the soils is gamma-HCH followed by alpha- and beta- HCH. This result can also be retrieved from Figure 26, which shows the isomeric pattern of the pollution of the areas sampled and the influence of the main wind direction on the pollution of the region of Basel. The predominant presence of gamma- HCH can be explained as a background contamination originating from the use of the pesticide in the past. Some areas such as the Neuerteich is a groundwater protection zone where no deliberate use of Lindane has occurred since several decades. However, the gamma- isomer is present at these sites which points to spray application of Lindane having taken place in the past. Figure 26 shows a low abundance of delta- and epsilon- HCH. As production of Lindane leads to only small amounts of these isomers, these low values are to be expected. The analysis of soils in the city Basel showed similar results.

Statistical analysis of the data has also been made. The tool “R-Studio” was used to assess any correlation of the sampling area with the concentration and the isomeric ratios in the soils. As a threshold for significance, a p-value of 0.05 was taken. Several statistical analysis have been carried out to investigate the difference between the two groups (within the main wind direction and outside the wind-influenced area) on the concentration of each isomer, the sum

of all isomers and the isomeric ratio. Furthermore, the two groups have been enlarged with the areas in the second main wind direction. All analysis leads to the same conclusion. There is no significant influence of the main wind direction on the pollution of sampled areas. The results of the statistical analysis are identical with the observations made through interpretation of the graphs.

6.3.2. Influence of Wind on the Pollution of Basel-city.

The influence of the main wind direction on the pollution in a closer distance to the ARA STEIH was investigated by sampling areas in the city Basel. 21 different green areas, mostly parks were sampled. The results are shown in Figure 27. Since the pollution of some areas were significantly higher than other areas, a standardisation by the square route was necessary.

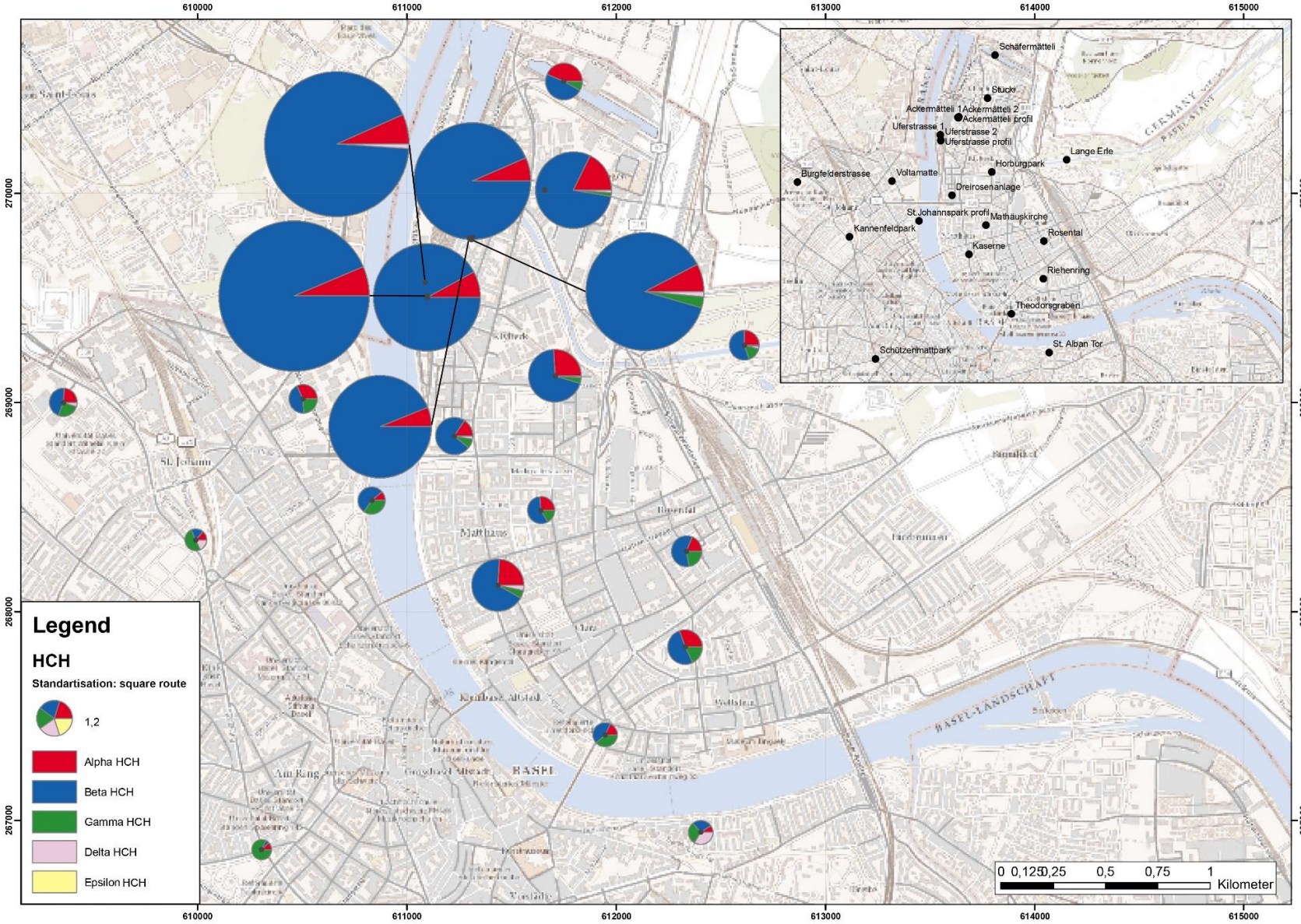


Figure 27. GIS of Basel-city.

The Pollution of the city Basel is displayed as a GIS graph. The values were standardised by the square route to be able to display all data. The Pollution is significantly increased for beta- and alpha- HCH in the close area to the ARA STEIH indicating a strong influence of the wind direction.

In general the concentration of the pollutants are rather low. However, some areas do show increased alpha- and beta- HCH. These highly contaminated areas are close to the ARA STEIH and are in the main wind direction. Thus an influence of the wind is present, but is strongly dependant on the distance to the pollution source. When comparing the results with the pollution of the further region of Basel, the predominant isomer has changed. Generally, the beta- isomer is the predominant isomer followed by alpha- HCH. However, there is a rather small background contamination of Lindane similar to the pollution of the further region of Basel. Delta- and epsilon- HCH do not influence the pollution significantly.

The extent of the wind influence has been investigated for other close areas. An explanation for the slightly elevated contamination of the areas Horburgpark, Dreirosenanlage, Kaserne, Schäfermätteli and Riehenring is difficult to make. The sampling areas are all on the eastern side of the river Rhein. The sampling area Rosental would also prove this phenomenon of the wind influence, however, the sampling area Mathäuskirche, which is in between all of the mentioned locations, does not fit into the pattern. Whether the soil has been perturbed or not cannot be proven. Looking further, other areas such as Lange Erlen and St. Alban Tor and Teich do not support this observation. Regarding the pollution of the highly contaminated areas Uferstrasse, Ackermätteli and Stücki, the pattern does not fit either. Furthermore, the three sampling areas are close to the ARA STEIH in an eastern direction. With increased distance, the pollution concentration decreases rapidly, which is also true for the sampling area Stücki.
A further important finding is the isomeric ratio. The sampling areas Ackermätteli and Uferstrasse show very high abundance of beta- HCH, up to 92 % followed by 7 % alpha- HCH. The Stücki area exhibits a slightly lower beta- HCH concentration, yet more alpha- HCH. Thus there is a trend considering alpha- and beta- HCH. With increasing distance, the concentration of these isomers decrease down to a background level as observed in the surroundings of Basel. Statistical analysis of the data has proven the interpretation of the graphic. A difference between areas in the main wind direction and others, not in the main wind is true for the alpha- and beta- HCH. The analysis has been carried out with all the data collected in the city of Basel.

Difference between the pollution in the city of Basel and the surroundings are shown in the GIS diagram below.

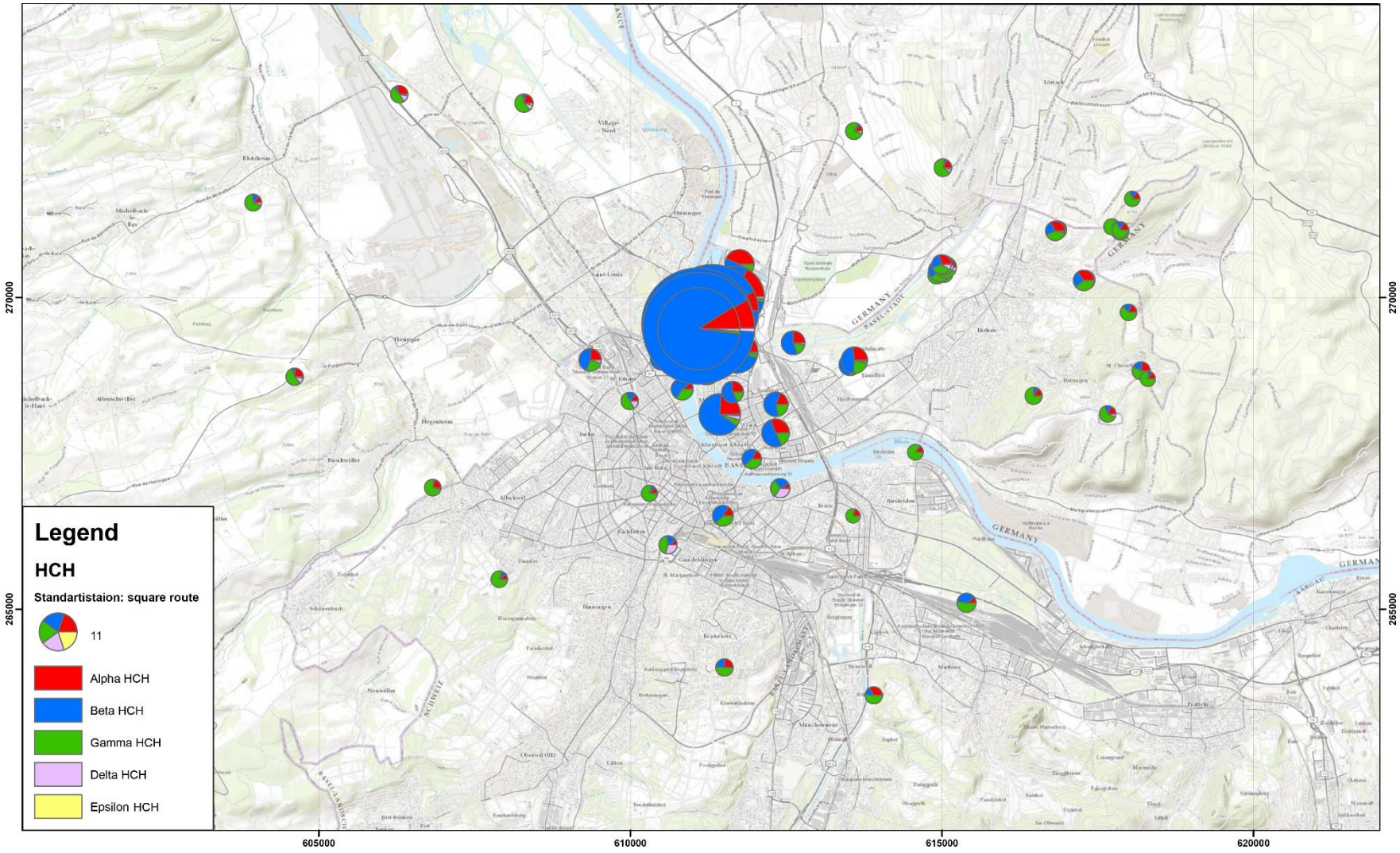


Figure 28. GIS of the pollution of Basel-city and surroundings

The graph shows the pollution in the city of Basel and its surrounding areas. The graph was normalised by the square route to be able to display all the data points. In the city of Basel beta- HCH is the predominant isomer. In the further region this changes to the favour of gamma- HCH.

6.3.3. Influence of Wind on the Pollution in Dependence of the Distance.

In the previous section an influence of the main wind direction on the pollution concentration in the closer area to the ARA STEIH was concluded. The influence of the distance on the pollution concentration has been investigated by the analysis of areas in the main wind direction with increasing distance to the pollution source.

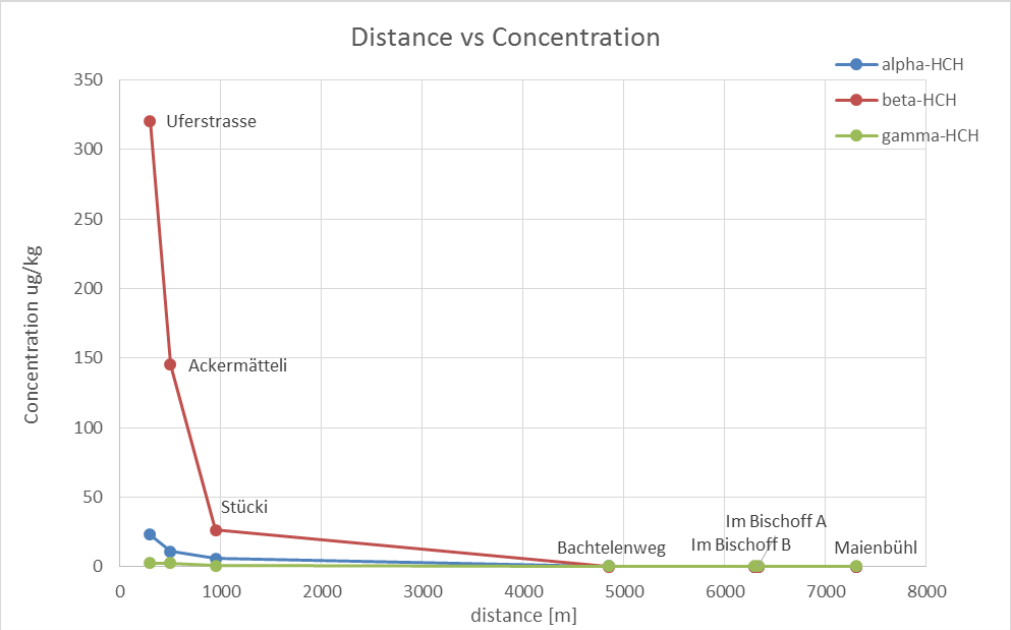


Figure 29. Influence of the distance on the pollution.

The pollution concentration of areas in the main wind direction with increasing distance are shown in this figure. There is a significant influence of the distance on the pollution concentration. Analysis have shown a strong dependence for beta- and alpha- HCH, whereas gamma-HCH remains constant.

In Figure 29 the areas close to the ARA STEIH show very high pollution concentrations for beta- HCH and lower concentrations for alpha- HCH. These pollutions decrease strongly with further distance to the ARA STEIH. For beta- and alpha- HCH the regression analysis show exponential decrease, whereas the gamma- HCH remains constant.

In conclusion, pollution occurs very locally, but in high concentrations.

6.4. Origin of Pollution

Investigations on the pollution in the surroundings of Basel and also in the city of Basel concerning the pollution time and source have been carried out. Also it was investigated, whether the pollution originate from newly deposits due to the remediation process. Therefore migration of the pollutants have been investigated.

6.4.1. Profile Analysis

In the surroundings of Basel a number of Profiles have been carried out to investigate the distribution of the HCH- isomers in the soil.

The analysis of soil profiles was carried out simultaneously with the sampling of soils in the further region of Basel. The profiles sampled have been taken roughly in the same distance to the pollution source and all derive from unperturbed soils. Furthermore, three profiles were sampled in the city of Basel. For the interpretation graphically and statistically they have not been included, since perturbation of these soils cannot be excluded.

The data shown in Figure 30 summarises the results for all profiles, to a depth of 20 cm and in slices of 5 cm, together with their corresponding pollution concentrations for each isomer. Furthermore the isomeric pattern of the profiles has also been interpreted and illustrated in Figure 31, showing the abundance of each isomer in percent. The predominant isomer throughout the sampling depth is the gamma- isomer followed by alpha- and beta- HCH, with the delta- and epsilon- isomers present only in lower concentrations.

The concentrations of all isomers as a function of the depth show no constant trend. Some show a slight increase, whereas others a decrease in concentration. The impressions of the graphic of the profiles were substantiated by statistical analyses. However, the analysis reveals, that the isomers are present in similar concentrations relative to one another and spread throughout the soil evenly. Reports in the past have shown, that HCH- isomers have been found in milk from the region of Basel. Therefore, initially it was thought, that a greater contamination would be found in the top layers: but this thesis can be rejected. Because the concentrations of the isomers relative to one another are approximately constant, it follows that the mobilities of the isomers are roughly the same and degradation of the contaminants must occur very slowly.

For the further region of Basel it can is concluded, that a pollution has occurred in the past. New pollution due to the remediation process can be excluded since no significant increase of HCH has been found in the top layers. The predominant gamma- HCH leads also to the conclusion that the contamination is probable to originate from the use of Lindane in the past.

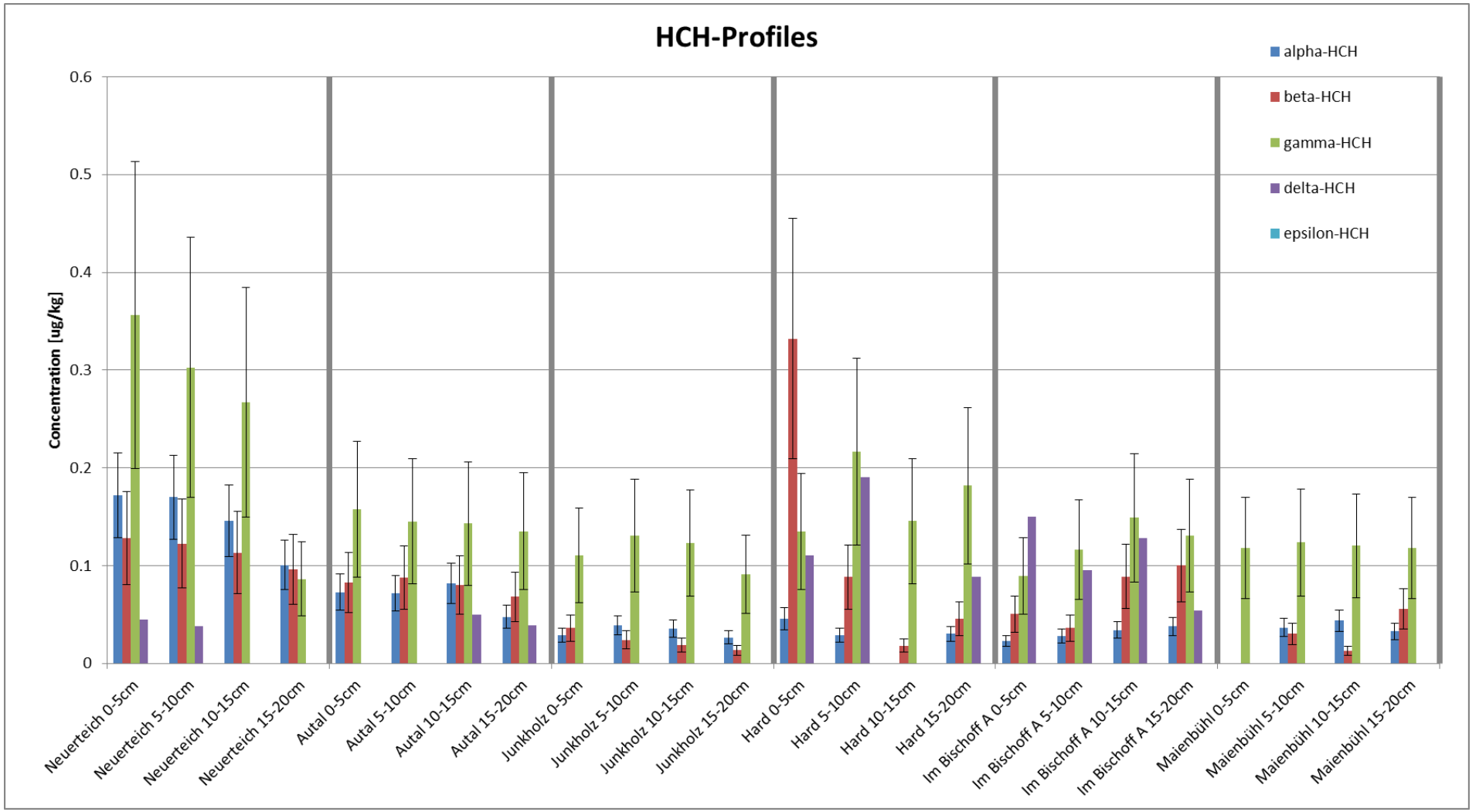


Figure 30. Profiles of the further region of Basel.

Six profiles from the surroundings of Basel have been sampled to a depth of 20 cm and divided into 5 cm slices. The pollution concentration is spread evenly throughout the sample depth. A correlation between the pollution concentration and the depth cannot be seen.

In Figure 30 all profiles from the further region of Basel are shown. Visual interpretation of the graph leads to the conclusion that no significant influence of the depth on the concentration of each isomer and the sum of all isomers is found.

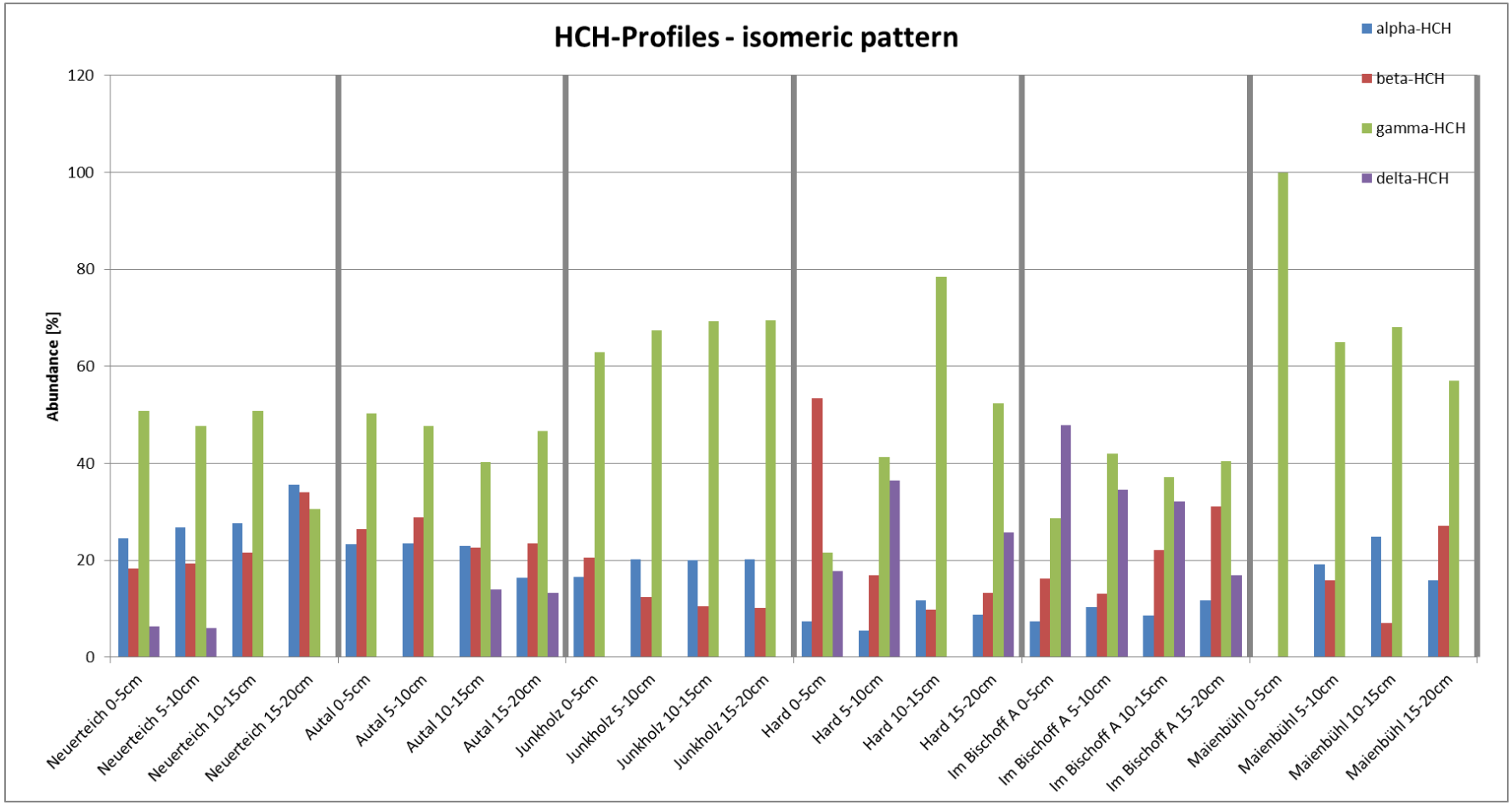


Figure 31. Isomeric pattern of the profiles of the further region of Basel.

Figure 31 displays the isomeric pattern of each profile giving the relative abundance for each isomer. Visual interpretation of the figure leads to the conclusion that the isomeric ratio does not change throughout the sample depth.

In addition two profiles were sampled to a depth of 40 cm to investigate the pollution to an ever greater depth. The profiles are shown in Figure 32 and Figure 33.

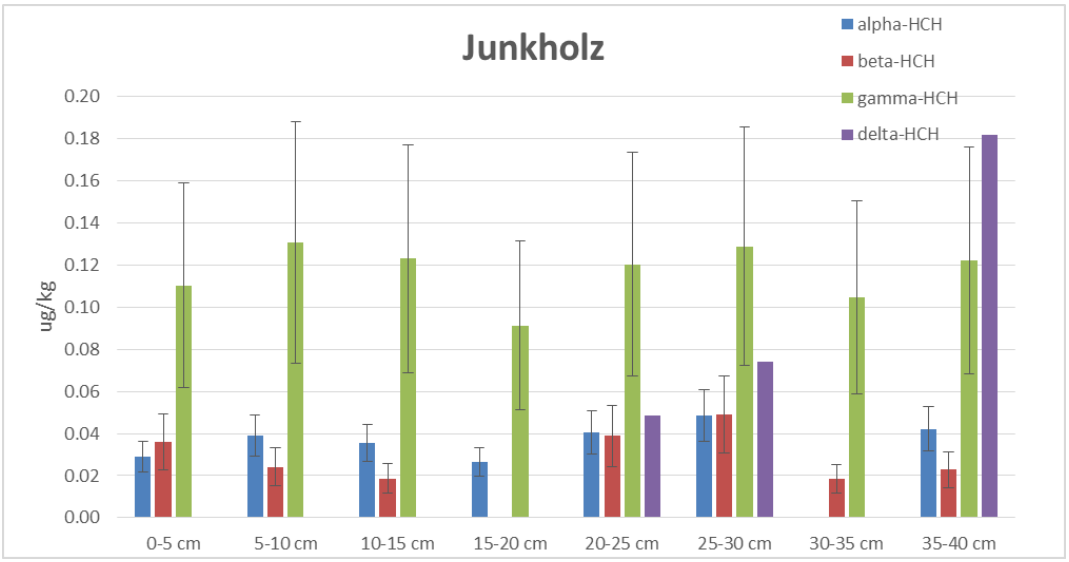


Figure 32. Junkholz profile to a depth of 40 cm.

In total the profile of two areas have been sampled to a depth of 40 cm. Epsilon- HCH could not be determined, since the concentrations were too low. The isomeric distribution remains similar throughout the sampled depth with no decrease of HCH- isomers. Thus a migration of the HCH- isomers over time has occurred. Other influences such as bioturbation are also to be considered.

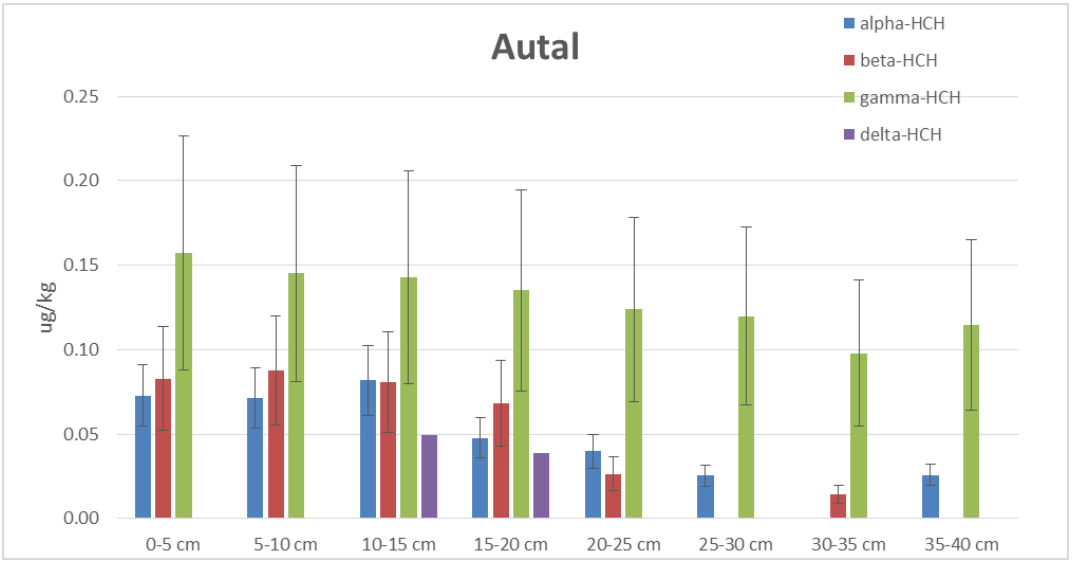


Figure 33. Autal profile to a depth of 40 cm.

Epsilon- HCH could not be determined, since the concentrations were too low. Both 40 cm profiles result in the same conclusions: pollution occurs also in deeper depth showing the same concentrations. Further a mobility for all isomers is present.

In Figure 32 and Figure 33 the profiles of the Autal and Junkholz to a depth of 40 cm are displayed. Since only two profiles were taken to a depth of 40 cm the results are regarded as informative. Nevertheless, out of the two graphs conclusions can be drawn that the pollution

concentrations reach similar levels also at depth. Thus a certain mobility of the HCH-isomers is given.

Interpretation of the data must nevertheless be done with caution, as the concentrations of the HCH- isomers in the soils are low, especially for delta- and epsilon- HCH and some data points are lower than the LOQ. However, selection of the corresponding chromatographic peaks was carried out using confirming ions and their ratios. Thus small peaks were able to be detected and reliably interpreted.

Higher variation of the results is encountered with regards to the sampling of cores. Soil sampling using a core sampler is only possible if there are few and only small stones present. Already smaller stones lead to an inaccuracy of the sampling. When slicing the sections it is important to slice as accurately as possible. Stones between slices increase the difficulty in slicing the core accurately and lead to inaccuracy in sampling. Also sampling cores with less loam tend to crumble. Therefore slicing the samples without contaminating the following slice, is to be done with great care. During the workup the stones are removed. Thus samples with very stony slices result in less soil compared to slices without stones and give a less representative sample. However, profiles are the result of five cores averaging such slices.

During the sampling procedure it has been observed, that such stony sections differ from one core to another. Thus the sampled soil has by nature a rather high inhomogeneity. Nevertheless, the interpretation of the data revealed reliable and interesting results.

In conclusion, the pollution is similar throughout a depth of 20 cm and might contaminate even deeper soils. A correlation of the concentration or isomeric ratio of any HCH- isomer with regards to the sampling depth could not be proven by interpretation of the graph or by statistical analysis of the data. The even distribution throughout the depth might have several reasons. For instance, the mobility of the HCH- isomers is a contributing factor. The mobilities of the HCH- isomers are rather small compared with other pollutants. However, with the passage of time and with the influence of rain the isomers migrate. Furthermore, natural mixing due to organisms must have an influence. The bioturbation has been already investigated by Charles Darwin and describes the mixing of soil due to diverse organisms. In the time elapsed since the production of Lindane, the bioturbation might be a contributing factor in redistributing the HCH- isomers which is difficult to quantify.

6.4.2. Profiles of Basel-city

Three profiles have been sampled in the city of Basel to investigate the origin and the migration of the pollutants closer to the ARA STEIH. The results obtained from the samples from the city of Basel are generally green space, which may have been perturbed. If the data is to be compared with the results of untouched soils sampled in the further region of Basel caution must be exercised.

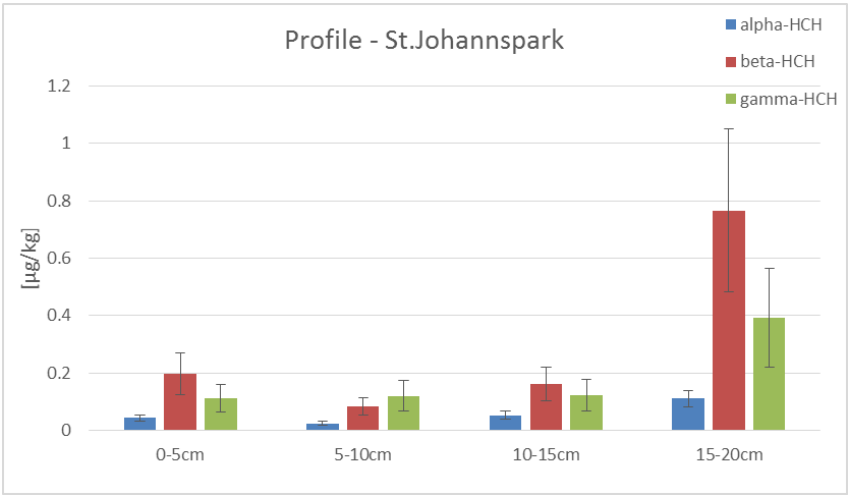


Figure 34. Profile - St. Johannspark.

Delta- and epsilon- HCH could not be determined since the concentrations were too low.
In total three profiles have been taken in Basel city. St. Johannspark shows a pollution throughout the sampled depth. According to the BVD Basel-Stadt the top 10 to 15 cm had been renewed more than a decade ago. Thus the pollution of the top layers is possibly a result of bioturbation.

The profile of St. Johannspark is to be regarded as informative. Whilst sampling, information from the city gardener was received, that the top soil (roughly 10 to 15 cm) had been renewed more than a decade ago. Nevertheless, some pollution of the soil was detected. The higher concentrations in the slice 15-20 cm might derive from the older soil. The pollution of the top layers is probably a result of bioturbation. The presence of gamma- HCH in the top layers, which must originate from production or usage in the past, supports this thesis.

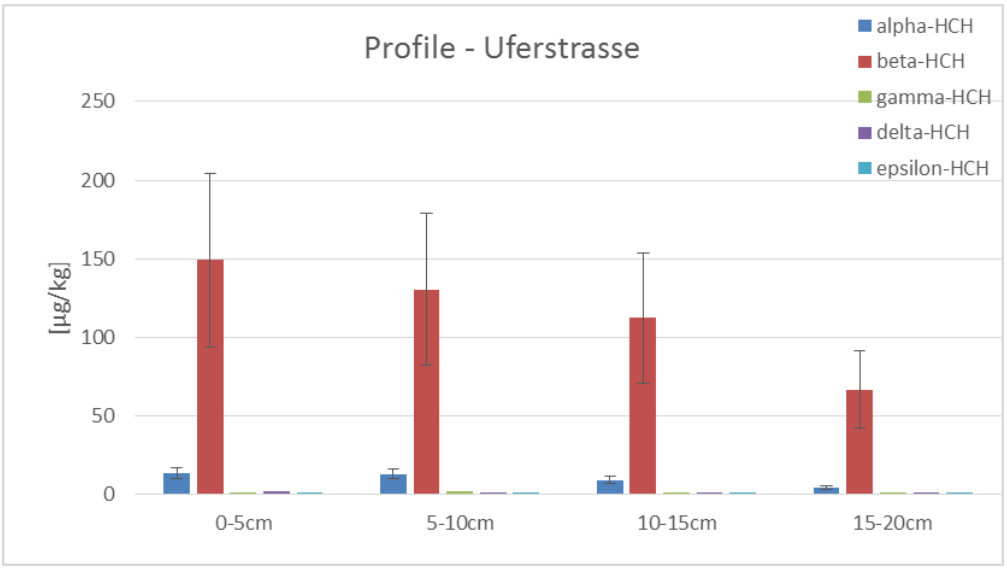


Figure 35. Profile – Uferstrasse.

The profile of the area Uferstrasse shows a high contamination to the sampling depth of 20 cm. Furthermore, the isomeric ratio stays the same throughout the sampled depth. Due to the low mobility of the HCHs, the pollution must have occurred in the past, probably from the production of Lindane.

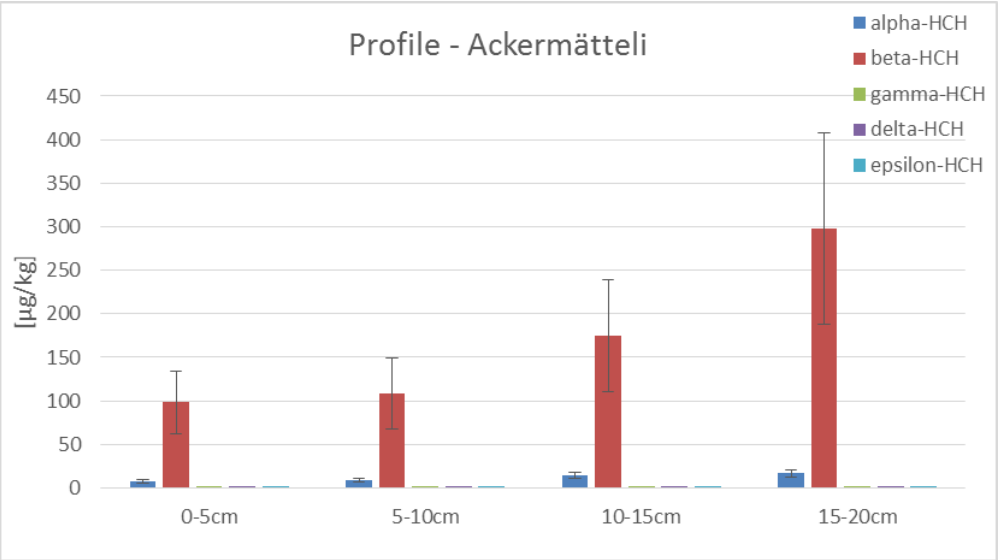


Figure 36. Profile – Ackermätteli.

The profile of the area Ackermätteli confirms the conclusion drawn from Figure 35.

Higher concentrations have been found throughout the depth of the profiles of Ackermätteli and Uferstrasse. The high beta- HCH concentrations are spread throughout the sampled depth, supporting the results of the interpretations of section 6.4.1. Thus not only the top layer is affected by the pollution. Taking into account the results of the profile sampling to 40 cm depth (Figure 32 and Figure 33), pollution an even greater depth is to be expected. The evenly distributed contamination of all the HCH- isomers leads to the conclusion that the pollution has migrated over a longer time period through the soil. Therefore, the main pollution originates from the time of the Lindane production. The remediation process in 2013 could have slightly contributed to the pollution. However, it is not possible to prove this by the soil analysis which have been carried out. Since the citizens have noticed the smell of the HCHs due to the remediation process, air and dust depositions are analysed in a recurring manner. These analysis are more suited for the determination of the new pollution extent.

6.4.3. Mobilities of HCH- isomers

The profile analysis has shown, that the HCH- isomers migrate through the soil. This migration was investigated in a laboratory experiment by a column test similar to a lysimeter experiment. The column test is similar to a chromatography except using soil as stationary phase and water as mobile phase. Thus, natural conditions of the draining of rain has been simulated.

Results are shown as absolute values, since several methods are involved and different amounts of soil and sand have been processed. A mass balance has been carried out resulting in 72 % recovery over all processes.

All the water fractions eluted from the chromatography column using soil as solid phase and water as mobile phase were collected in fractions of 0.9 l.

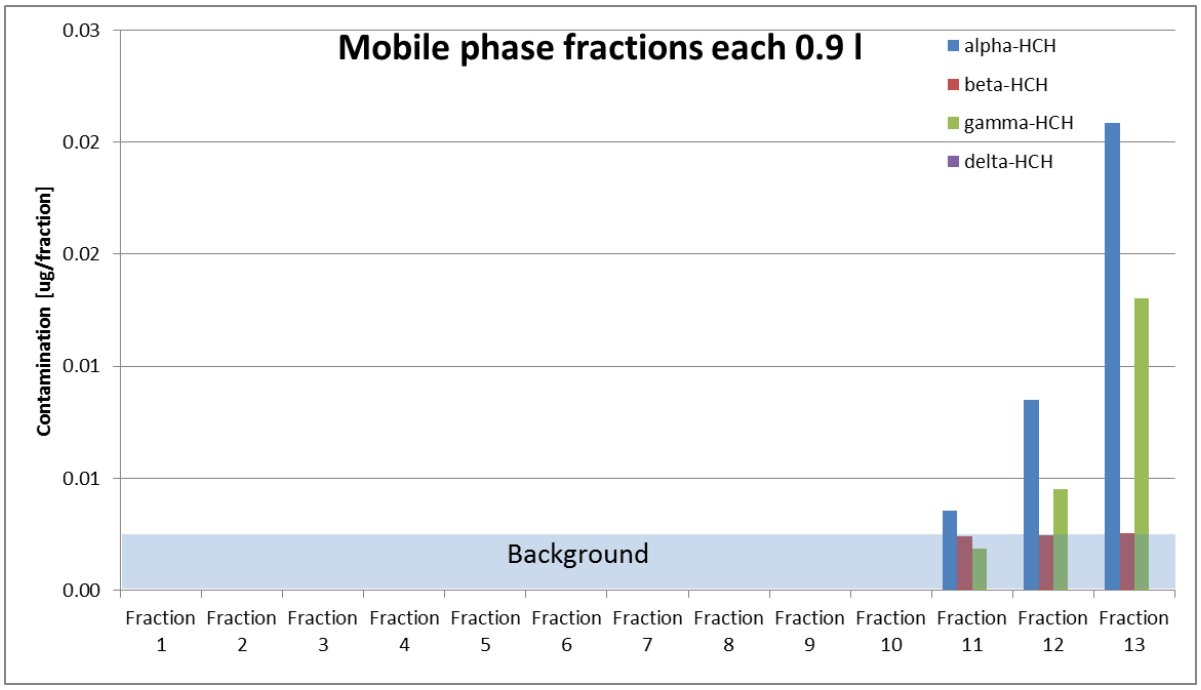


Figure 37. Analysis of the mobile phase fractions from the column test.

Water fractions from the column experiment have been analysed and show low concentrations of the HCH- isomers. Increased concentrations of alpha- and beta- HCH lead to the conclusion that a slight increased mobility for these isomers is possible.

The analysis has revealed that the presence of the HCH- isomers in the water fractions is very low. In the first ten fractions from the column test, the concentrations are roughly similar and possibly originate from a low homogenous pollution of soil used (Laubertal) in the column test. In Figure 37 some isomers, predominantly alpha- and gamma- HCH, were present in the last two fractions. Thus it can be seen that these isomers possess a certain mobility.

Soil sections were extracted and analysed. Results are shown in the figure below.

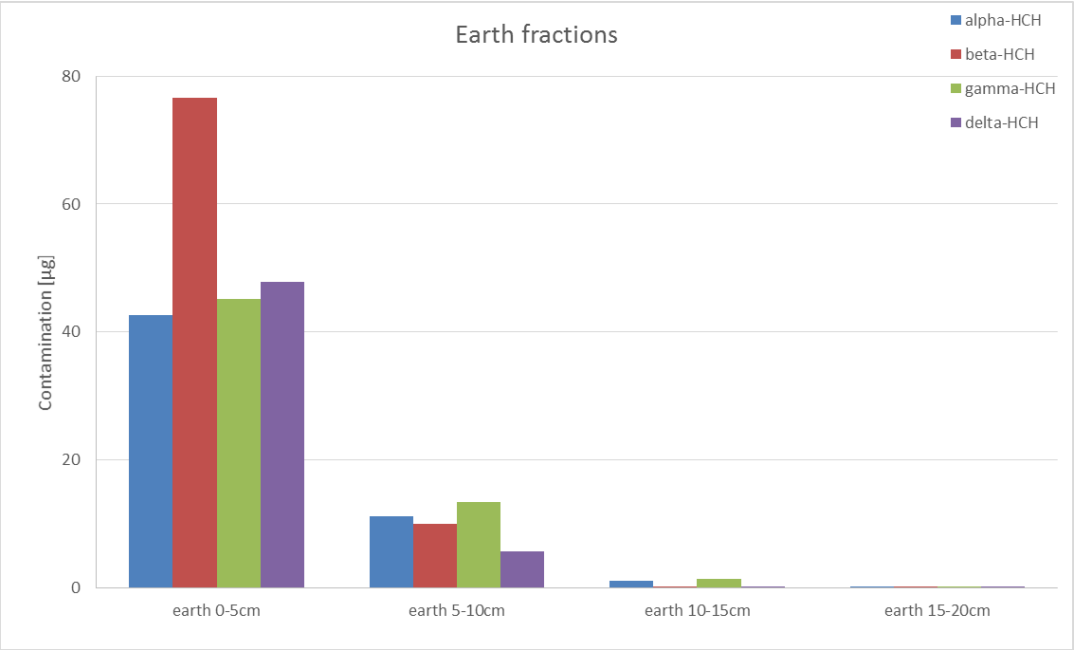


Figure 38. Earth fractions from the column test.

Analysis of the earth sections from the column experiment show that the vast majority of the HCH remains in the first slice (0-5 cm). However, slight contamination of the second slice (5-10 cm) proves mobility of the isomers.

In Figure 38 the results are shown as absolute values. Since all the slices consist of roughly the same amount of soil, they are comparable. Furthermore, due to the experimental setup cross contamination can be excluded. The experiment shows, that most of the hexachlorocyclohexanes has been retained in the first 5 cm. A significant concentration decrease is found in the subsequent section 5-10 cm. In the section 15-20 cm there is only a very small amount present, too little to be displayed in the diagram. Nevertheless, a certain mobility of the isomers is apparent. The beta- HCH is retained slightly more than alpha- and gamma- HCH. These two isomers are the predominant isomers in the following slices 5-10 cm and 10-15cm. It is concluded that the mobilities of the isomers are dissimilar.

As a filler and as spacer between earth slices sand has been used. These sand fractions have been dried and extracted in the same manner as the soil samples.

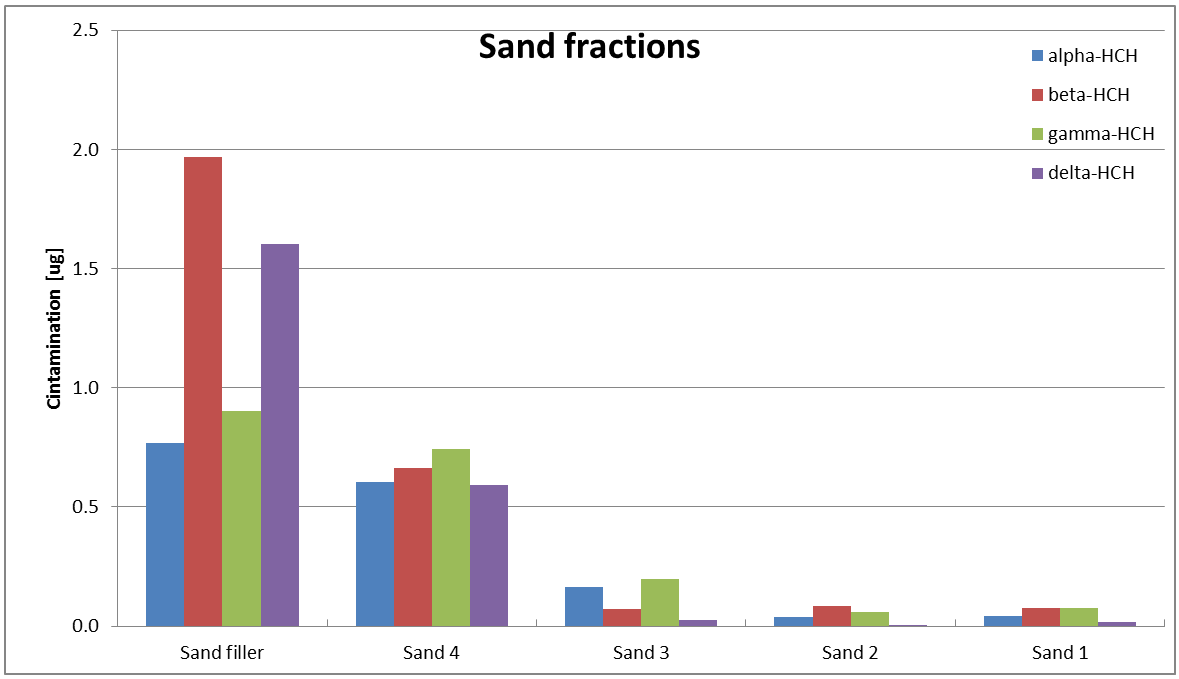


Figure 39. Sand fractions from the column test.

Analysis of the sand fractions from the column test show only low concentrations compared to the concentrations in soil. Thus the absorption ability is very low in sand sections and high in soil.

A comparison of Figure 38 and Figure 39 show that the scale of the contamination is of vital importance. Thus the contamination of the sand is significantly lower than the contamination of the soils. Therefore, the adsorption of the pollutants is predominantly on the earth fractions and not on the sand fractions. This is an important fact, since only the top soil layer contains loamy sections. At depth more sand and stones are present in the soil. Thus absorption will not take place in deeper strata. This might have an influence on ground water in regions with highly polluted soils. Ground water analysis in areas of highly contaminated soil is highly recommended.

The column experiment was carried out under laboratory conditions with one selected soil with homogeneous particle size, excluding stones, roots and invertebrates. In the region of Basel there are many different soils with different properties. For the experiment a homogenous loamy soil has been used and experiments with different soils may lead to further conclusions. Furthermore, the experiment has been conducted under laboratory conditions whereas in nature soils undergo cycling between wet and dry periods. The drying of the soil leads to crack formation in a natural environment and therefore a more rapid spreading of the pollutants could be possible.

In total, a volume of approximately one years of rain (region Basel) has been used in the experiment. However, the pollutants have been present since several decades and therefore have been probably washed down over time. Notwithstanding this, the experiment does con-

firm the mobility of the HCH-isomers, which explains the distribution of the pollutants throughout the examined sampling depth. Furthermore the experiment has been designed to investigate a single pollution event. For the region of Basel the pollution has occurred in a recurring manner over a long time period. Pollution which has occurred from the production time, could have migrated through top soil into deeper strata and might not have been sampled. Thus further models may be required to give a definite explanation of the spreading of the contaminants.

The experiment has shown, that the HCH- isomers migrate through the soil. With time, the concentration of the HCH- isomers should decrease in top layers. However, the beta- HCH would be more retained. Analysis of profiles have not proven this hypothesis, thus further natural processes have an influence on the distribution. One explanation for the even distribution is the bioturbation.

6.5. Old and New Contamination

Profile analysis and also profiles from Basel city show that the pollutants are distributed throughout the top 20 cm of soil evenly. The column test has further shown, that the HCH-isomers have a slight mobility with water. None of the profile slices showed a significant increase of HCH- contamination. The pollutants also in deeper layers must therefore have originated from pollution in the past not only by a single event.

The pollution in the further region of Basel is characterised by an abundance of gamma- HCH pointing to Lindane usage in the past.

Results for the city Basel show that the polluted areas have been contaminated by the former production site of Ugine-Kuhlmann. Profiles of the city of Basel have shown, that the concentration distribution is similar to the profiles mentioned above. Hence, the pollution must also have originated in the past. Contamination of the top 5 cm are similar to the concentrations in deeper sections. Hence a defined pollution duration dated back to the production of the Lindane would result in a decrease of HCH- isomers in the top layers. Such an effect was not observed. There are several explanations for this: bioturbation and other natural effects could result in a homogenous contamination of the soil. Further it is possible, that most of the HCH has already migrated through the soil, whereas the observed contamination is a broad band originating from the washing down effect. A combination of these possibilities is also probable. A constant pollution due to the remediation process of the ARA STEIH cannot be confirmed, but also not discounted. Since the remediation process in 2013 air and dust depositions are analysed constantly and the possibility that contaminated dust is responsible for this contamination can be ruled out.

In the theoretical part it has been mentioned, that the production of Lindane leads to isomeric wastes which vary in composition in the range of: α-HCH (55 – 80 %), β-HCH (5 - 14 %), γ-

HCH (Lindane, 8 - 15 %). Water analysis of the Rhein below the ARA STEIH as far back as the time of production resulted in: 2700 ng/l α-HCH, 300 ng/l β-HCH and 1100ng/l γ-HCH. It is noteworthy that a high concentration of alpha- HCH was found.
The pollution of the areas close to the production site have revealed high abundance for beta-HCH, up to 92 % followed by 7 % alpha- HCH. Therefore, an altering of the isomeric ratio has occurred.
Several explanations are possible: for instance, the alpha- HCH has been converted naturally in the soil to beta- HCH, increasing the value for beta- HCH and decreasing the concentration for alpha- HCH. Furthermore, washing out most isomers except beta- HCH from the soils due to their mobilities is also likely as contribution.
Constant pollution of the areas close to the ARA STEIH since the termination of the production is not probable. The wastes produced were disposed of in a large dump and mixed with concrete. Thus HCHs bound to the concrete are unlikely to be spread in such high concentrations. Therefore, the majority of the pollution concentrations of the highly contaminated soils originate from the production time of Lindane.

6.6. Further Organochlorines

Initially the analysis of further organochlorines (OCl) has been undertaken to see whether the pattern of pollution in the surroundings of Basel is similar to Lindane. The analytical method was validated in the AUE BS laboratory for the determination of organochlorines in suspended matter (analysis of river Rhein). It can be assumed, that the method gives reasonable results for the soils.
A number of different contaminants have been analysed in one analytical method. Similar pollution patterns to the HCH-pollution have been found for PCB-101 and PCB-180. However, blank analysis have revealed, that there are interferences. Thus no definite comparison with the pollution of gamma- HCH could be made.
Nevertheless, analysis of these further OCl have shown interesting results. For instance, elevated concentrations of 1,2,4-trichlorobenzol have been found for the higher HCH- contaminated areas in Basel. 1,2,4-trichlorobenzol is a product of the biotic degradation of alpha- HCH, as described in Figure 5.

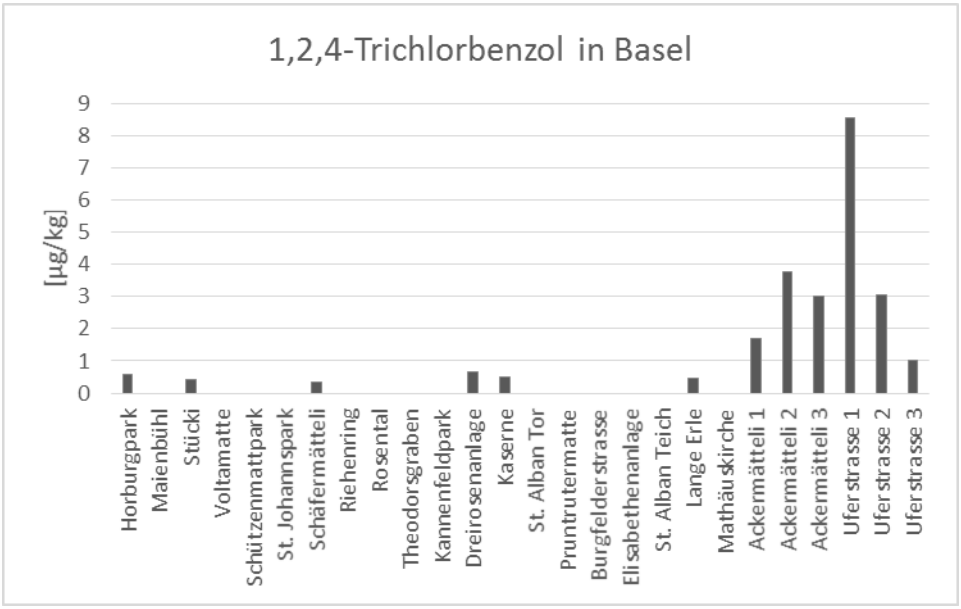


Figure 40. 1,2,4-Trichlorbenzol in Basel-city.

Detection of 1,2,4-trichlorobenzol in Basel-city shows increased values for the highly HCH polluted areas.

The origin of 1,2,4-trichlorobenzol can not be determined definitly by the carried out analysis. However, it is hypothesised that the contamination of 1,2,4-trichlorobenzol for the highly HCH-contaminated areas originates from the degradation of HCH. A similar, but lower pollution is also true for 1,2,3-trichlorobenzol.

In the further region only the Autal shows increased values for 1,2,4-trichlorobenzol. This sampled area is close to the beck Aubach and shows more elevated values for several PCBs, 1,2,3-trichlorbenzole and 2,4'-DDT. Further up the valley Au, there is a waste disposal which could be the source of the elevated values for all the OCls. Further investigations in this area could prove this hypothesis.

More OCl-pollutions are shown in the following figures.

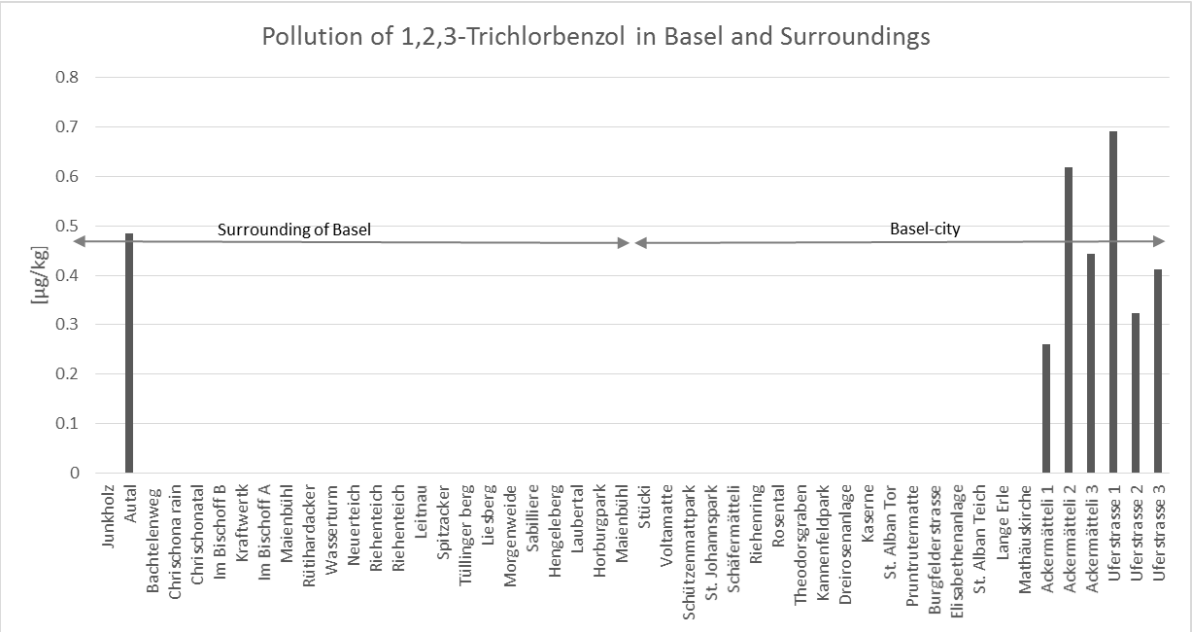


Figure 41. 1,2,3-Trichlorbenzol pollution in Basel and surroundings.

Detection of 1,2,3-trichlorobenzol in Basel-city and surrounds shows increased values for the highly HCH polluted areas and Autal.

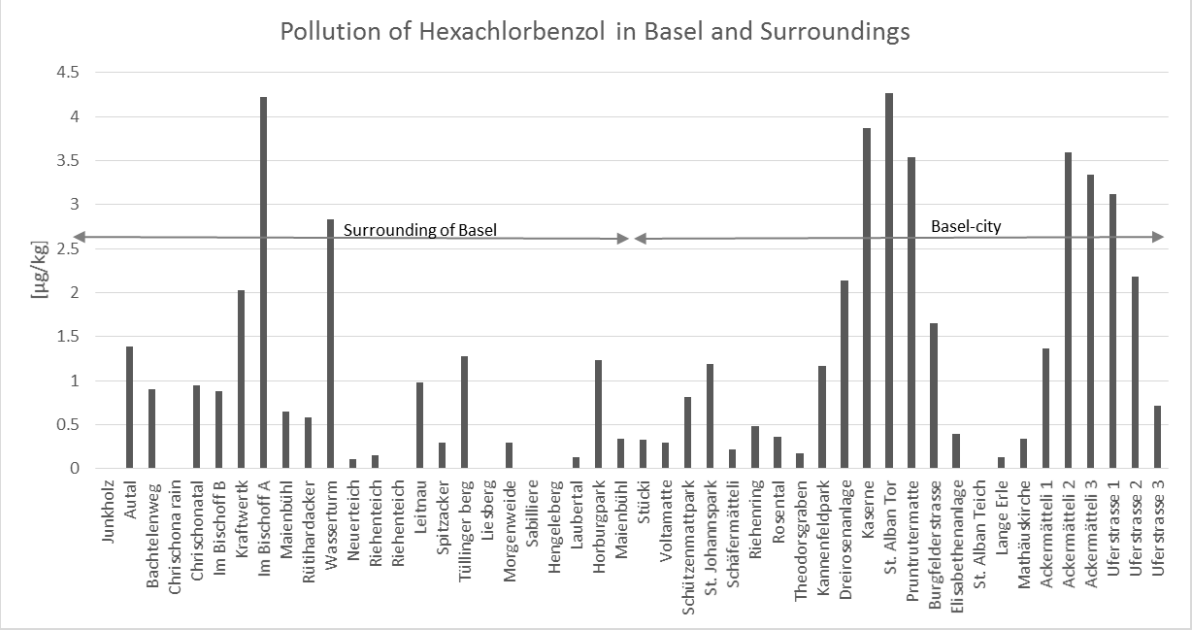


Figure 42. Hexachlorbenzol pollution in Basel and surroundings.

Detection of hexachlorobenzol in Basel-city.

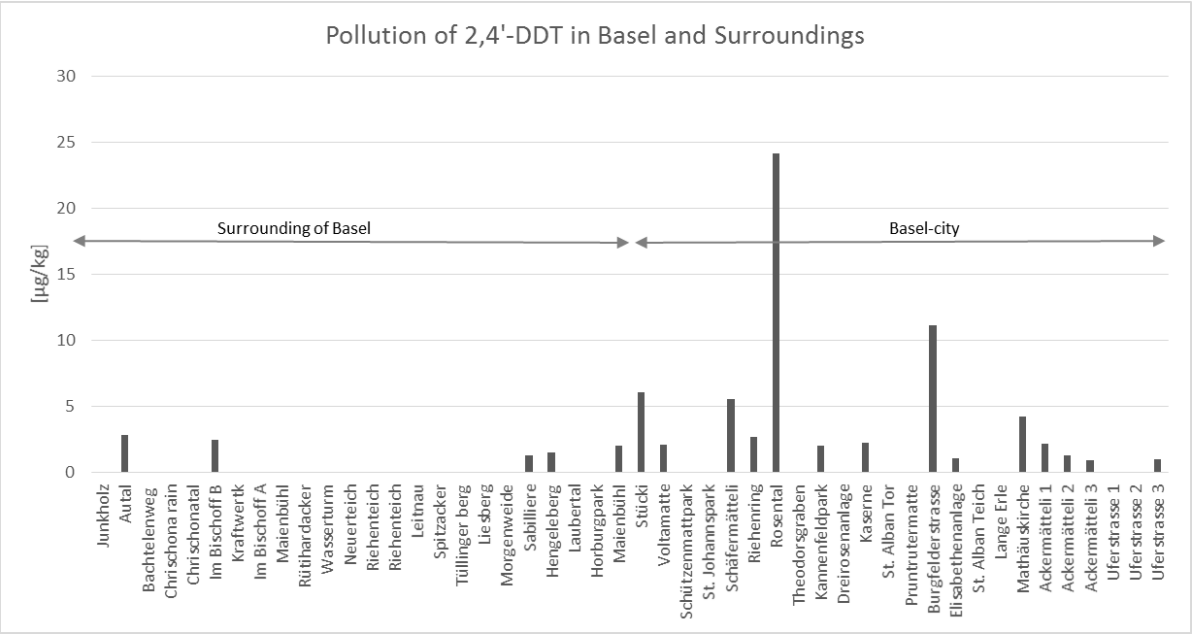


Figure 43. 2,4'-DDT pollution in Basel and surroundings.

Detection of 2,4'-DDT in Basel-city.

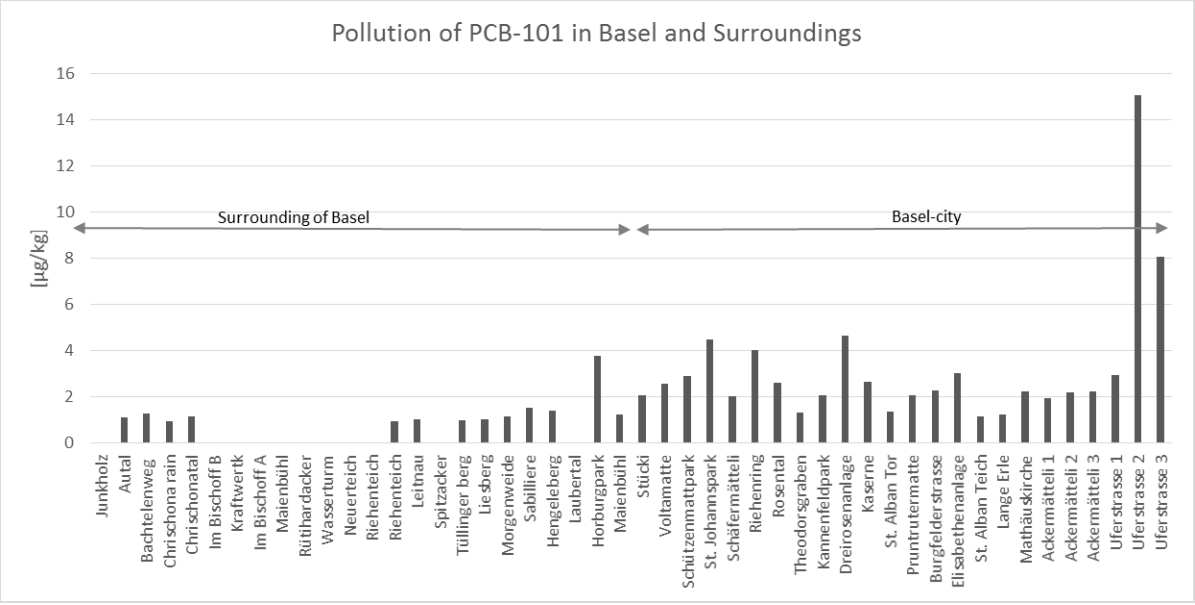


Figure 44. PCB-101 pollution in Basel and surroundings.

Detection of PCB-101 in Basel-city.

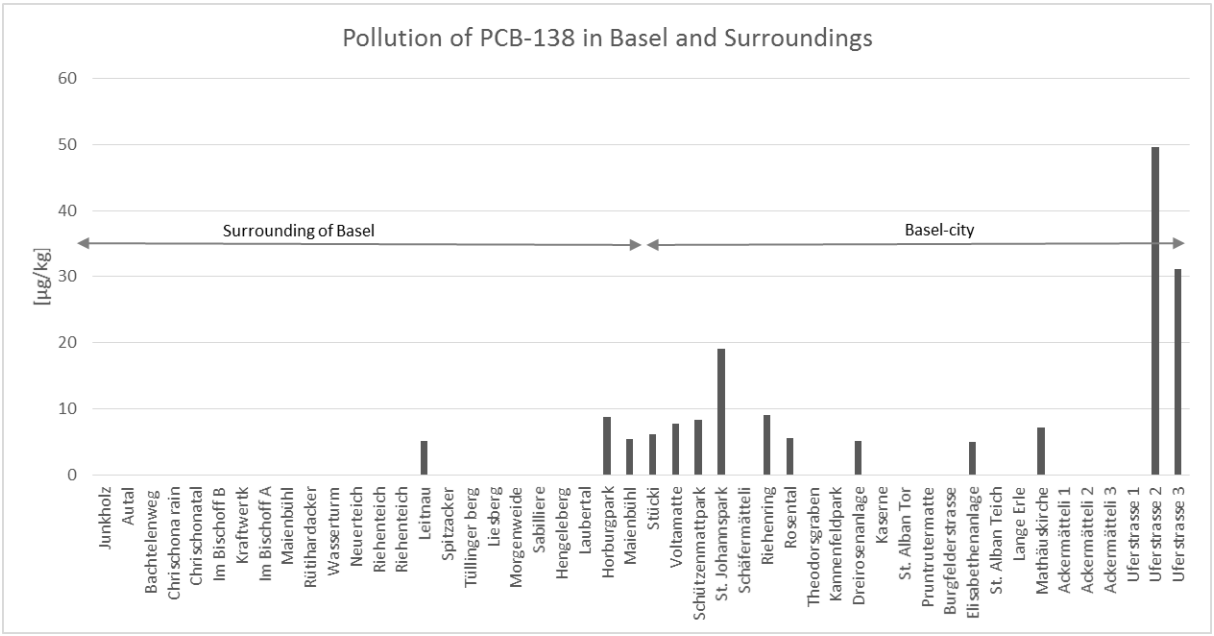


Figure 45. PCB-138 pollution in Basel and surroundings.

Detection of PCB-138 in Basel-city.

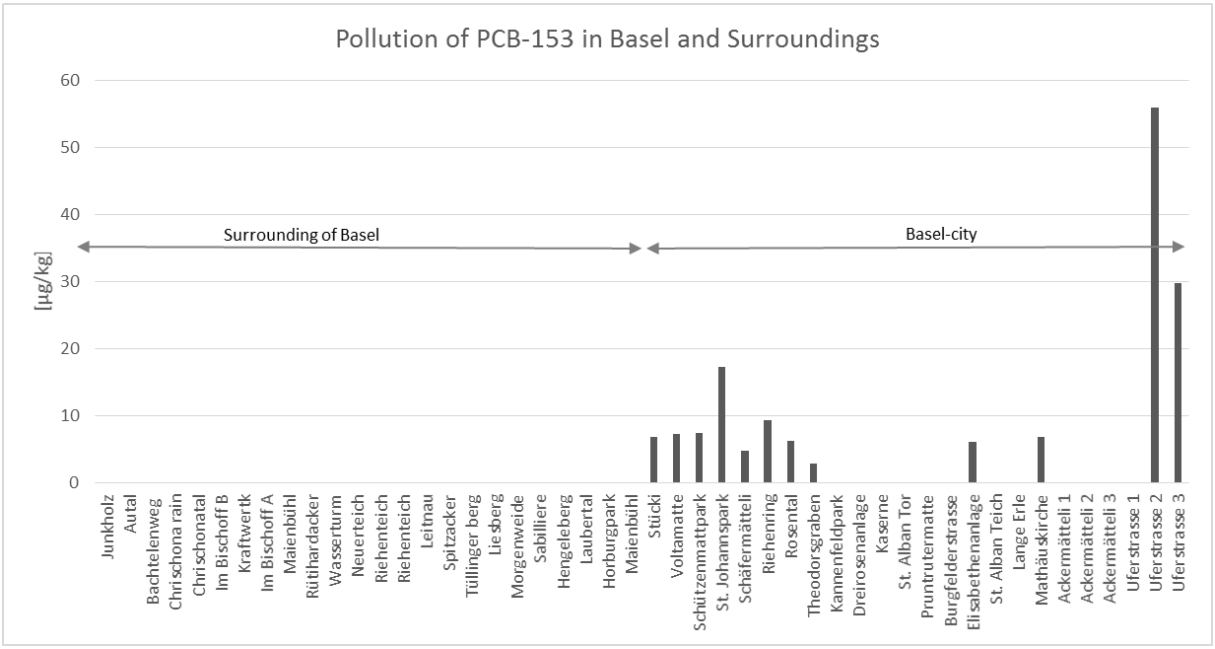


Figure 46. PCB-153 pollution in Basel and surroundings.

Detection of PCB-153 in Basel-city.

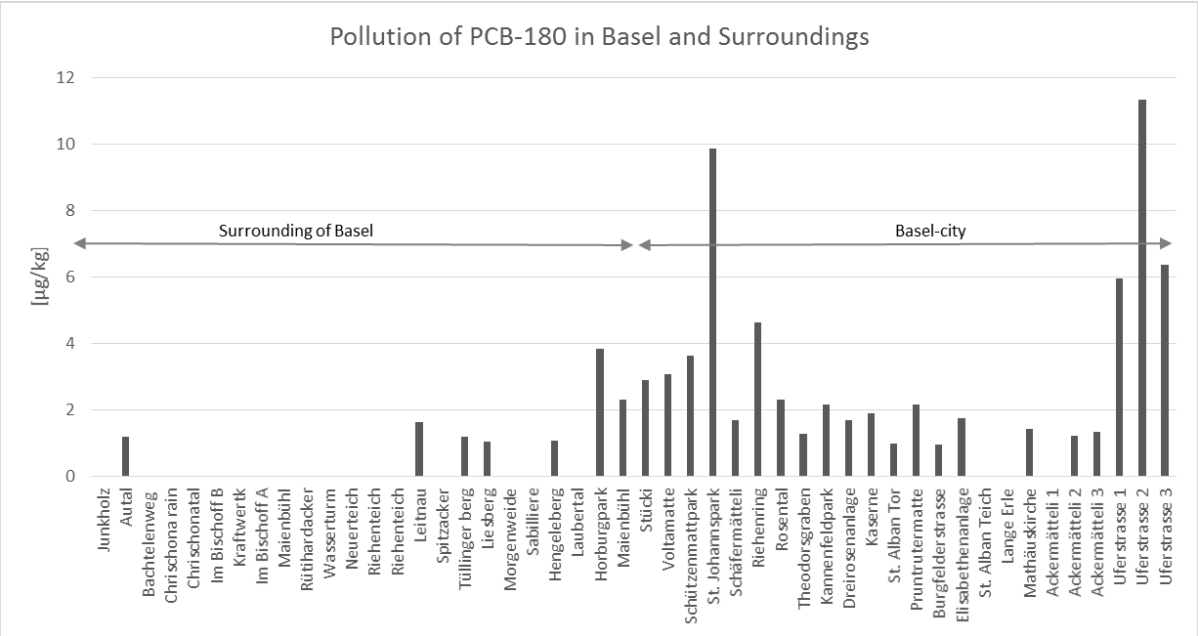


Figure 47. PCB-180 pollution in Basel and surroundings.

Detection of PCB-180 in Basel-city.

Along with hexachlorocyclohexane several other banned OCl have been found in the soils in the city of Basel and its surroundings as the figures above show. The results give a broad overview on the pollution in the soils. The results should be confirmed by further analysis, for example by a detailed analysis of the Au valley and its beck.

7. Conclusions

Several goals were addressed in this study. Extraction methods and their suitability for the evaluation of the pollution of hexachlorocyclohexanes in soils and environmental influences on the pollution were investigated. The extraction of hexachlorocyclohexane in soils was carried out by the evaluation of three extraction methods: accelerated solvent extraction, sonication extraction and hot Soxhlet extraction. Evaluation of the extraction methods by defined criteria lead to the conclusion, that 30 min hot Soxhlet extraction suffices to complete the extraction and is best suited for this study.

Validation of the method involved the investigation of a number of parameters and error sources and resulted in a well suited and verified method. Additionally, the robustness of the sampling procedure has been established. Thus specific sampling has shown that large objects, such as trees, do not produce local concentration hot spots due to rain washing down the HCH- deposits. A six-fold work up of one sampling area gave in-depth information on the homogeneity of the soil and its contaminants. The sampling method has proven to be robust and enables samples to be taken anywhere.

Investigation of the main wind influencing the pollution has been carried out by sampling soils in two rough circles around the ARA STEIH site. The larger circle covers the surroundings of Basel, whereas the smaller circle focused on the pollution in the city of Basel. Graphical and statistical analysis have shown that there is no influence of the main winds on the surroundings. Also, results have shown that the pollution in the further region of Basel is very low, with gamma- HCH (Lindane) as the predominant isomer. Results for areas in the city of Basel have shown increased concentration for beta- and alpha- HCH, which originate from the production wastes. It is therefore proposed, that the pollution in the surroundings of Basel originate also from usage of Lindane in the past.

A strong influence of the main wind direction on the pollution of beta- and alpha- HCH in areas closer to the ARA STEIH (Basel-city) has been proven graphically and statistically. Furthermore, a strong dependency of the distance from the pollution source on the beta- and alpha- HCH concentration has been confirmed. In total 21 different sampling locations, mostly park areas, have been sampled in the city of Basel. The majority of these areas have only slightly increased HCH- isomer concentrations, mostly beta- and some alpha- HCH. However, three sampling areas showed significantly higher concentrations with predominantly beta- and some alpha- HCH. These sampling areas are located close to the pollution source and lie in the main wind direction. Two of the sampling areas have been sampled three times to confirm the results. The legal limit of 1000 μg/kg was not exceeded.

Analysis of the river Rhein in 1974 showed a high abundance of alpha- HCH and lower concentrations of beta- HCH at that time. The soil analysis has revealed that the predominance

of alpha- HCH has changed over the years in favour of the beta- isomer. An explanation is the higher stability of beta- HCH compared with the other isomers. This result leads to the conclusion that the pollution originates from wastes from the former production site.
The origin of the pollution in the city Basel has been studied by sampling and analysing soil profiles in these areas. These have shown that the pollution is distributed throughout the sampled depth evenly. Since the mobilities of the HCHs are low, the major contamination originates from the time of the production of Lindane.

Pollution profiles of unperturbed soil have been sampled to investigate the mobility and the distribution of the hexachlorocyclohexanes. Although the HCH- isomers have very hydrophobic properties, a distribution throughout the sampled depth of 20 cm was observed. Statistical and graphical interpretation of the data lead to the conclusion, that the concentration of each isomer is not dependant on the depth; at least not to a depth of 20 cm. Furthermore, two profiles have been collected to a depth of 40 cm, which show similar concentration of all isomers to the measured depth. It is concluded that the pollutants are mobile in the soil. A column test was therefore made to investigate the mobility of the HCH- isomers in more detail. The laboratory experiment has shown, that all HCH- isomers have slight mobilities with water. However, the beta- HCH was retained more than the other isomers. This could also contribute to the altering of the isomeric abundance over time and can explain the higher concentration of beta- HCH in the soils of Basel. Further results show, that the adsorption on loam is much greater than the adsorption on sand layers.
The sampled soils have also been semi-quantitatively analysed for further organochlorines. Results for areas highly contaminated by HCH wastes, have shown an increased concentration of 1,2,4-trichlorbenzol, which is an alpha- HCH degradation product. This result reinforces the theory that degradation of alpha- HCH over time results in a high abundance of beta- HCH.

8. Outlook

The pollution by hexachlorocyclohexanes is an explosive topic discussed in media and politics. Recommendations on further actions have not been addressed in this project. Moreover, this thesis has been conducted to gather more information on the pollution. The scientifically based results are to be used as basis for further actions and discussions.

The sampling of the city Basel has shown that fortunately the pollution is very local. The pollution concentrations close to the ARA STEIH show vast pollution differences to other areas. In order to precisely localise all polluted areas, further samples should be taken nearby the highly contaminated areas.

The profiles sampled, also to a depth of 40 cm, show an even distribution of the pollutants. The sampling to even greater depth could lead to further interesting results on the mobilities of the HCH- isomers.

The carried out column has shown, that the pollutants could migrate into the leachate in the region of highly contaminated soils. Thus analysis of the ground water in these regions must be investigated.

New contamination due to the remediation process of the ARA STEIH is already routinely surveyed by the analysis of dust depositions and air pollution and should be continued.

The analysis of further OCl has revealed, that several other pollutants are present in the soil. Elevated values for several OCls has been found in the area of the beck Aubach. Thus further analysis on these pollutants should be considered.

The soil extraction method gives interesting results for more than just hexachlorocyclohexane. Once the samples are taken and homogenised, further data should be gathered after verifying of the analytical method.

9. Acknowledgement

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Especially I would like to thank Dr. Jan Mazacek who enabled the realisation of my master thesis. The confidence he has shown in me, the constructive discussions and superb guidance throughout this study have been a great motivation for me.

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During the entire study I was fortunate to have great support and understanding by my “personal research assistant” Marina Wetzel who has encouraged me and endured many late returns from work.

10. Abbreviations

AUE	Amt für Umwelt und Energie Basel-Stadt
ASE	Accelerated solvent extraction
DDT	Dichlorodiphenyltrichloroethane
FHNW	University of Applied Sciences Northwestern Switzerland
GC	Gas chromatography
HCH	Hexachlorocyclohexane
MS	Mass spectroscopy
OCl	Organochlorine
OPC	Organochlorine pesticide
PFE	Pressurised fluid extraction
PLE	Pressurised liquid extraction
POP	Persistent organic pollutant
PSE	Pressurised solvent extraction
QqQ	Triple quadrupole mass spectrometer
UV	Ultraviolett
VBBo	*Verordnung über Belastungen des Bodens*

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12. Safety

“Safety does not happen by accident.”

Therefore several evident measures are to be considered.

When entering a laboratory it is to be assured, that safety glasses, lab-coat and closed shoes are to be worn. Furthermore the clothing must be adequate. Thus ankles must also be covered.

For the sampling, safety precautions are to be taken.

Risks and Safety issues concerning chemicals:

n-Hexan

Classification	Highly flammable, harmful, irritant, dangerous for the environment
Flash point	-26.0 °C - closed cup

Hazard statements

H225	Highly flammable liquid and vapour.
H304	May be fatal if swallowed and enters airways.
H315	Causes skin irritation.
H336	May cause drowsiness or dizziness.
H361f	Suspected of damaging fertility.
H373	May cause damage to organs through prolonged or repeated exposure.
H411	Toxic to aquatic life with long lasting effects.

Precaution statements

P210	Keep away from heat/sparks/open flames/hot surfaces. - No smoking.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P273	Avoid release to the environment.
P281	Use personal protective equipment as required.
P301 + P310	If swallowed: Immediately call a poison centre (Tel. 145) or doctor/physician.
P331	Do NOT induce vomiting.
Personal protection	eye protection (safety glasses), skin protection (lab coat and gloves), respiratory protection (fume hood), body protection (flame retardant antistatic clothing and shoes).
Special notes	Fire measures: Call 118, use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Aceton

Classification	Highly flammable (F), irritant (Xi),
Flash point	-17.0 °C - closed cup
Hazard statements	
H225	Highly flammable liquid and vapour.
H319	Causes serious eye irritation.
H336	May cause drowsiness or dizziness.
Precaution statements	
P210	Keep away from heat/sparks/open flames/hot surfaces. - No smoking.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P273	Avoid release to the environment.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
EUH066	Repeated exposure may cause skin dryness or cracking.
Personal protection	eye protection (safety glasses), skin protection (lab coat and gloves), respiratory protection (fume hood), body protection (flame retardant antistatic clothing and shoes).
Special notes	Fire measures: Call 118, use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

HCH-isomers (α, β, γ and δ)

Classification	Toxic (T), harmful (Xn), dangerous for the environment (N)
Hazard statements	
H301	Toxic if swallowed.
H312	Harmful in contact with skin.
H332	Harmful if inhaled.
H351	Suspected of causing cancer.
H362	May cause harm to breast-fed children.
H373	May cause damage to organs through prolonged or repeated exposure.
H410	Very toxic to aquatic life with long lasting effects.
Precaution statements	
P263	Avoid contact during pregnancy/ while nursing.
P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing.
P301 + P310	If swallowed: Immediately call a poison centre (Tel. 145) or doctor/physician.
P501	Dispose of contents/ container to an approved waste disposal plant.
Personal protection	eye protection (safety glasses), skin protection (lab coat and gloves), respiratory protection (fume hood).
Special notes	HCH-isomers have different toxicities. Any kind of contact is to be avoided.

13. Attachment

Precision:

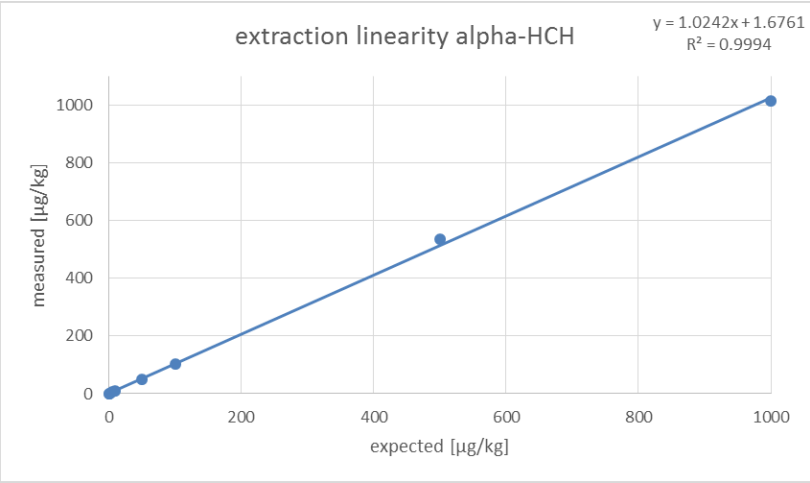
The detailed data for the precision is shown in the table below.

	alpha-HCH	beta-HCH	gamma-HCH	delta-HCH	epsilon-HCH
percision 1 [µ/kg]	5.5	5.2	4.7	4.7	10.9
percision 2 [µ/kg]	5.6	5.2	4.6	4.9	10.5
percision 3 [µ/kg]	5.2	5.2	4.7	4.5	10.2
percision 4 [µ/kg]	5.6	5.0	5.0	5.0	10.6
percision 5 [µ/kg]	5.7	5.1	4.8	4.6	11.0
percision 6 [µ/kg]	5.2	4.9	5.0	5.0	10.5
Average [µ/kg]	5.5	5.1	4.8	4.8	10.6
Standarddev. [µ/kg]	0.2	0.1	0.2	0.2	0.3
rel. Std [%]	3.6	2.0	3.8	4.4	2.7
recovery rate [%]	111.3	100.0	96.0	96.8	106.0

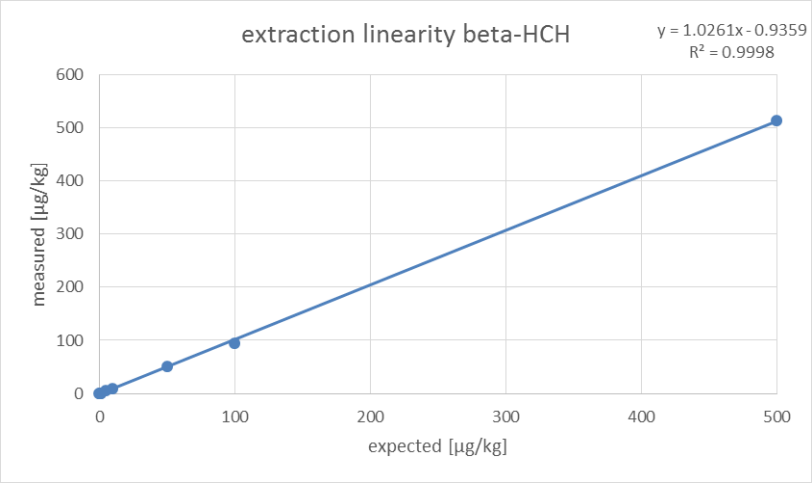
Linearity:

The Linearity of the extraction method has been evaluated and results are shown in the following illustrations:

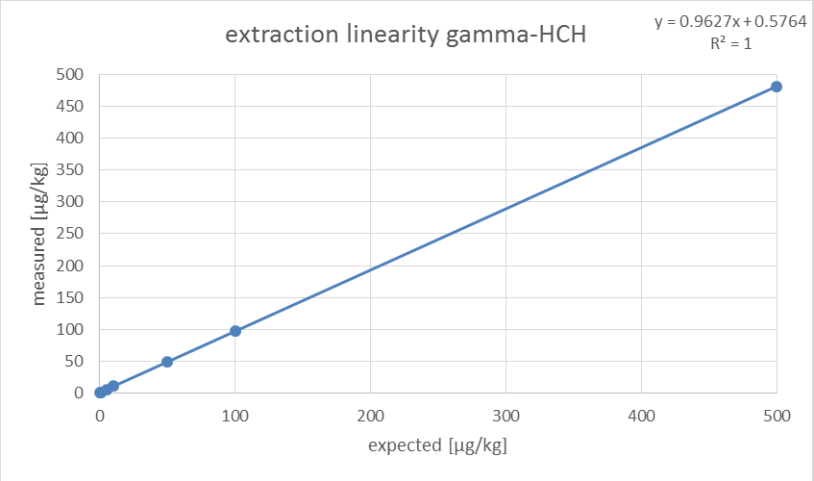
Extraction linearity of alpha- HCH



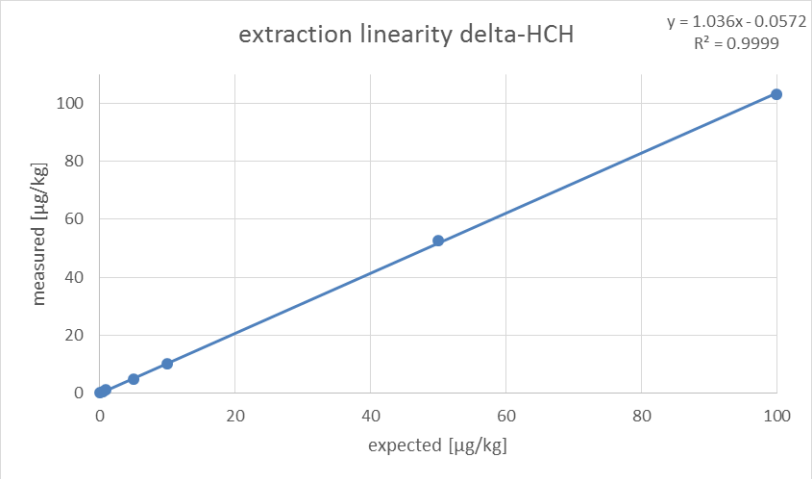
Extraction linearity of beta- HCH



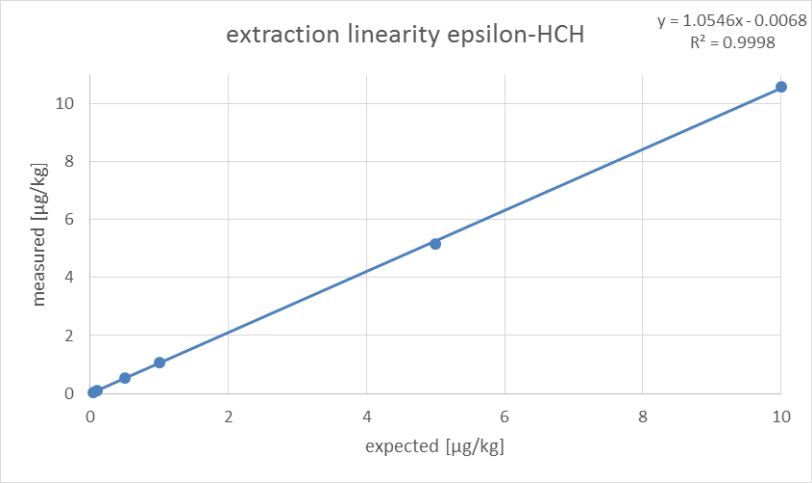
Extraction linearity of gamma- HCH



Extraction linearity of delta- HCH



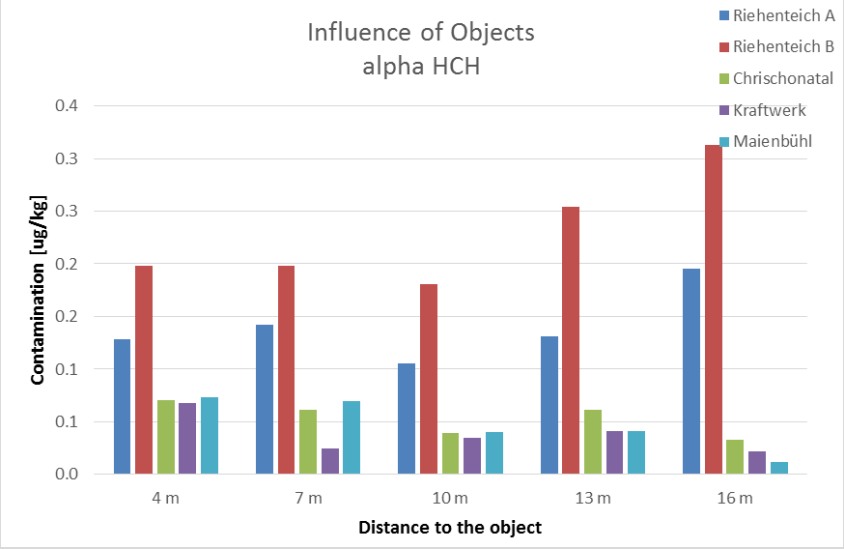
Extraction linearity of epsilon- HCH



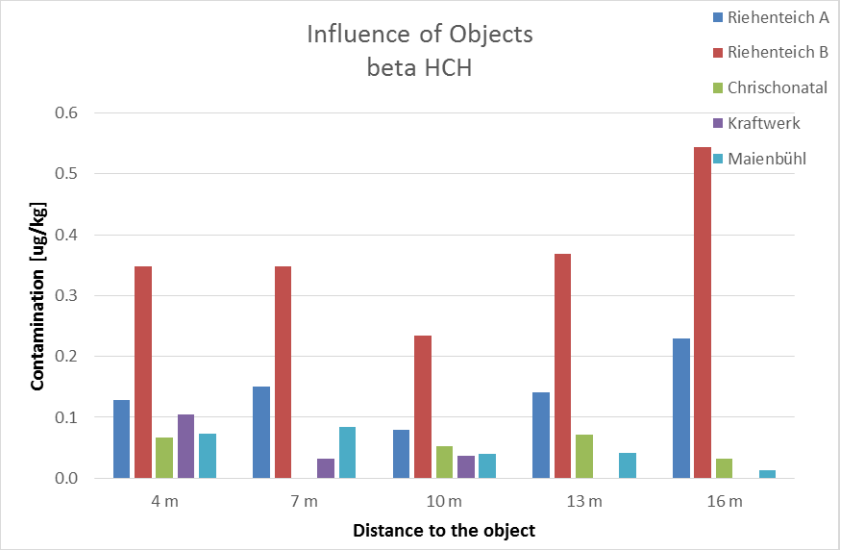
Objects influencing the pollution concentration

The influence of the sampling close to objects has been already shown for the sum of all isomers. The figures bellow show the results for each isomer:

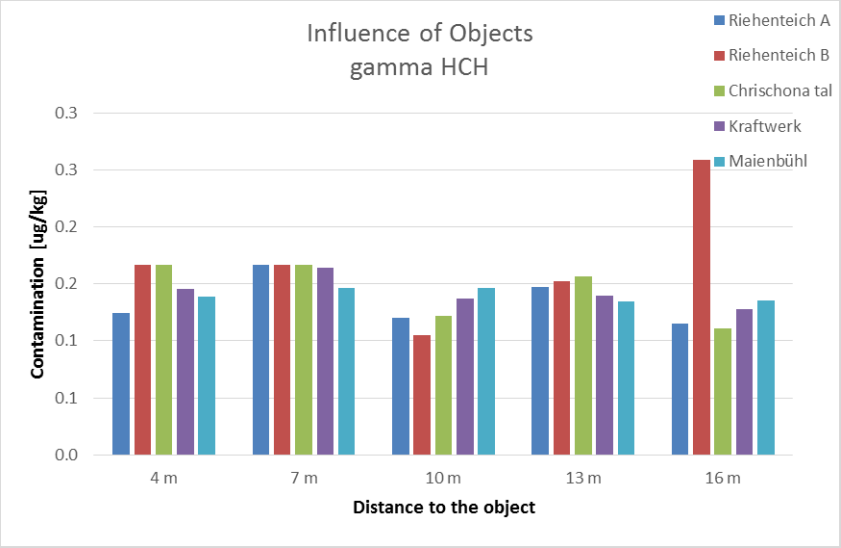
Influence of Objects on alpha- HCH



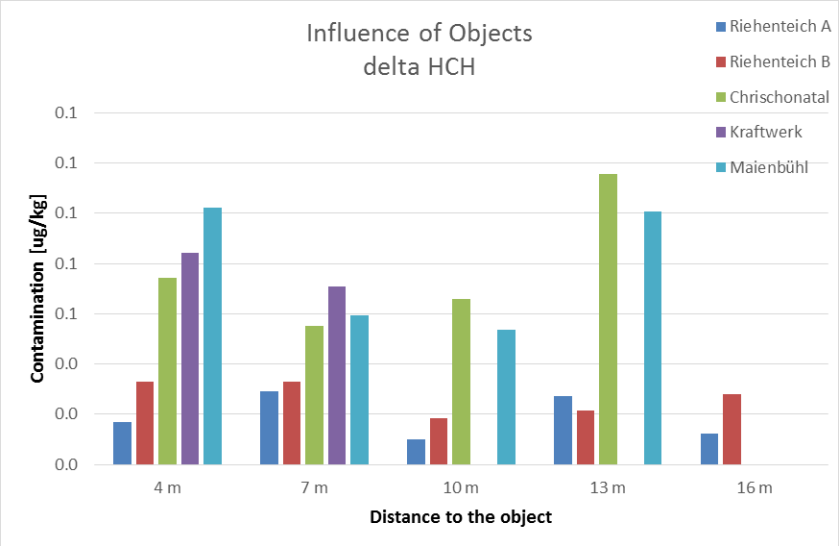
Influence of Objects on beta- HCH



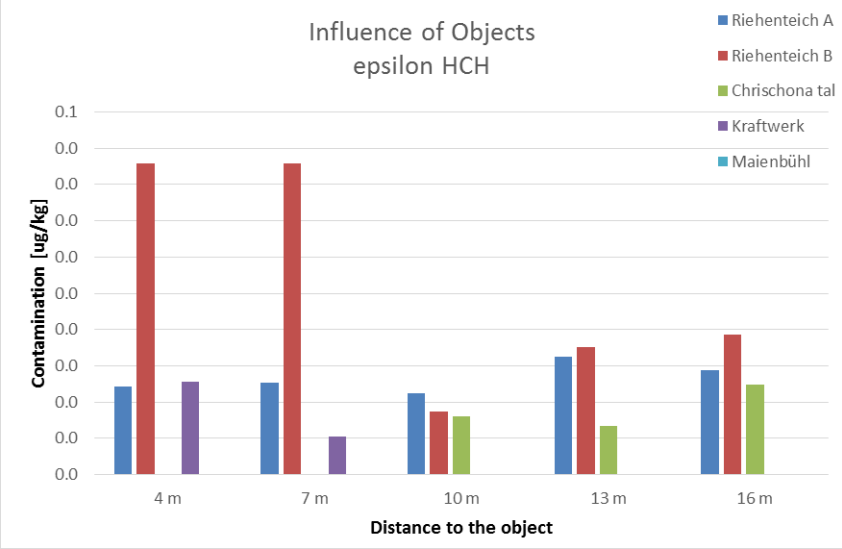
Influence of Objects on gamma- HCH



Influence of Objects on delta- HCH



Influence of Objects on epsilon- HCH



Declaration of Academic Honesty

I hereby affirm that the master thesis at hand is my own written work and that I have used no other sources and aids other than those indicated.
All passages, which are quoted from publications or paraphrased from these sources, are indicated as such.
This thesis was not submitted in the same or in a substantially similar version, not even partially, to another examination board and was not published elsewhere.

Place, Date Signature