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Differences in the selectivity of the metal uptake in perch and pike in the shoreline regions of a limed, acidified and neutral lake

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Sebastien Rauch helped with creating the project. He also did a lot of planning of the thesis and gave valuable guidance in the valuation of the results. He supervised the thesis. Sebastien also made vital contributions with the running of the ICP-MS (and everything around it).

Greg Morrison brought the fish and water samples from Anten.
Greg also contributed with creating the problem. Greg examined the report.

Both Sebastien and Greg helped with achieving the fulfillment of the report both by giving opinions and ideas.



Abstract

Current research regarding acidification mainly deals with the elements most commonly considered toxic. The researches are mainly carried through in acidified or contaminated environments. This paper investigates the presence of metals in fish and water in three different water systems; acidified, limed and neutral water systems. The fish species selected for the investigation were the pike and the perch with the exception for the neutral lake where only perch was caught. Measurements were performed in both the liver and the tissue for both fish species; the liver were chosen because the metals associated to the metallothioneins were of special interest (they are known to preferably accumulate in the liver) and the tissue because that is also interesting in the fish species.

The measurements performed were pH, alkalinity and several occasions when metals were analysed with ICP-MS (Inductively Coupled Plasma - Mass Spectrometer) technique. Two different analytical techniques were performed with the ICP-MS: first total quantitative analysis second, the more precise quantitative technique.

With the total quantitative technique most metals in the periodic table of the elements were measured simultaneously. Metallic elements that were found to have similar concentrations in the water samples in the total quantitative analysis were selected, along with several additional interesting elements, for the quantitative analysis. Within the total quantitative measurement the lakes were investigated separately and for the quantitative measurement the lakes were compared.

The total quant measurement indicated that the bioavailability of metals in the limed lake was maintained at high levels; the liming does not seem to decrease the bioavailability of metals. Almost all metals that were found in the water were also found in the fish in the limed lake. Biomagnification of metals is not considered a problem in the limed lake since only two elements biomagnified in the liver and tissue (Zn and Zr). This brings about the possibility that the perch binds metals more efficiently than the pike, or simply that different species binds metals more or less efficient. The exposure of metallic elements for the fish was found to make larger connections to the rate of accumulation than the dietary regime in the limed lake.

Metallic elements were not very available to the perch in the neutral lake (in relation to the limed and acidified lake). Most metals found in the water samples did not bind to the perch from this lake.

In the acidified lake the results were as expected; the metals were available to biota. The similarities to the limed lake were however surprisingly many (with a few unexpected exceptions). Biomagnification was not found to be a problem in the acidified lake since only two elements biomagnified in the liver and tissue (Pb and Hg).

From the quantitative analysis it was found that in the limed lake the bioavailability of metals actually increases to an extent larger than in the acidified lake compared to the neutral lake. The most surprising result was Aluminium, which was only found in the fish from the limed lake. The water concentrations of this element were however higher in both the acidified and neutral lake. It was found that the bioavailability of metallic elements in the acidified lake were, in many cases, maintained at a level similar to what was found in the neutral lake. It was also found that the concentrations of many toxic metallic elements were higher in the limed lake compared to the acidified lake, which was unexpected.



Table of Contents

| | |
|---|-----------|
| 1. Introduction..... | 3 |
| 2. Description of the problem | 3 |
| 2.1. Description of the lakes | 3 |
| 2.2. Sources of Pollution..... | 6 |
| 2.3. Differences in the ecosystem of Sandsjön, Rammsjön and Anten | 6 |
| 2.3.1. General Characterisation of the Pike and Perch | 6 |
| 2.3.2. Description of the ecosystem in the lakes..... | 7 |
| 2.4. Aims and Objectives..... | 7 |
| 3. Water chemistry: emphasized on the metals..... | 8 |
| 3.1. Introduction..... | 8 |
| 3.2. The Carbonate System..... | 8 |
| 3.2.1. Chemistry of Metals in Unacidified Lakes | 9 |
| 3.2.2. The Chemistry of Metals in Acidified Lakes | 9 |
| 4. General Description of the Cell: Its Potential as a Metal Binding Site..... | 10 |
| 4.1 Metallothioneins | 12 |
| 5. Pathways for the uptake of metals in fish..... | 12 |
| 5.1. The selectivity of the uptake..... | 14 |
| 6. Sampling | 14 |
| 6.1. Fish sampling strategy | 14 |
| 6.2. Water sampling strategy | 15 |
| 6.3. Measurement strategy..... | 16 |
| 7. Measurement techniques..... | 16 |
| 7.1. ICP-MS | 17 |
| 7.1.2. Total Quant Analysis..... | 17 |
| 7.1.3. The Quantitative Analysis..... | 18 |
| 7.2. Alkalinity and pH | 18 |
| 7.2.1. Alkalinity | 18 |
| 7.2.2. pH | 19 |
| 8. Results..... | 19 |
| 8.1. Results from pH and Alkalinity Measurements | 19 |
| 8.2. Results from Total Quant | 20 |
| 8.2.1. Results from water measurements (total quant analysis) | 20 |
| 8.2.2. Selection of elements for the quantitative analysis..... | 22 |
| 8.2.3. Three ways with which the elements are analyzed..... | 22 |
| 8.2.3.1. Analysis of results from Sandsjön | 23 |
| 8.2.3.1.1. Analysis of results in the first way | 23 |
| 8.2.3.1.2. Analysis of results in the second way..... | 26 |
| 8.2.3.1.3. The elements analysed in the third way..... | 27 |
| 8.2.3.2. Analysis of results from Anten | 28 |

| | |
|---|-----------|
| 8.2.3.2.1. Analysis of results in the first way | 28 |
| 8.2.3.2.2. Analysis of results in the second way..... | 30 |
| 8.2.3.2.3. The elements analysed in the third way..... | 31 |
| 8.2.3.2.4. Analysis of the perch milt..... | 31 |
| 8.2.3.3. Analysis of results from Rammsjön | 32 |
| 8.2.3.3.1. Analysis of results in the first way | 32 |
| 8.2.3.3.2. Analysis of results in the second way..... | 34 |
| 8.2.3.3.3. The elements analysed in the third way..... | 35 |
| 8.2.4. Summary from tot quant analysis | 37 |
| 8.3. Results from Quantitative Analysis | 37 |
| 8.3.0. Initial comments | 37 |
| 8.3.1. Results from water measurements (quantitative analysis)..... | 38 |
| 8.3.2. About the following Analysis of the elements | 41 |
| 8.3.3. Analysis of the elements | 41 |
| 8.3.3.1. Analysis of Copper | 41 |
| 8.3.3.2. Analysis of Zinc..... | 43 |
| 8.3.3.3. Analysis of Tin | 44 |
| 8.3.3.4. Analysis of Lead | 45 |
| 8.3.3.5. Analysis of Cadmium | 47 |
| 8.3.3.6. Analysis of Strontium..... | 48 |
| 8.3.3.7. Analysis of Sodium..... | 50 |
| 8.3.3.8. Analysis of Lithium | 51 |
| 8.3.3.9. Analysis of Magnesium | 52 |
| 8.3.3.10. Analysis of Iron | 53 |
| 8.3.3.11. Analysis of Cobalt | 55 |
| 8.3.3.12. Analysis of Antimony..... | 56 |
| 8.3.3.13. Analysis of Manganese..... | 58 |
| 8.3.3.14. Analysis of Nickel | 60 |
| 8.3.3.15. Analysis of Silver | 61 |
| 8.3.3.16. Analysis of Aluminium..... | 62 |
| 8.3.3.17. Analysis of Tellurium..... | 63 |
| 8.3.3.18. Analysis of Chromium..... | 64 |
| 8.3.4. Summary from quantitative analysis | 64 |
| 9. Reference list | 66 |

1. Introduction

The lakes that are used in this project are a part of a large fish treatment area in which several lakes are included. Most of the lakes are limed to prevent acidification, but some lakes have remained unlimed, so called reference lakes. These lakes are simply used as an indicator for showing how liming affects the water, environment etc of a lake. In this project the reference lake is acidified, but not to the extent that the fish are extinct, and therefore there are possibilities to make investigations of how the composition of different elements varies between water and fish between the two watersystems.

The study will give an assembled picture of most of the metallic elements in the periodic table of the elements. Their behaviour and presence in water and the accumulation of them in biota will be examined. There will be an investigation that compares a limed and acidified lake and the investigations performed will make it easy to understand the difference in behaviour between metals in the two most common types of water system in Sweden. A neutral lake has, for reference, also been examined.

This report first gives a description of the problem and then the “tools” needed for the investigation is included. Theoretical knowledge with emphasis on metals is also included. This refers to water chemistry and the description of the cell, proteins and the pathways for accumulation of metals in biota. In chapter two it was decided to include only relevant details. Therefore for pike and perch a complete description of the fish species is not developed; only information useful for the understanding and analysis of the results.

The information this project yields can for instance be useful for waste treatment of computers, electronic components and any other thing that contains metals, which accumulation on biota are not known and which concentrations in the lakes for this investigation are so high that any conclusions can be made.

2. Description of the problem

To give a complete picture of the project there will initially be a description of the lakes that have been used for the investigation, their sources of pollution and the similarities and differences they possess. After that the aims and objectives will be described in detail and also what should be investigated and obtained from the study.

2.1. Description of the lakes

Sandsjön and Rammsjön

The two lakes, Sandsjön and Rammsjön, see Figure 2.1, which are used for this investigation are situated in the western part of Småland, 40 km east of Halmstad. The two lakes are both acting as a startpoint for two different watersystems, meaning that no other large lakes are draining into Sandsjön or Rammsjön. Despite the small distance between the lakes (about 600 metres), the water they “produce” does not interact in any way; they are both the product of the same nature, but interactions of any kind with water exchange does not exist. This has been confirmed from the detailed maps by the environmental protection agency over the area, which shows the groundwater movement in the ground. The area around Sandsjön and Rammsjön, including the lakes, is part of an environmental protection area. The decision to make it an environmentally protected area started around 1950 when large quantities of

uranium were found in the moss areas. There were considerations about starting mining for uranium around this time, but then the environmental agency took over the area, and in that way protected it.

Sandsjön, is the bigger of the two lakes and it is being limed on a regular basis. Liming of the lake started around 1985 and since then the water quality has improved, which has supported several sensitive species, like roach, to return. The main inlet to Sandsjön is situated in the south of the lake and is unfortunately not shown on the map. This inlet drains from the moss and peat areas north of the lake. The inlet from Stensjön is smaller and waters only flows in this inlet at high water levels in Stensjön.

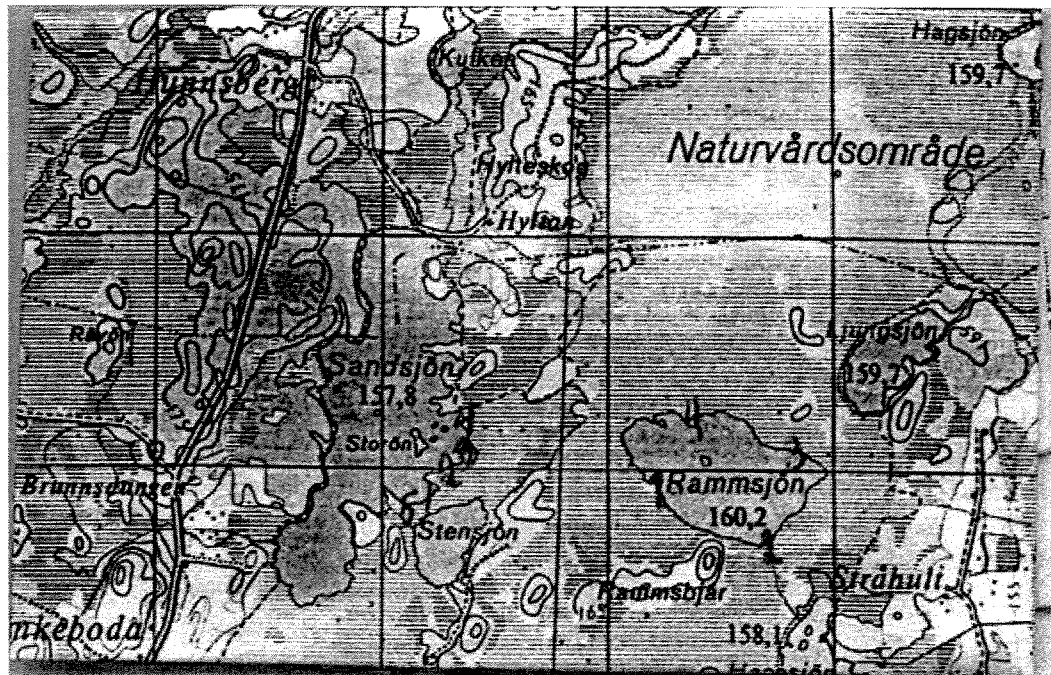


Figure 2.1.: the map shows the location of the lakes and the spots where water samples were taken

Both of these inlets can be considered to have a catchment area that is naturally acidified, and they are responsible for a charge to the alkalinity of Sandsjön. The environments around Sandsjön consist of either moss/peat areas (on the eastern side) or coniferous forest on the western side. In other words, poor-buffering environments surround the lake. The water in the lake flows in a south-north direction, the outlet in the north drains towards Kulken and eventually Bolmån. The discharge of the outlet is much larger than the sum of the known inlets, which suggests that there might be some groundwater upwelling in the lake. When investigating the topography, this might indeed be possible on especially the West Side where large slopes towards the lake are found. Sandsjön is quite shallow; the deepest known is around 5 metres. The sediments of the lake up to 1 metres depth is almost entirely consisting of sand and stones, exception is where water and fish samples were taken, where peat and mud is the main sediment. Sandsjön is a nutrient-poor forest lake. The bedrock around Sandsjön consists of fine-grained gneiss.

Rammsjön is the unlimed reference lake in the area. There is no single inlet to Rammsjön, instead there are approximately 15 smaller ditches that drain from the surrounding moss/peat areas into the lake. The outlet in the southern part of the lake drains towards Hagasjön, Krokån and eventually Lagan. During summertime in dry periods this outlet might run dry. When comparing the sum of the discharges from the inlets to the outlet, it can be concluded that approximately all water in the lake origins

consist of forest environments, and where the bedrock is gneiss and gravel. The remaining inlets to Anten originate from surrounding areas consisting of the same type of environment as previously mentioned. None of these inlets originate from another water system that might affect Anten in any way. The outlet in Anten is situated in the south of the lake, where it drains towards Åsjön and Mjörn. There are no moss or peat areas around Anten. The surrounding area consist mainly of forest and the shoreline often consists of large slopes towards the lake where the bedrock reaches the surface of the earth. The bedrock consists of gneiss and gravel. The whole area around Anten is considered to have a poor resistance against acidification. Anten is about 30 metres deep.

2.2. Sources of Pollution

General

The precipitation of sulphur and nitrogen oxides over the selected lakes is similar according to data from 1994. Results from any measurements on the average concentrations of metals in the precipitation over the lakes have not been found. This matter is further investigated in section 8.2.2. and 8.2.

Sandsjön and Rammsjön

In both Sandsjön and Rammsjön there are only natural sources of acidification. This means that in the area there are no human activities that affect the water in any way, i.e. industries, roads etc. All the inlets origins from areas that are naturally acidified, and the drainage areas are protected by law to remain that way. The roads on Figure 2.1. are small country roads used by people in the neighbourhood. The closest main road is situated 4 km south of the lakes, but the traffic of that road is not as dense as it could be regarded as a problem. There are no farming activities in the area releasing additional nutrients to the lakes.

Anten

There are no industries releasing contaminants to Anten in the surrounding area. The main source of pollution is the roads that follow the lake on the west side. There is a groundwater outtake in the north of the lake. The catchment area to this outtake is approximately the same as the main catchment area to Mellbyån. The area is protected from additional acidifying substances. I expect no contaminants from Sollebrunn, the town north of the lake, since there is a groundwater and protection area situated downstream the town. There is however a sewage treatment plant with discharge into Mellbyån. The catchment to Mellbyån mostly consists of cultivated land; the addition of nutrients and lime added to the lands drains into the river and thereafter the lake.

2.3. Differences in the ecosystem of Sandsjön, Rammsjön and Anten

2.3.1. General Characterisation of the Pike and Perch

The perch

The perch generally lives in large shoals, and exist in both the shallow and deep parts of lakes; the young fish usually resides in the shallow parts. As fry the perch feeds solely on different plankton species, but at the age of a couple of months they get a more varied dietary regimen. The perch feed on larvae, wateranimals, fishroes and fry and the big perch feed on smaller fishes and fry as well, preferably roach fry; it does

not feed on invertebrates. The perch can become 12-15 years old and reach a weight of about 2 kg (Camm, Christensen 1981).

The pike

The pike lives in solitude and does not live in shoals. The pike is known to live within a radius of 100 meter during its lifetime. The pike is a predator that feeds on almost all kind of fresh water fish species (including itself); it prefers roach and perch. In addition to fish it also feeds on frog fry (small pike) and it is common that bigger pike can attack small wild ducks at the surface. The pike can become 10-12 years old and reach a weight of 30 kg (Camm, Christensen 1981).

2.3.2. Description of the ecosystem in the lakes

Rammsjön

In Rammsjön, the only species existing are the pike and the perch. The perch exist in large quantities, and species at a weight above 100 gram are fairly rare. When dissecting them small insects and water animals were found in their stomachs. The pike, which probably feeds solely on these perch, exists rather sparsely, and therefore the ones caught seldom weigh less than 1 kg.

Sandsjön

In Sandsjön the fish species present are pike, perch, roach, bream and eel. In this lake, perch exist more sparsely, probably due to competition with other species. However the current fairly diverse composition of species can probably be attributed to the liming, and as a first consequence thereof. However, the pike still remains the top predator as in Rammsjön, but the perch should not be considered as the lowest fish specie in the food chain, as it is in Rammsjön. In Sandsjön it probably feeds on roach and small bream as well. The perch caught for this investigation was at the size at which they could feed on roach fry easily. However, the pike still remains at the level above the perch.

Anten

Anten contains a diverse composition of species like perch, pike, bream, pikeperch, burbot, roach, tench and trout. The main species present are perch, pike, burbot and roach. In this lake pike still remains the top predator but the pike is equal to species like pikeperch and trout but their presence is fairly low. From the rich content of perch and roach, it can be concluded that the pike feeds mostly on these species. Regarding the perch, it probably feeds on a larger array of fish fry than in Sandsjön and Rammsjön. However, since roach is one of the main species of the lake I suspect that it mostly feeds on these fry (with the addition of small water animals that the perch feed on in all three lakes).

2.4. Aims and Objectives

From the current literature there are several papers dealing with accumulation of a specific metal in a fish species in fresh waters. The main parts of those investigations concern mercury, cadmium, copper, zinc and lead and its accumulation and toxicity potential. In general, measurements of these metals are performed in waters that are contaminated or acidified, and therefore there has been an interest in investigating toxic metals. Based on this conclusion, decisions were made to make a quantitative

analysis of selected metals, see section 7.1.2., in three water systems that possess different properties. To see the effect of liming one limed lake and one acidified lake have been chosen, which are the ones described above, and to see the effect of liming one neutral unlimed lake has been selected. The investigations will include presence of metals in the water, and two fish species, pike and perch of which the pike is in the level above the perch in the food chain of the water systems. The study will investigate the following:

- The difference in metal content in the water in a limed, acidified and neutral lake
- Examine the difference in metal accumulation in pike and perch in a limed, acidified and neutral lake. Both liver/tissue and fish/water will be examined. The ratio Water : Perch : Pike will be examined.
- Investigate the behaviour of the metals associated to metallothioneins between pike and perch in a limed, acidified and neutral lake
- Try to gain some understanding from the results by looking at certain common properties of the metal ions
- Investigate how metal accumulation differs between pike and perch in a limed, acidified and neutral lake.
- Correlation between water and fish in the limed, acidified and neutral lake

The investigation will be based on comparing ratios between the different species and water, and as stated in point four there will be an examination of the common properties of the accumulated species. These properties are shown in section 5.1. The lakes were investigated separately and then there has been a comparing study between the lakes. The last point show the correlation between liver, tissue and water, which should reflect the metal binding site that most obviously reflects the environment the organism is exposed to. This study also shows to which of the selected organs (liver and tissue) different metals preferably binds. What is expected to be obtained from the study is to see how the selectivity of the metals, that are incorporated into biota, differs between a limed, acidified and neutral lake.

The study will not examine the toxicity potential of the elements selected. The aim is to see the difference in the bioavailability of metals in the three water systems selected.

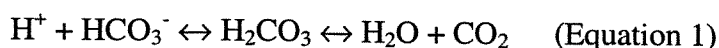
3. Water chemistry: emphasized on the metals

3.1. Introduction

The two water systems under investigation in this report are the limed water system and the acidified water system. Since the investigation will be based on finding relations between metal storage in fish in these two water systems, the emphasis in the water chemistry will be based on the behaviour and presence of metals in the separated systems. For the description of metals in unacidified water, references to the neutral lake is most certain. The report will investigate if the metals behave in a similar way in a limed lake as in a neutral lake.

3.2. The Carbonate System

The carbonate system is the main buffering system in lakes. Equation 1 describes the system:



If acidified water, containing hydrogen ions, will be discharged into a lake, the acidified water will be neutralized by the HCO_3^- -ions. Consequently, this system provides the water with an ability to resist acidification. However, this ability is limited, depending on the environment, i.e. the volume of the lake, the waters renewal time and physical properties of the surrounding environment. If the charge of acidifying substances becomes too high the pH will drop, because the buffering agent, HCO_3^- , is no longer present. This is the reason why there are unacidified and acidified waters, and in each of the systems the chemistry regarding i.e. metals will be different. (Jackson, 1996)

3.2.1. Chemistry of Metals in Unacidified Lakes

In all waters the matters contained therein will strive for equilibrium. This also appeals to the metal ions and they form complexes with different ligands in the water. These ligands can be both inorganic and organic. The main ligands that form complexes with metal ions are inorganic species like SO_4^{2-} , OH^- and CO_3^- and dissolved organic complexes. Many of these metal complexes precipitate to the bottom of the lake and they are removed from bioaccumulation in the organisms this way, mainly because the ability of the metal complexes to react and form metalloproteins is lowered due to the stability of the metal complexes formed. Regarding the formation of complexes between metals and organic ligands it has been found that Cu most easily ties to organic compounds. If for instance a large discharge of Cu appears into a lake, the Cu-ions will replace other metals in the complexes that they have formed with organic ligands. This in turn affects the equilibrium concentrations of other metal complexes. (Stumm and Morgan, 1996)

3.2.2. The Chemistry of Metals in Acidified Lakes

As stated earlier with the interactions between Cu, organic ligands and other metals, there is to an even larger extent interdependence happening between metals and ligand in an acidified lake. In an acidified lake there is one more species that compete with the metals in forming complexes and that is the addition of hydrogen ions. Within the acidified lake there is an abundance of cations simultaneously as the negatively charged ligands gets less and less (i.e. HCO_3^- and CO_3^-). This in turn means that the cation that forms the most stable complex with the ligands will most easily form complexes. Therefore in acidified waters hydrogen ions more or less takes over the metal ions previous function as a complex forming cation. Equations 2-5 in figure 1 show that hydrogen ions can successfully compete with metal ions for the available ligands and equations 6-8 in figure 1 shows that the proportion of free metal ions (or complexes containing a metal ion) increases markedly with lowered pH.



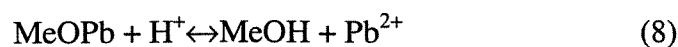
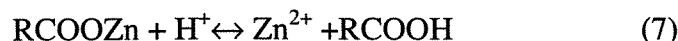
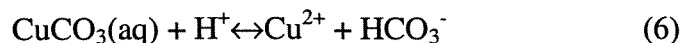


Figure 1: The effect of excessive hydrogen ions in acidified waters on the metal distribution in the water (Stumm and Morgan, 1996)

The metal ions that will be released from their complexes, due to excess hydrogen ions, is now available for biota and can be accumulated in the inner structure of cells in organisms (Stumm and Morgan, 1996).

4. General Description of the Cell: Its Potential as a Metal Binding Site

Before going in to more biological effects and metal complex formation in the organism it might be useful to give a founding description of the cell and its actors, which is where metals are finally incorporated in the living system. Figure 2 shows the animal cell and figure 3 shows the formation of a protein in a cell. The DNA stores the genetic information and is made up of nucleic acids, of which the somewhat negatively charged phosphate group is of importance as a metal binding site. The RNA is also made up of nucleic acids and their task is to translate the genetic information (mRNA) and bring amino acids (tRNA) to the ribosome (rRNA). (Hecht, 1996)

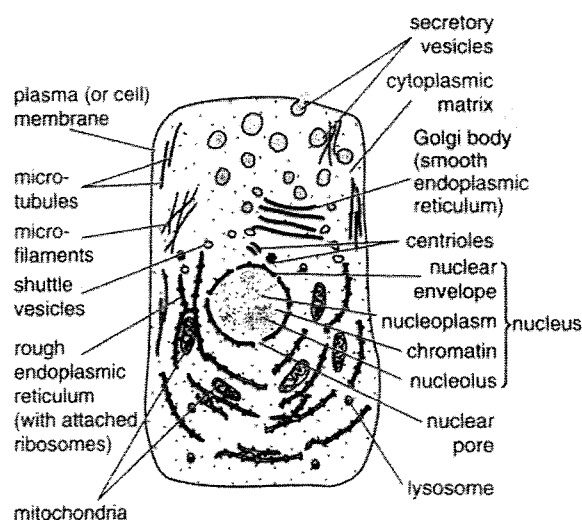


Figure 2: The structure of an animal cell (Jackson, 1996)

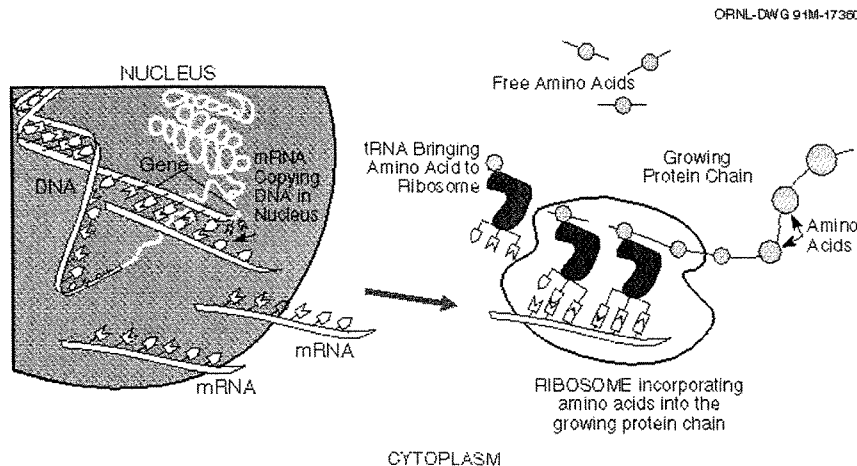


Figure 3: The interactions between DNA, RNA and the manufacturing of proteins (internet 1).

The process of protein manufacturing is called protein synthesis and is an important process for the cell. Within the protein synthesis a lot of the essential biochemical elements needed are produced. There are 20 different amino acids and they have a general form like shown in figure 4.

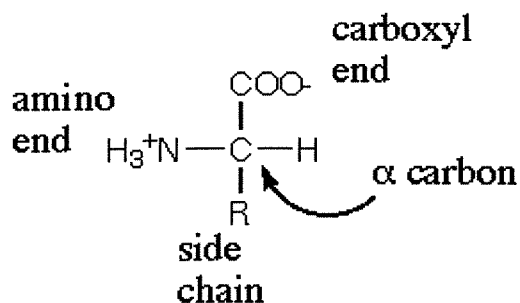


Figure 4: The general formula of amino acids (internet 2).

It is within the side chain that variation occurs and due to its composition metals can be more or less attracted to form complexes with amino acids. All 20 amino acids are pictured in appendix 1. A peptide is a protein containing 2-50 amino acids residues; a protein contains more than 50 amino acid residues. A residue is the name of each amino acid in a peptide molecule (internet 3).

Enzymes are proteins tailored to catalyze specific biological reactions between chemically reactive amino acids. The active site is the part of the enzyme that binds the substrate molecule of the enzymatic reaction and performs the conversion of the substrate to the product molecule. A cofactor is i.e. a metal ion that enzymes incorporate into their active sites to function properly. When the substrate is converted to a product both the product and the cofactor fall off the enzyme. The cofactor then is ready to repeat the process. A few cations are transported into and out of cells in this way, acting as cofactors. This type of transport, called active transport, is important for H^+ , Na^+ , K^+ , Ca^{2+} and Mg^{2+} . The activities of humans can seriously affect this type of transport. Ions not normally present in the environment, which are similar in charge and size to the natural substrate ions, can interfere with active

transport. Ions such as Cd^{2+} and Ba^{2+} are not normally abundant enough in the water to pose a threat, but can interfere with Ca^{2+} and Mg^{2+} uptake in polluted waters (Kustin et al., 1983)

4.1 Metallothioneins

One kind of protein that naturally binds metals are the metallothioneins. They are low molecular weight proteins and polypeptides of extremely high metal and sulfur content. Metallothioneins are thought to play roles both in the intracellular fixation of the essential trace elements zinc and copper, in controlling the concentrations of the free ions of these elements and neutralize the harmful effects of cadmium and mercury exposure. Metallothioneins are the only protein known that normally binds cadmium. However, cadmium is the only one of several optional metallic components, the others being most commonly zinc, copper, mercury and platinum. Metallothioneins, MT's, are mostly made up of cysteine and bind bivalent metal ions (internet 4). Metallothioneins occur throughout the animal kingdom and are most abundant in liver and kidney. There are wide variations in concentrations in different species and tissues, reflecting effects of age, stage of development, dietary regimen and other not yet fully identified factors. The binding of toxic metals to MT represents a sequester function associated with the cellular protection against metal toxicity (Linde et al., 1999)

5. Pathways for the uptake of metals in fish

When an organism is exposed to metals there is a potential for its accumulation in the fish body. The uptake of metals can take place in several ways and for fish the main ways are through the gills and the food. Figure 5 shows the pathways for the uptake and incorporation of elements into an organism. The initial chemical reactions, which are the basis for the uptake, are primarily equilibrium formulas between a ligand connected to the organism and an ion that appears in the water. These ligands can be dissolved organic material i.e. amino acids, fatty acids and carbohydrates. Once a metal ion has penetrated the organism/environment interface, it soon appears in the blood and the homogenous phase of blood called plasma. The difference between the water and the plasma is that the dissolved organic substances are of more significant concentrations and present to a larger extent as macromolecules, namely proteins, in addition to being present organic acids. This makes biological tissues very attractive for metal complex formation (K. Kustin et al. 1983)

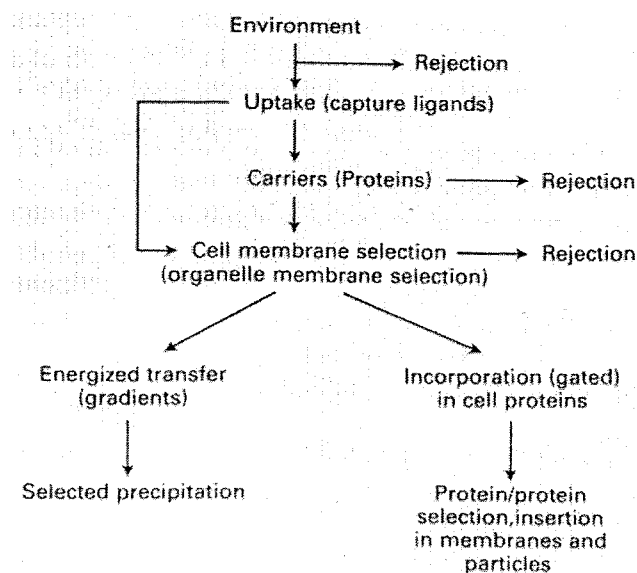
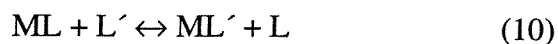


Figure 5: The pathways for the uptake and incorporation of elements into an organism (Frausto da Silva and Williams, 1991)

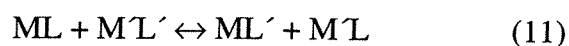
In equations 9-11 below M and M' are metal ions and L and L' represent ligands. The consequences for metal ion complex formation in making the transition from free water to cell are the shift in equilibrium from simple aqua metal ions to complexes (see equation 9 below). Equation 9 is determined by the occurrence of the reactive metal ions in the water, which are supposed to increase when pH decreases. However, these chemical species are not very stable, and the complex can be dissolved again if the organism and its surrounding media encounter a new equilibrium regarding the participating reactants.



Apart from the reactions between metals and ligands in the free water, the reactions in the organism are more a matter of metal exchange between two complexes. Equation 10 and 11 below are not obtained because of the striving for establishing chemical equilibrium, these are complexing reactions under thermodynamic control. This means that if an ion is present as a complex in the fish (equation 9), but the metal atom in this complex can form a more stable complex with another ligand that it encounters in the fish, then equation 10 will occur.



Equation 10 obviously is a matter of metal exchange between complex, but the stability of the complexes formed never gets as stable as they could be considered as irreversible. However, the stability of the complexes increases through equation 9-11. Equation eleven is the final site of the metal atom in the organism. It has hereby been incorporated into proteins and it does no longer take part in any diffusion reactions between other ions.



In this way metals are biomagnified, meaning that the higher you get in the food chain, the higher the concentrations of metals in the organisms should be (Frausto da Silva and Williams, 1991).

5.1. The selectivity of the uptake

The ability of an element to form complexes is mainly determined by the size of its ionic radius, its valence and by the structure of the electronic shells of the atoms. An increase in valence and decrease in ionic radius results in more stable complex formation (Singer 1973). Unfortunately, in this study conclusions on which ligands the metal atoms form complexes with cannot be made. From literature it has been found that certain metals prefer a certain geometry in the complexes they form with ligands as well as they prefer specific ligands to form complexes with. This means that if the concentration of a specific ligand is high, then the concentration of the metal that prefers to form complex with this ligand might also be high. These results can however only be speculated on. Figures 5, 6 and table 1 (see appendix 2) are general knowledge of the atoms and they will be useful when analysis of the results are performed. Also, the ratio between valence and metallic radius of the transition metals in table 2 (see appendix 2) have been included in the appendix, and in table 3 and 4 the elements are divided into groups based on their increased ratio (table 3) or increased radii (table 4).

6. Sampling

The collecting of samples in Rammsjön was done around the lake both regarding water and fish. This will give a uniform picture of the shoreline regions of the lake as a whole and there will be no site around the lake that deteriorates in any way, that samples will not have been collected from.

In Sandsjön sampling was only performed at shoreline regions closest to Rammsjön and that consisted of the same type of environment as in Rammsjön. This was done because it would be easier to make conclusions on environments that were very similar in most ways. By doing so the only difference between the water systems will be the lime in Sandsjön.

6.1. Fish sampling strategy

General

The fish was caught in a traditional way with a fishing rod. The fish was dissected within two hours after the catch. The liver and the white tissue were saved. The liver and the fish was then divided into three equally sized proportions and put into plastic bags, which were placed in the freezer. The liver and tissue were mixed separately and one liver cannot be connected to one (or two) tissues. It is expected that each of these plastic bags represent a uniform result of what metals are stored in the tissue and liver of the fish.

Rammsjön

The fish was taken at a uniform rate around the lake. Fishing for perch was performed on the 1:st of September, the weather was cloudy but no rain, temperature around 18 degrees. Fishing for pike was performed on the 6th of October, the weather was cloudy but no rain, temperature around 12 degrees. The average weight of the perch

was approximately 50 gram. Approximately 30 perch were caught; none weighed more than 100 grams. The average weight of the pike was approximately 600 grams. 4 pike were caught; one weighed approximately 1500 gram, the others between 300 and 600 gram.

Sandsjön

The fish was taken between spots 2 and 4, see Figure 2.1. Fishing was performed on the 6th of October. Both perch and pike were caught simultaneously. The average weights of the pike were 400 gram and the average weights of the perch were 100 gram (one was in the range of 500 grams). 4 pike were caught, all weighed approximately 400 grams. Approximately 20 perch were caught of which two weighed above 100 grams.

Anten

The perch samples from Anten were collected at the end of October. Five perch were caught, they all weighed below 100 grams. The perch were caught outside the inlet of Mellbyån in the north of the lake (see Figure 2.2.).

6.2. Water sampling strategy

General

Water samples were taken on the 2nd of September and 22nd of October in Rammsjön and 6th of October in Sandsjön. 1 litre water was collected at each place and the plastic bottles were stored in a frozen condition. The water was taken approximately on 0.5 metres depth in the shoreline regions of the lake. The water level in the lakes was at its normal level for the season, and there could be no reason for suspecting any momentary physical factors to affect the samples.

Rammsjön

The water samples were taken at points 1-4, see Figure 2.1, to give a uniform picture of the results in the lake. The day the water samples were taken it was raining and the air temperature was about 15°C. For pH and alkalinity measurements samples 1 and 3 were taken out to defrost. This was done because there would be a long time delay until the metals were measured and it was desired to keep at least two of the samples undisturbed in the fridge until measurements of metals were performed. The samples chosen were the ones situated most far away from each other, and they represent two different environments of the shoreline; site number 1 consist of more sand and stone type of environment, while site 3 represent purely bog and sphagnum environment. The same type of difference was also found when comparing the environments of site 2 and 4, so the results obtained from site 1 and 3 should be equal to those in site 2 and 4.

Additional samples collected on the 22nd of October were collected during a rainy period, and the water in the lake was approximately 40 cm above its normal level. These water samples were collected because of the results from the initial analysis where specific metals were found which were considered to require some more water samples. 5 samples were taken and they were collected at the same spots as shown on the map, with the exception of sample 5, which were collected at the east side of the lake, between spot 1 and 4. These results have however been decided to be treated with the same attention as the samples collected earlier. High water levels in the lake

is a natural phenomenon for the season, and the result will only to an larger extent contribute to give a better picture of the environmental situation in the lake.

Sandsjön

The water samples were collected in point 1-5, see Figure 2.1.. They were collected on the 6th of October; weather was cloudy but no rain, temperature around 12 degrees.

Anten

Water samples were collected at the beginning of November during a period of heavy raining. The water samples were collected at the inlet of Mellbyån in the north of the lake (see Figure 2.2:).

6.3. Measurement strategy

Sandsjön

From Sandsjön measurements were performed from seven different perch livers (randomly selected) and seven different perch tissues (randomly selected). Seven different measurements have been performed from the pike liver and tissue. Since only four pike were caught, unfortunately 3 samples origin from those four pike for the liver and the tissue.

Rammsjön

From Rammsjön measurements were performed from seven different perch livers (randomly selected) and seven different perch tissues (randomly selected). Seven different measurements has been performed from the pike liver and tissue. Since only four pike were caught, unfortunately 3 samples origin from those four pike for the liver and the tissue.

Anten

From Anten seven different measurements were performed from perch and seven different perch tissues. Since only five perch were caught, unfortunately 2 samples origin from those five perch for the liver and the tissue.

7. Measurement techniques

The procedure for the preparation and analysis of the samples are shown in figure 7. The water samples were acidified with HNO₃ to preserve the sample and then analysed by the ICP-MS. The fish samples had to be liquified before measurements in the ICP-MS could be performed. Approximately 0.2 gram of liver and tissue were put in the dryer. I took 7 samples from each species and each lake. After the dryer the samples were weighed more carefully (the dry weight was used for the concentration calculations). The samples were then acidified with HNO₃ before digestion in the microwave. The digestion in the microwave was carried through at a temperature around 200°, so the proteins would be decomposed as well. After the microwave the samples were diluted with nanopure water and put in the ICP-MS.

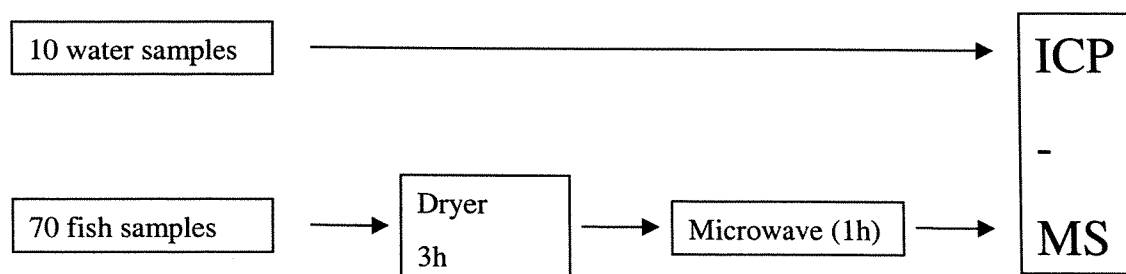


Figure 7: The procedure for the preparation and analysis of the samples

The concentration in the water samples was given direct by the ICP-MS. The concentration for the fish samples was calculated by equation 12:

$$C(\text{mg / kg}) = \frac{C * V}{m} \quad (\text{Equation 12})$$

Where

C = Concentration in the diluted fish sample from the ICP-MS in ppm

V = The volume to which the sample was diluted (100 ml)

m = The dry weight

7.1. ICP-MS

The ICP-MS is a very precise analytical technique that performs elemental analysis with a high sensitivity and a low detection limit. The ICP-MS can perform semi-quantitative analysis and quantitative analysis. The ICP-MS can simultaneously measure most elements in the periodic table. The ICP (inductively coupled plasma) is the ionisation source in the instrument and the MS (mass spectrometer) filters the ions produced in the plasma. The procedure for the ICP-MS in more detail is as follows: The liquid samples are introduced by a pump to the nebulizer where the sample aerosol is formed, which is then introduced to the plasma. In the plasma, Argon gas is introduced, via the ICP torch (a series of concentric quartz tubes). The collisions between Argon atoms in the torch generates a high-energy plasma, and when the sample aerosol is introduced to this plasma it will instantaneously decompose, and form analyte atoms which are simultaneously ionized. The ions produced are extracted from the plasma into the mass spectrometer region which is held at high vacuum and the ions are separated based on their mass/charge ratio. Finally, the ions are measured using an electron multiplier, and are collected by a counter for each mass number (Houk, 1985).

7.1.2. Total Quant Analysis

The Total Quant analysis is a semi-quantitative method for investigating what elements are present in the samples. With this method most elements in the periodic table of the elements can be measured simultaneously. The results found are not as reliable as the ones obtained from the quantitative analysis and should therefore not be considered as definite. The main reason for this is that the ICP-MS correlates the

concentration for those elements against an internal standard. The Total Quant analysis can analyse the following metallic elements (Table 7.1.):

Table 7.1: The elements analysed in the tot quant technique

| | | | | | | |
|----|----|----|----|----|----|----|
| Li | Cr | Y | Sn | Gd | W | Th |
| Be | Mn | Zr | Sb | Tb | Re | U |
| Na | Fe | Nb | Cs | Dy | Os | |
| Mg | Co | Mo | Ba | Ho | Ir | |
| Al | Ni | Ru | La | Er | Pt | |
| K | Cu | Rh | Ce | Tm | Au | |
| Ca | Zn | Pd | Pr | Yb | Hg | |
| Sc | Ga | Ag | Nd | Lu | Tl | |
| Ti | Rb | Cd | Sm | Hf | Pb | |
| V | Sr | In | Eu | Ta | Bi | |

7.1.3. The Quantitative Analysis

The quantitative analysis is more precise since the elements selected for this analysis have a specific standard solution for different concentrations of the metallic element programmed to the ICP-MS. This means that the ICP-MS does not have to correlate the concentration for a specific element and therefore produces more accurate results. For the quantitative analysis, the only elements selected for analysis are those to which a standard solution is available.

7.2. Alkalinity and pH

7.2.1. Alkalinity

Method: Titration method was used. 50 ml of the sample was taken out, to which three drops of liquid indicator were added. The sample was then stirred and carbon dioxide was added to keep the alkalinity prevailed and not be changed from reactions with the surrounding air. Then the hydrochloric acid was added to the sample. When the indicator shifted colour from grey to greyish red the alkalinity of the water was zero. The required amount of HCl was then observed.

For calculating the alkalinity the following equation was used (equation 13):

$$A = \frac{c(\text{HCl}) * (V5 - V6) * 1000}{V4} \quad (\text{Equation 13})$$

Where

A is the alkalinity or the concentration of hydrogen carbonate in millimoles per litre

c(HCl) is the concentration of the hydrochloric acid used for titration. c(HCl) is 0.02 moles per litre.

V4 is 50 ml. This is the volume of the sample used for titration.

V5 is the required amount of HCl for the titration in millilitres.

V6 is the average value of the required amount of HCl from three titrations, where nanopure water was used for volume V4. This volume was calculated to 0.3 ml.

7.2.2. pH

Method: About 100 ml of the sample were taken out and put into a glass beaker and an electrode was put into the water, which read the pH-value. The water was stirred while the electrode read the pH.

8. Results

8.1. Results from pH and Alkalinity Measurements

General

The results found from the water measurements in the limed acidified and neutral lake were very much as expected and therefore the hypothesis about the lakes selected really being a limed, acidified and neutral lake can be confirmed.

Sandsjön

Table below shows the results from pH and water measurements. The results are as expected, perhaps the pH is higher than what was expected. The alkalinity however seems to follow an opposite trend compared to the pH and is the highest when the pH is the lowest. The results are however propitious for continued analysis. Average pH is 7.4 and average alkalinity is 0.16 mM/l (see table 8.1.).

Table 8.1: The results from water measurements of alkalinity and pH from Sandsjön.

| | Site 1 | Site 2 | Site 3 |
|--|--------|--------|--------|
| pH | 7.31 | 7,42 | 7,45 |
| Alkalinity (mM/l of HCO_3^-) | 0,248 | 0,132 | 0,1 |

Rammsjön

I only measured pH and alkalinity in two sites but the values obviously are very similar. The alkalinity is as expected 0 and the pH is just above the level at which the perch can maintain reproduction. I can hereby conclude that this water is very poor in negatively charged complexes (the carbonate system is the main buffering system in the selected lakes). Average pH is 5.16 (see table 8.2.).

Table 8.2: The results from water measurements of alkalinity and pH from Rammsjön.

| | Site 1 | Site 3 |
|--|--------|--------|
| pH | 5.15 | 5.17 |
| Alkalinity (mM/l of HCO_3^-) | 0.0 | 0.0 |

Anten

The results from Anten, table below, are fairly similar to the ones in Sandsjön. The pH is somewhat lower while the alkalinity is fairly similar to what was found in Sandsjön. Average pH is 6.8 and average alkalinity is 0.19 mM/l (see table 8.3.).

Table 8.3: The results from water measurements of alkalinity and pH from Anten.

| | Site 1 | Site 2 | Site 3 |
|---|--------|--------|--------|
| pH | 6,8 | 6.82 | 6,76 |
| Alkalinity (mM/l of HCO ₃ ⁻) | 0,12 | 0,26 | 0,18 |

8.2. Results from Total Quant

In the following chapter the elements will be analysed based on the concentration of metallic elements in the three lakes from the total quant analysis. These results will be used for the selection of which elements will be investigated more carefully in the quantitative analysis. This investigation is however included in this part also .

Due to the unknown presence of metals in the area around the lakes (and in the lakes) that origin from either anthropogenic sources or natural sources the analysis of the lakes performed here is carried through separately. The chemical difference between the lakes (regarding acidification) still remains but the result found may not only be a consequence thereof. However it is worth pointing out that the difference between Sandsjön and Rammsjön should probably be due to this difference mentioned, since this is what was aimed for when the sampling was performed (see section 6). For further investigation in comparison between the three lakes, see analysis of results from quantitative analysis.

The unit for all the fish samples is mg/kg and the unit for the water samples is µg/kg. These units have been kept to eliminate all the zeros and large numbers that would be present in quotas and water concentrations otherwise. Whenever a ratio between fish and water is below one it does not mean that the water concentrations are higher; the ratio should be multiplied by a thousand. However, there is no sample where a ratio between fish and water exist that admits that the water concentration of metallic elements is higher than the concentrations found in the fish.

Firstly, the water results will be discussed and then the analysis of the fish results will be performed.

8.2.1. Results from water measurements (total quant analysis)

The results from the water measurements will later in the study give the preconceptions for which elements that will be chosen for the comparison between the lakes. The concentrations of metals in the water also reflect the degree to which the pike and perch is exposed to different metallic elements. The findings from the water measurements are however somewhat contradictory. They show that the most diverse composition of metallic elements, as well as the largest concentration of metallic elements are found in the neutral lake. Table 8a show the lake that contains the highest and lowest concentration of the metallic elements measured. The two most surprising findings is that the aluminium concentration is remarkably higher in the neutral lake than in the acidified lake, and the calcium concentrations is higher in the

unlimed, neutral lake, than in the limed lake. There are no larger differences in the concentrations between the limed and acidified lake; the concentrations of metallic elements in general are somewhat higher in limed lake. The concentration of aluminium is zero in the limed lake and the calcium concentrations is about 10 times higher in the limed lake; the calcium probably originates from the lime. The acidified lake contains the highest concentration of Zn, Pb, Cd and Ag, which is what I expected from the acidified lake. Referring to the chapter of chemistry, and especially equation 2-8, it is possible that the metals in the neutral and limed lake are more tightly bound to inorganic negatively charged complexes. This means that the metal ions will not be incorporated in to biota to the same extent as in the acidified lake, where negatively charged complexes are more or less absent. Therefore these results, from the water, are found to be very propitious, especially when the rate at which the elements are incorporated in to biota is considered.

Another point of interest here can be referred to what was said in section 5.1., about the importance of the ratio between valence and radius when a metal ion is forming a complex. It is possible that there is a clearer connection between increasing ratio and the concentrations of metallic elements in the fish and water in the acidified lake than in the neutral and limed lake. This would in turn mean that the metals are more available to biota in the acidified lake than in the limed and neutral lake.

Table 8a: The elements sorted after the concentration in the water samples (N=neutral lake, L=limed lake, A=Acidified lake)

| Element | Highest Conc. | Middle Conc. | Lowest conc. | Element | Highest Conc. | Middle Conc. | Lowest conc. |
|---------|---------------|--------------|--------------|---------|---------------|--------------|--------------|
| Li | N | L | A | Rh | N | L | A |
| Be | N | - | - | Pd | N | - | - |
| Na | N | L | A | Ag | A | - | - |
| Mg | N | L | A | Cd | A | L | - |
| Mg | N | A | L | In | N | - | - |
| K | N | L | A | Sn | L | A | - |
| Ca | N | L | A | Sb | N | A | L |
| Sc | N | L | A | Cs | N | A | - |
| Ti | N | L | A | Ba | L | N | A |
| V | N | - | - | La | N | L | A |
| Cr | - | - | - | Ce | N | L | A |
| Mn | N | A | L | Pr | N | L | - |
| Fe | N | A | L | Nd | N | L | A |
| Co | N | A | L | Sm | N | - | - |
| Ni | N | L | - | Gd | N | - | - |
| Cu | N | L | A | Dy | N | - | - |
| Zn | A | N | L | Er | N | - | - |
| Ga | N | A | - | Yb | N | - | - |
| Rb | N | L | A | Hf | N | - | - |
| Sr | N | L | A | Pt | N | - | - |
| Y | N | L | A | Tl | N | L | - |
| Zr | N | - | - | Pb | A | N | L |
| Nb | N | A | L | Bi | L | A | - |
| Mo | N | - | - | Th | N | A | L |
| Ru | - | - | - | U | N | L | A |

8.2.2. Selection of elements for the quantitative analysis

For the comparing study between the lakes selected, the metals were chosen that have similar concentrations in water samples from the three lakes. This selection was done because it will be easier to make conclusions on the difference in the selectivity of the metal accumulation of the fish species. This selection was based on the elements present in the water samples of which the ratio between the water samples did not exceed 10. Thereby it has been taken into account that the natural presence, as well as the metal composition of rainwater, of specific metals might differ. This is especially true between the neutral lake and the limed/acidified lake because of the large difference in distance between this lake and the others.

The selection of which elements, that will be chosen to be analysed in the quantitative method, is based on the concentrations of metallic elements found in the water samples from the total quantitative analysis. The elements that were found not to have a ratio between the water samples that exceeded 10 are written in table below. The ratio is regarding the maximum and minimum concentration.

Table 8b: The elements selected for the quantitative analysis and the ratio between the average value on the concentrations of the elements in the water samples from the three lakes (max conc./min conc).

| Element | ratio | Element | ratio |
|---------|-------|---------|-------|
| Li | 8,9 | Zn | 5,3 |
| Na | 2,4 | Rb | 6,8 |
| Mg | 4,2 | Sr | 7,4 |
| K | 7,1 | Rh | 3,5 |
| Mn | 3,4 | Sb | 1,2 |
| Fe | 3 | Ba | 1,9 |
| Co | 7,4 | Pb | 7,5 |
| Cu | 2,6 | | |

Table 8b shows that Sb is very similar between the three lakes and therefore it is very favourable for making comparative analysis between the lakes. In all elements above the water concentrations were highest in the neutral lake with the exception for Zn and Pb, which had the highest concentrations in the acidified lake, and Ba, which had the highest concentration in the limed lake.

8.2.3. Three ways with which the elements are analyzed.

There are three ways in which the elements are analysed. Firstly the elements are sorted based on the increasing logarithm on the ratio between water and fish. Secondly, the elements are sorted after the increasing value on the logarithm for the water concentrations. In the second way it has been taken into account the possibility that diffusion affects the rate at which the elements are incorporated to biota. In the second way elements that were only found in the fish have been excluded, because of difficulties of finding a valid ratio for those elements between the water and fish. In these two ways it has been assumed that the accumulation of metals is not depending on what the species feed on (biomagnification is neglected). These tables assume that the environment the organism is exposed is of greater importance for accumulation of metals than the accumulation potential of the metals in the food the organism feed on. The third way with which the elements are analysed is a comparison between the concentrations of metals in the organs selected. In this way it has been assumed that

the most important factor for accumulation of metals is dependent more on the dietary regimen than the surrounding medias content of metals. In this way it has been taken in account biomagnification, and the study is more of a biological matter than of a chemical investigation where ions are more or less able to react with organic matter due to their properties. These tables do not give any reference to concentrations (those are shown in the correlation graphs, see text below and appendix). These tables show how different metallic elements are biomagnified between pike and perch, as well as where the largest concentrations of metallic elements are found between tissue and liver. Whenever an element is written in italics in these tables it means that the element is only present in the stated organ. The elements written in brackets are the three strongest binding elements to the stated organ. The number reveals how many times higher the concentration is in the organ. There are no intentions to speculate in the results found in this kind of analysis because of the author's limited knowledge on fish anatomy.

Correlation graphs in the appendix between all possible measurements performed are also included. These show the most abundant elements and they also give an easy overview of how the metals are accumulated in the fish and the relation between concentrations. One important note about these graphs are all the elements that are found on the axis (where the x- or y-value are zero); these elements are only present in either what is stated on the x- or y-axis, dependent on which axis they are found on. Decisions were made to keep them there because it was not considered correct to assume a ratio for them. If analysis of trends etc. of the elements between water and fish are performed, these elements are best left out. Some comments to these graphs are however given in the appendix.

Decisions were also made not to refer to any specific concentrations in the analysis of these results; the uncertainties from the total quant measurement are considered to be too large. The analyses performed here are based on rough classifications of the results obtained. The reason why the correlation graphs are put in the appendix is simply because those results cannot be confirmed because of the uncertainties present.

8.2.3.1. Analysis of results from Sandsjön

In Sandsjön both pike and perch have been examined, and the results found are as confident as the ICP-MS allowed it to be (28 fish samples and 5 water samples). In this lake some elements were found which most likely should not be there. These elements are subjected to the limitations of the analytical technique and their presence might be the result of other elements interfering during the measurement. Since the MS measure ions based on their mass/charge ratio it is possible that bivalent metal ions will interfere with monovalent metal ions with half the mass of the bivalent metal ions. However the following elements were found in the samples and most likely they should not be there: Ba, Rh, Nb, Pd, Cs, Hf, W and Nd. These elements are very rare in the earth crust.

8.2.3.1.1. Analysis of results in the first way

Table 8c shows how different metals preferably bind to the fish and how strong they bind to the fish. The elements with a logarithmic value below -2 are only present in the water. These elements do not seem to bind very strong to the fish. As can be seen in table 8e, the perch appears to be the strongest metal binding site from the two fish species selected. The elements not incorporated into the fish are common for both fish

species, and most of them belong to the Lanthanides and Actinides, especially for pike, in the periodic table of the elements.

Table 8c: The elements divided after increasing number on the logarithmic value on the ratio between water and fish

| C= perch Liver/water | C= perch tissue/water | C= pike liver/water | C= pike tissue/water |
|--|---|---|--|
| Log C =<-2. These elements do not exist in perch liver | log C =<-2 These elements do not exist in perch tissue | log C =<-2 These elements do not exist in pike liver | log C =<-2 These elements do not exist in pike tissue |
| Pr Nd | Pr Th | Sb Nd Ce Th Pr U | Sc Nd Y Bi Sn Th Ce U Pr Fe |
| Log C =(-2)-(-1) Ca Sr | log C =(-2)-(-1) Ba Bi | log C =(-2)-(-1) Ca Ba Sr La | log C =(-2)-(-1) Mn Sr |
| Log C =(-1)-(0) Li Rh Na Ba Mg La Sc Ce Mn Bi Y U | log C =(-1)-(0) Na Sb Sc La Mn Ce Fe Nd Sr U Y | log C =(-1)-(0) Li Sc Na Ti Mg Mn Y | log C =(-1)-(0) Li Co Na Rh Ca La Ti Pb |
| Log C =(0)-(-1) Ti Sn Fe Sb Cu Pb Th | log C =(0)-(-1) Li Co Mg Cu Ca Rh Ti Sn | log C =(0)-(-1) Pb Rh Fe Sn Co Pb Nb | log C =(0)-(-1) Mg Nb Ni Cd Cu Sb |
| Log C =(1)-(-2) K Rb Co Nb Zn | log C =(1)-(-2) K Nb Zn Tl Rb | log C =(1)-(-2) K Zn Ni Rb Cu Cd Tl | log C =(1)-(-2) K Zn Rb Tl |
| Log C =(2)-(-3) Ni Cd Tl | log C =(2)-(-3) Ni Cd | | |
| Log C =>(3) These elements only exist in perch liver (not found in the water) | log C =>(3) These elements only exist in perch tissue (not found in the water) | log C =>(2) These elements only exist in pike liver (not found in the water) | log C =>(2) These elements only exist in pike tissue (not found in the water) |
| Al Pd V Ag Cr Cs Ga Hf Zr W Mo Hg | Al Ag Cr Cs Ga W Zr Hg Mo Pd | Al Ag Cr Cs Ga Hf Zr Hg Mo Pd | Al Hf Zr Hg Mo Ag Cs |

Uranium only binds to the perch in this lake, while antimony do not bind to the pike liver only. The second, third and fourth group ($\log C = (-2) - 1$) (elements that bind weakly to the fish) mostly the hard metals with a low valence and a high abundance in the water and fish are found. I strongly suspect that many of these elements are controlled by homeostasis. I think so because the, on average, high concentrations of the elements in the water is not as high in the fish, which could be due to a biological control of the uptake of these elements. In general from these result, one can say that elements with a high water concentration do not bind as strongly to the fish as elements with less concentration in the water. The elements with high concentration are also known to nature and biological systems and these elements are not to be considered toxic (Williams, Frausto da Silva, 1996). In the third, fourth and fifth group, the intermediate metals are found, of which the elements associated to the metallothioneins belong. Among the metallothioneins copper binds the weakest to the fish, while cadmium binds the strongest to the fish, except for the pike tissue where zinc binds the strongest (for the presence of highest concentration, see also table 8e). Lead follows approximately the same channels as Zn, it binds in much the same ways as zinc and replaces zinc in certain aminoacids (Williams, Frausto da Silva, 1996). Lead also reacts with the sulfhydryl group of the aminoacids, see chapter 4 and appendix 1. Lead binds almost as strong as zinc to the fish. In the fifth and seventh group the elements with the largest volume are found (K, Rb and Cs); these tend to bind strongly to the fish. Potassium is an essential element, and it is possible that Rb and Cs are incorporated to the organism via the same channels as potassium. From literature I have found that both these elements can go in via the same channels as potassium (Williams, Frausto da Silva, 1996). Also, if any of the correlation graphs are studied, there seem to be a straight line between these elements, which suggests that there is no difference in the selectivity of the uptake between these three elements. This is also applied to the result for Tl, which is an element that enters the cell via the same route as potassium. However, regarding rubidium there are large uncertainties from the ICP-MS since it is possible that there are large interferences from other elements in the measurements.

The elements in the seventh group are only present in the fish samples, and therefore they tend to bind very strongly to the fish or any organic matter. Most of these elements belong to the soft metals, and they are elements that nature has had little or no experience of. It is obvious that the elements that are incorporated into the fish the strongest, are very similar between the species and organs. The most unexpected result here is the aluminium which tend to bind very strong to the fish, even if the liming has made aluminium precipitate from the water. It is however possible that when liming is performed, the toxic precipitate $\text{Al}(\text{OH})_3$ is formed. This precipitate is known to react very strongly with organic matter, and it can cause great aluminium toxicity when liming is performed (aemt lecture Sep-Oct 1999). Molybdenum and Pd also binds very strong to the fish; it is only found in the fish samples.

In general the majority of the metals present in the examined water environment are found in the fish, which concludes that metals are still very available to biota, despite the liming. It also seems possible that elements with the largest ratio between valence and radius are the ones that bind strongest to the fish. The average ratio between valence and radius is highest among the elements that are only present in fish compared to any of the other groups. This would in turn mean that many metals are still available to the fish in an ionic form. This would also support the theory of the

selectivity of the metal uptake based on the size and charge of ions. However, since it is not possible to analyse the complex with which the metal is present, it can also, perhaps most likely, be a coincident.

8.2.3.1.2. Analysis of results in the second way

In table 8d the elements are sorted after similar concentrations of metallic elements in the water samples. There is a trend that elements with low water concentrations are accumulated in the fish to a larger extent than elements with higher water concentrations. Also if the ratios at which the elements are incorporated into fish are investigated, it seems as if the bivalent metal ions (Cu, Zn, Cd, Ni, Co) are the ones that most strongly bind to the fish.

Table 8d: The metallic elements divided after the concentration in the water samples. The ratio between fish and water is also provided (C= water concentrations).

| Log C = (-3)-(-2) | | | | | Log C = (0)-(1) | | | | |
|------------------------|-----------------------|-------------------------|------------------------|-----|------------------------|-----------------------|-------------------------|------------------------|------|
| Perch liver / water | pike liver / water | perch tissue / water | pike tissue / water | | perch liver / water | pike liver / water | perch tissue / water | pike tissue / water | |
| Nb | 53 | 2,8 | 23 | 1,6 | Ti | 2 | 0,2 | 4,3 | 0,8 |
| Pr | 0 | 0 | 0 | 0 | Rh | 0,7 | 1,3 | 2,8 | 0,6 |
| Tl | 102 | 30 | 50 | 14 | Ba | 0,4 | 0,1 | 0,05 | 0,01 |
| Th | 8 | 0 | 0 | 0 | | | | | |
| Log C = (-2)-(-1) | | | | | Log C = (1)-(2) | | | | |
| Perch liver / water | pike liver / water | perch tissue / water | pike tissue / water | | perch liver / water | pike liver / water | perch tissue / water | pike tissue / water | |
| Li | 1 | 0,6 | 1,2 | 0,8 | Mn | 0,6 | 0,3 | 0,4 | 0,06 |
| Co | 15 | 3,5 | 2,3 | 0,6 | Sr | 0,03 | 0,01 | 0,3 | 0,08 |
| Ni | 160 | 46 | 125 | 9,7 | | | | | |
| Cd | 455 | 62 | 305 | 5,5 | Log C = (2)-(3) | | | | |
| Sn | 1 | 2,4 | 2,6 | 0 | perch liver / water | pike liver / water | perch tissue / water | pike tissue / water | |
| Sb | 2 | 0 | 0,7 | 5,9 | Mg | 0,7 | 0,4 | 1,4 | 1,44 |
| La | 1 | 0,1 | 0,2 | 0,4 | K | 33 | 25 | 51 | 51 |
| Ce | 0,5 | 0 | 0,5 | 0 | Fe | 1,2 | 3,7 | 0,1 | 0 |
| Nd | 0 | 0 | 0,2 | 0 | | | | | |
| U | 0,1 | 0 | 0,3 | 0 | Log C = (3)-(4) | | | | |
| Log C = (-1)-(0) | | | | | perch liver / water | pike liver / water | perch tissue / water | pike tissue / water | |
| Perch liver / water | pike liver / water | perch tissue / water | pike tissue / water | | Na | 0,7 | 0,4 | 0,5 | 0,5 |
| Sc | 0,4 | 0,1 | 0,2 | 0 | Ca | 0,01 | 0,01 | 1,8 | 0,4 |
| Cu | 6 | 23 | 3,7 | 1,4 | | | | | |
| Zn | 44 | 71 | 17 | 50 | | | | | |
| Rb | 70 | 69 | 73 | 60 | | | | | |
| Y | 0,4 | 0,1 | 0,2 | 0 | | | | | |
| Pb | 6 | 2,3 | 3,9 | 0,3 | | | | | |
| Bi | 0,1 | 0,1 | 0,01 | 0 | | | | | |

The essential element K is having the highest ratio between fish and water among elements with higher water concentrations, and it is possible that this explains why Rb so strongly binds to the fish; Rb can enter the cell in the same channels as K

(Williams, Frausto da Silva, 1996). However, regarding rubidium there are large uncertainties from the ICP-MS since it is possible that there are large interferences from other elements in the measurements.

It is possible that diffusion affects the rate at which metals are incorporated in the limed lake. As can be seen in table 8d many elements are accumulated in the fish, even if the ratio is not so high at all times. The elements that are accumulated are not always essential, and almost all elements found in the water also were present in the fish. It is possible that the uptake is more based on the presence of an element in the water, than the necessity of incorporating it into the organism to make the organism carry out its functions properly

8.2.3.1.3. The elements analysed in the third way

Table 8e shows where the largest concentrations of specific metallic elements were found.

Table 8e: Differences in the metallic elements preferences for a metal binding site in pike and perch from the limed lake.

| | |
|--|--|
| Following elements have higher concentrations in the perch liver than in the perch tissue | Following elements have higher concentrations in the perch tissue than in the perch liver |
| Na, Al, Sc, Cr, Mn, Fe, Co, Ni, Cu, Zn, Y, Nb, Mo, Ag, Cd, Sb, Ba, La, W, Hg, Tl, Pb, Bi, <i>Th, V, Hf, Cr, Ga, Pd</i> (Fe (11), Bi(9), Co(6,5)) | Li, Mg, K, Ca, Ti, Ga, Rb, Sr, Zr, Rh, Pd, Sn, Cs, Ce, U, <i>Nd</i> (Ca (100), Rh(4), U(3)) |
| Following elements have higher concentrations in the pike liver than in the pike tissue | Following elements have higher concentrations in the pike tissue than in the pike liver |
| Mn, Fe, Co, Ni, Cu, Zn, Rb, Nb, Mo, Rh, Ag, Cd, Ba, Tl, Pb, <i>Sc, Y, Bi, Cr, Ga, Pd</i> , (Fe(4100), Cu (16), Ag(28)) | Li, Na, Mg, Al, K, Ca, Ti, Sr, Zr, Cs, La, Hf, Hg, <i>Sb</i> (Sr(9), Ca(33), La(4)) |
| Following elements have higher concentrations in the perch liver than in the pike liver | Following elements have higher concentrations in the pike liver than in the perch liver |
| Li, Na, Mg, Al, K, Ca, Sc, Ti, Cr, Mn, Co, Ni, Ga, Rb, Sr, Y, Nb, Pd, Cd, Cs, Ba, La, Hg, Tl, Pb, <i>In, W, Sb, V</i> (Ti (8), Nb(19), Ce(8)) | Fe, Cu, Zn, Zr, Mo, Rh, Ag, Sn, Bi (Cu (4), Ag (6), Fe(3)) |
| Following elements have higher concentrations in the perch tissue than in the pike tissue | Following elements have higher concentrations in the pike tissue than in the perch tissue |
| Li, Na, Al, Ca, Ti, Mn, Fe, Co, Ni, Cu, Rb, Sr, Nb, Mo, Rh, Ag, Cd, Cs, Ba, Tl, Pb, <i>Bi, W, Nd, U, Pd, Ce, Y, Sc, Ga, Sn, Cr</i> (Fe (120), Nb(15), Cd (55)) | Mg, K, Zn, Zr, Sb, La, Hg, <i>Hf</i> (Zn(3), Zr (15), Sb(56)) |

In the right column of row three and four the elements that are biomagnified are found. There are only two elements that biomagnify in both the liver and tissue which is Zn and Zr. This result is somewhat contradictory to what was expected since the pike feed on the perch and therefore should contain everything that is found in the perch. It is however possible that there is a difference in the metabolism of metals between the pike and perch. Also interesting is the strong preference for antimony to bind to the pike tissue (it is 56 times higher than in the perch tissue), while antimony is only found in the perch liver and not in the pike liver where the concentration is zero. Judging from this result it seems not completely true to say that there is a danger that metallic elements are biomagnified in species higher in the food chain. From these results it seems more to be a question of the fact that different species binds metals more or less efficient.

It is also possible that there is a difference in the selectivity between the liver and tissue for both species based on the valence. Most bivalent metal ions are in the liver, while there is a mixture of ions in the tissue. In general, the concentrations of metallic elements are lower in the tissue than the liver (see table 8e).

Regarding the metallothioneins, Copper and Zinc are the only elements that are biomagnified in the liver, while cadmium is not elevating its concentrations in the food chain. If row 1 and 2 in table 8e are examined it is easy to see that the concentration of the metals associated to the metallothioneins are highest in the liver of both pike and perch, which is as expected.

8.2.3.2. Analysis of results from Anten

From Anten I only got perch to analyse. Attempts to include the pike were abandoned due to time restrictions. The male perch included contained milt in which two measurements were made. These results are however not intended to be included in the report as a specific investigation, mainly due to the low number of measurements and therefore uncertain results. In this lake some elements were found which most likely should not be there. These elements are subjected to the limitations of the analytical technique and their presence might be the result of other elements interfering during the measurement. Since the MS measure ions based on their mass/charge ratio it is possible that bivalent metal ions will interfere with monovalent metal ions with half the mass of the bivalent metal ions. However the following elements were found in the samples and most likely they should not be there: Ba, Rh, Nb, Pd, Pt, Au, Cs, In and Nd.

8.2.3.2.1. Analysis of results in the first way

Table 8f the elements are sorted after increasing value on the ratio between the fish and water. As can be seen there is a wide composition of metals in the water that do not bind to the fish (group 1 where $\log C = < -2$). Most of the lanthanide and actinides are found here. Most metals bind to the liver, and therefore the liver most certain reflect the content of metals the perch is exposed to.

Table 8f: The elements divided after increasing number on the logarithmic value on the ratio between water and fish

| C= perch Liver/water | C= perch tissue/water | C= perch liver/water | C= perch tissue/water |
|-------------------------|--------------------------|-------------------------|--------------------------|
|-------------------------|--------------------------|-------------------------|--------------------------|

| | | | | | | | |
|---------------------------------|----|----------------------------------|----|---------------------------|----|---------------------------|----|
| Log C <=-2 | | log C <=-2 | | log C =(-1)-(0) | | log C =(-1)-(0) | |
| These elements do | | These elements do | | | | | |
| Not exist in perch liver | | not exist in perch tissue | | Li | Mn | Li | Mg |
| Be | Pr | Be | La | Na | Fe | Na | Ni |
| Al | Nd | Al | Ce | Mg | Ni | | In |
| Sc | Sm | Sc | Pr | | Sb | | |
| V | Gd | V | Nd | | | | |
| Zr | Dy | Fe | Sm | log C =(0)-(1) | | log C =(0)-(1) | |
| Nb | Er | Ga | Gd | K | Rh | K | Cs |
| Mo | Yb | Zr | Dy | Co | Cs | Cu | Tl |
| Pd | Hf | Nb | Er | Cu | Pb | Zn | Pb |
| In | Pt | Mo | Yb | Rb | | Rb | |
| Ce | Th | Rh | Hf | log C =(1)-(2) | | log C =(1)-(2) | |
| | | Pd | Pt | Zn | | | |
| | | Sb | Th | Tl | | | |
| | | Ba | U | | | | |
| | | Y | | | | | |
| Log C =(-2)-(-1) | | log C =(-2)-(-1) | | log C =>2 | | log C =>2 | |
| | | | | These elements do | | These elements do | |
| | | | | not exist in water | | not exist in water | |
| Ca | Y | Ca | Co | Ag | Sn | Sn | |
| Ti | Ba | Ti | Sr | Cd | Au | Hg | |
| Ga | La | Mn | | | Bi | | |
| Sr | U | | | | | | |

One interesting element found in group 1 is Aluminium that does not bind to the fish at all in the neutral lake. It is also worth pointing out that the concentrations of aluminium in the water samples were in the range of 350 µg/l, which is superior the other lakes (5 and 0 µg/l in the acidified resp. limed). This brings about the possibility that aluminium is strongly bound to complexes in the water that are very stable and unavailable to biota. The same conclusion can also be drawn for molybdenum and Pd, which also did not bind to the perch.

In the neutral lake platinum was found in the water samples; it does not seem to accumulate in perch in neutral lakes. I have understood from literature that platinum forms complexes with ammonium, and the large concentrations of nutrients in Anten might therefore explain the preference for platinum to form complexes in the water (Williams, Frausto da Silva, 1996). However, regarding platinum there may be large uncertainties from the ICP-MS since it is possible that there are other elements interfering during the measurements.

Antimony, uranium and iron do not bind to the perch tissue; they are only found in the perch liver.

In group two and three (log c=(-2)-0) many of the abundant hard metals with a low valence are found. Antimony is found in group three and its concentrations are approximately 300 times greater in the liver than in the water. The only fish sample in which indium was detected are found in group three; Indium only tends to bind to the perch tissue. Indium is however uncertain from the ICP-MS. Indium is a very rare element and most likely Indium should not be found here. In the fourth group (these elements bind strongly to the perch), the elements with the largest volume, potassium, rubidium and caesium are found. They seem to bind with the same strength to both liver and tissue. Tl also binds to the liver and tissue with about the same strength as potassium. Tl can enter the cell via the same channels as potassium (Williams, Frausto da Silva, 1996). However, regarding rubidium there are large uncertainties

from the ICP-MS since it is possible that there are large interferences from other elements in the measurements.

In the sixth group the elements that bind the strongest to the fish are found. All of these elements belong to the soft metals, which are considered toxic to biological systems. Elements that are only present in the liver is silver, cadmium, gold and bismuth, while mercury only seems to bind to the perch tissue.

Regarding the metallothioneins; cadmium binds strongest to the liver, second strongest zinc and least strongly copper. Copper and zinc bind with the same strength in the tissue (group 4).

There is no clear connection between the ratio between valences and radii of the elements and the uptake of metals in perch; this concludes the possibility that the metals are not present as free ions, but incorporated into complexes in the water which are more or less stable.

8.2.3.2.2. Analysis of results in the second way

In table 8g the elements are sorted after similar concentrations of metallic elements in the water samples. The elements that binds the strongest to the perch liver is Tl. It is having one of the lowest concentrations in the water samples. Zinc binds the second strongest to the liver; zinc binds the strongest to the perch among the metallothioneins. Cadmium is excluded since it was only found in the fish (see table 8f). Potassium, rubidium and cesium binds with approximately the same strength to both liver and tissue. However, regarding rubidium there are large uncertainties from the ICP-MS since it is possible that there are large interferences from other elements in the measurements.

Table 8g: The metallic elements divided after the concentration in the water samples. The ratio between fish and water is also provided (C=water concentrations).

| Log C = (-3)-(-2) | | | Log C = (-1)-(0) | | | Log C = (1)-(2) | | |
|------------------------|-------------------------|-----|------------------------|-------------------------|------|------------------------|-------------------------|------|
| Perch liver / Water | Perch tissue / Water | | Perch liver / Water | Perch tissue / Water | | Perch liver / Water | Perch tissue / Water | |
| Dy | 0 | 0 | Li | 0,4 | 0,1 | Ti | 0,06 | 0,08 |
| Er | 0 | 0 | Sc | 0 | 0 | Mn | 0,3 | 0,02 |
| Yb | 0 | 0 | Co | 5 | 0,02 | Sr | 0,02 | 0,02 |
| Hf | 0 | 0 | Ni | 0,5 | 0,3 | | | |
| Pt | 0 | 0 | Ga | 0,02 | 0 | Log C = (2)-(3) | | |
| Tl | 22 | 4,2 | Nb | 0 | 0 | | | |
| Be | 0 | 0 | La | 0,05 | 0 | Perch liver / Water | Perch tissue / Water | |
| Log C = (-2)-(-1) | | | Ce | 0 | 0 | Al | 0 | 0 |
| | | | Nd | 0 | 0 | K | 5 | 8,5 |
| | | | Pb | 4,4 | 1,4 | Fe | 0,3 | 0 |
| Perch liver / Water | Perch tissue / Water | | U | 0,03 | 0 | | | |
| V | 0 | 0 | Log C = (0)-(1) | | | Log C = (3)-(4) | | |
| Mo | 0 | 0 | | | | | | |
| Pd | 0 | 0 | Perch liver / Water | Perch tissue / Water | | Perch liver / Water | Perch tissue / Water | |
| Sb | 0,2 | 0 | Cu | 4,5 | 1,4 | Na | 0,6 | 0,3 |
| Cs | 1,7 | 2,6 | Zn | 14,3 | 4,2 | Mg | 0,3 | 0,6 |
| Pr | 0 | 0 | Rb | 7,4 | 8,9 | Ca | 0,07 | 0,06 |
| Sm | 0 | 0 | | | | | | |

| | | | | | |
|----|---|---|----|------|-------|
| Gd | 0 | 0 | Y | 0,02 | 0,002 |
| | | | Zr | 0 | 0 |
| | | | Rh | 1,4 | 0 |
| | | | Ba | 0,04 | 0 |

By judging from these results, diffusion does not seem to affect the rate at the uptake of metals in the perch. The uptake seems more to be a question of which channels that are available through which an element can enter the cell. If diffusion did affect the rate of incorporation more metals should have been incorporated into the fish but as can be seen in table 8g the elements are in many cases only present in the water samples. Therefore, as mentioned, the uptake seems more to be a question of available channels through which an element can enter the cell.

8.2.3.2.3. The elements analysed in the third way

Table 8h: Differences in the metallic elements preferences for a metal binding site in perch liver and tissue from the neutral lake.

| Following elements have higher concentrations in the perch liver than in the perch tissue | Following elements have higher concentrations in the perch tissue than in the perch liver |
|--|--|
| Li, Na, Ca, Mn, Co, Ni, Cu, Zn, Sr, Y, Sn, Tl, Pb, Fe, Ga, Ge, Rh, Ag, Cd, Sb, Ba, La, Au, Bi, U, (Co (240), Sn (73), Mn (13)) | Mg, K, Ti, Rb, Cs, In, Hg, (Mg (1,8) K (1,7), Cs (1,5)) |

Table 8h shows where the largest concentrations of specific metallic elements were found. Most of the elements bind to the liver in the neutral lake. The general bivalent metal ions and the metallothioneins are found in the liver which is as expected. Interesting is the large amount of elements that were only found in the liver; there seem to be a difference in selectivity of the metal accumulation between the liver and tissue. As can be seen in the perch tissue, most elements are hard metals, with small toxicity potential. Indium and mercury are the only elements that were only found in the perch tissue.

8.2.3.2.4. Analysis of the perch milt

Decisions were made to investigate the content of metals in the milt of the perch. Two measurements of the milt were made. The roe had still not developed in the female species, so only the milt was selected. There are some limitations to these results that might be mentioned. These are:

- The milt was not fully developed (the fish was caught in November, and they spawn in the spring); It is possible that there will be alterations happening in the metal content until the milt is fully developed
- Only two measurements were made
- No reference material exists for the limed and acidified lake

What might be found by investigating in the milt is the following:

- Does any metal preferably bind to the milt and thereby;

- Is there any metal or metals that possess the property of being more resistant against biological removal from a species after a specific metal or metals has been removed from the water.

What can be found by this investigation is to see which metals that will remain in biological systems for the longest time after for instance liming of a polluted lake is performed (if liming removes metals from the water).

Table 8i: Differences in the metallic elements preferences for a metal binding site in the perch milt, liver and tissue from the neutral lake

| Following elements have higher concentrations in the perch milt than in the perch liver | Following elements have higher concentrations in the perch liver than in the perch milt |
|---|---|
| Na, Mg, K, Rb, Cs, Pb, Bi, <i>Te</i> (Cs (2), Pb (2), K (2)) | Li, Ca, Ti, Mn, Cu, Zn, Sr, Rh, Tl, <i>Fe, Co, Ni, Ga, Y, Ag, Cd, Sn, Sb, Ba, La, Au, U</i> , (Mn (18), Ca (10), Sr (15)) |
| Following elements have higher concentrations in the perch milt than in the perch tissue | Following elements have higher concentrations in the perch tissue than in the perch milt |
| Li, Na, Cu, Zn, Rb, Cs, Tl, Pb, <i>Rh, Te, Bi</i> , (Na (3), Tl (4), Pb (5)) | Mg, K, Ca, Ti, Mn, Sr, <i>Co, Ni, Y, In, Sn, Hg</i> , (Sr (13), Ca (9), Ti (2)) |

I have included the perch milt in table 8i. As can be seen the milt does not contain as many elements as the liver or tissue. However, the highest concentrations of lead was found in the milt and the only samples in which tellurium were found were in the milt. The concentrations of Bi were also highest in the perch milt. Lead and Bismuth are toxic metals and they can bring about big harm to biological systems (Williams, Frausto da Silva, 1996). This result shows that these elements are not easily removed from biological systems due to their elevated concentrations in the milt. Bi were not found in any water samples (see table 8f); it is possible that the Bi in the fish is "inherited" from earlier generations when the fish might have been exposed to Bi in the water. Also, there seems to be no difference between milt and flesh.

8.2.3.3. Analysis of results from Rammsjön

In this lake some elements were found which most likely should not be there. These elements are subjected to the limitations of the analytical technique and their presence might be the result of other elements interfering during the measurement. Since the MS measure ions based on their mass/charge ratio it is possible that bivalent metal ions will interfere with monovalent metal ions with half the mass of the bivalent metal ions. However the following elements were found in the samples and most likely they should not be there: Ba, Rh, Nb, Pd, Pt, Cs and Nd.

8.2.3.3.1. Analysis of results in the first way

Table 8j shows how different metals preferably bind to the fish and how strong they bind to the fish. In group 1 ($\log C \leq -2$) the elements that do not bind to the fish are found. There are four different elements that do not bind to either the pike or perch in the acidified lake. These are Al, Sc, Th and U. Interesting here is aluminium, which

does not bind to the fish in the acidified lake; Aluminium should be present as Al^{3+} -ions with a large affinity to bind to organic matter. It is possible that the aluminium-ions are bound to humic acids in the water; the water in the lake is very dark, which darkness probably originates from humic acids (see i.e. Figure 2.1. and chapter 2.1. for more environmental data).

Table 8j: The elements divided after increasing number on the logarithmic value on the ratio between water and fish

| C= perch Liver/water | C= perch tissue/water | C= pike liver/water | C= pike tissue/water |
|---|--|--|---|
| Log C =<-2 These elements do not exist in perch liver | log C =<-2 These elements do not exist in perch tissue | log C =<-2 These elements do not exist in pike liver | log C =<-2 These elements do not exist in pike tissue |
| Al Th Sc U | Al Nd Sc Th Ce U | Al Ce Sc Nd Ga Th Nb U Sb Ba | Al Nd Sc Th Ce U |
| Log C =(-2)-(-1) | log C =(-2)-(-1) Fe Sb | log C =(-2)-(-1) Sr | log C =(-2)-(-1) Mn Fe |
| Log C =(-1)-(0) Li Ba Mn Pb Sb Bi | log C =(-1)-(0) Li Y Na Ba Mn Pb Co Bi | log C =(-1)-(0) Li Y Na Pb Ca Bi Mn | log C =(-1)-(0) Li Nb Na Sb Co Ba Ga Pb Sr Bi Y |
| Log C =(0)-(-1) Na Sr Mg Y Ca Nb Fe Rh Co Cs Ga Ce Nd | log C =(0)-(-1) Mg Sr Ca Nb Cu Rh Zn Ag Ga Cd La | log C =(0)-(-1) Mg Rh Ti Ag Fe Cs Co La Zn | log C =(0)-(-1) Mg Rh Ca Cd Ti Sn Cu La Zn |
| Log C =(1)-(2) K Rb Ti Ag Cu Cd Zn Sn La | log C =(1)-(2) K Sn Ti Cs Rb | log C =(1)-(2) K Cd Cu Sn Rb | log C =(1)-(2) K Rb Cs |
| Log C =>2 These elements only exist in perch liver (not found in the water) | log C =>2 These elements only exist in perch tissue (not found in the water) | log C =>2 These elements only exist in pike liver (not found in the water) | log C =>2 These elements only exist in pike tissue (not found in the water) |
| Hg Tl | Hg Tl | Pd Tl Hg | Pt Tl Hg |

Also interesting here is the similarity between perch tissue and pike tissue regarding the elements, which are not incorporated into either the perch tissue or pike tissue.

Regarding perch liver and pike liver, more elements are incorporated into the perch liver, which is unexpected, since the pike feed on the perch.

Antimony and Barium are not found in the pike liver only. Barium however is a very unreliable result since it is interfered with Argon (with the same weight as Barium) molecules during measurements. In the third group, it can be seen that Li, Pb and Bi bind with the same strength to both the liver and tissue to both species; these elements do not seem to have selectivity for where they most preferably bind. In this group, the two strongest binding sites for Antimony are found; for the pike it binds the strongest to the tissue and for the perch it binds the strongest to the liver. The similarity mentioned earlier between perch tissue and pike tissue still remains if the elements in group two and three are compared. It is only Ga, Sr and Nb that binds stronger to the perch tissue.

In group three and four the abundant hard metals are present. Magnesium and calcium seem to be the hard metals that binds the strongest to the fish. One interesting point here is that both of these elements bind much stronger to the fish in the acidified lake, than in the neutral and limed lake where the water concentrations of these elements are much higher. In the fish however the concentrations are very similar especially for magnesium in all three lakes, but also for calcium in limed and acidified lake; these elements seem to be controlled by homeostasis.

In the fifth group, as usual, potassium, rubidium and cesium are found. These elements have the largest volume (see appendix), potassium is essential and rubidium and cesium seems to go in via the same channels as potassium (Williams, Frausto da Silva, 1996). There are however large uncertainties regarding rubidium from the measurements. Also notice the ratio between valences and radii for these elements; based on this ratio they should not bind to the fish as strong as they obviously do.

In the sixth group ($\log c \geq 2$), mercury and Tl are found in all samples. Pd only was found in the pike liver, while the only samples containing platinum was found in the pike tissue. Pd is a very rare element and its presence here is most likely due to limitations in the measurement and interferences from other elements.

Regarding the metallothioneins the perch liver seems to be the site that most strongly attracts these elements. Cu, Zn and Cd are all found in the fifth group for the perch liver. Cu and Cd binds the strongest to the pike liver, while for the tissue for both pike and perch, these elements are found in group three; these elements obviously do not bind so strong to the tissue.

Finally, the ratios between valence and radius for these elements have been examined. This investigation shows that elements with the highest ratio on average are found in group 5 and 6. This would in turn mean that the elements are more present as free ions in this water, than present as inorganic or organic complexes.

8.2.3.3.2. Analysis of results in the second way

In table 8k the elements are sorted after similar concentrations of metallic elements in the water samples. The elements that binds the strongest to the perch liver are Ga and Sn; two elements which are considered toxic and belongs to the soft metals (Williams, Frausto da Silva, 1996). Cadmium, Sn and potassium bind the strongest to the pike liver.

Table 8k: The metallic elements divided after the concentration in the water samples. The ratio between fish and water is also provided (C=water concentrations).

| Log C = (-3)-(-2) | Log C = (0)-(1) |
|-------------------|-----------------|
|-------------------|-----------------|

| Perch liver / water | Pike liver / water | perch tissue / water | pike tissue / water | | Perch liver / water | pike liver / water | perch tissue / water | pike tissue / water |
|--------------------------|-----------------------|-------------------------|------------------------|------------------------|------------------------|-----------------------|-------------------------|------------------------|
| Sc | 0 | 0 | 0 | 0 | Al | 0 | 0 | 0 |
| Nb | 1,1 | 0 | 1,7 | 0,7 | Zn | 13 | 9,3 | 3 |
| Sn | 69 | 21,5 | 19 | 5,7 | Sr | 2,8 | 0,05 | 4,33 |
| Cs | 4 | 4,3 | 11 | 22 | Ba | 0,5 | 0,003 | 0,2 |
| La | 16 | 2,3 | 2,5 | 2 | Pb | 0,5 | 0,7 | 0,5 |
| Ce | 6,5 | 0 | 0 | 0 | Log C = (1)-(2) | | | |
| Nd | 1,6 | 0 | 0 | 0 | | | | |
| Th | 0 | 0 | 0 | 0 | | | | |
| U | 0 | 0 | 0 | 0 | | | | |
| Log C = (-2)-(-1) | | | | | Perch liver / water | pike liver / water | perch tissue / water | pike tissue / water |
| | | | | Mn | 0,4 | 0,3 | 0,1 | 0,1 |
| | | | | Log C = (2)-(3) | | | | |
| Perch liver / water | Pike liver / water | perch tissue / water | pike tissue / water | | Perch liver / water | pike liver / water | perch tissue / water | pike tissue / water |
| Li | 0,3 | 0,7 | 0,8 | 0,6 | | | | |
| Co | 5,3 | 1,3 | 0,2 | 0,3 | Na | 1,4 | 0,83 | 0,5 |
| Ga | 107 | 0 | 1,5 | 0,4 | Mg | 1,2 | 1 | 2,7 |
| Y | 1,5 | 0,7 | 0,4 | 0,2 | K | 31,6 | 32,2 | 58,6 |
| Ag | 12 | 2,9 | 4,4 | 0 | Ca | 5,5 | 0,1 | 8,4 |
| Sb | 0,5 | 0 | 0,03 | 0,4 | Fe | 5,2 | 4,7 | 0,02 |
| Log C = (-1)-(0) | | | | | | | | |
| Perch liver / water | Pike liver / water | perch tissue / water | pike tissue / water | | | | | |
| Ti | 13,3 | 1,7 | 18 | 5 | | | | |
| Cu | 11,5 | 16 | 2,4 | 1,7 | | | | |
| Rb | 27 | 42 | 48,2 | 39,5 | | | | |
| Rh | 4 | 1,8 | 6,4 | 2 | | | | |
| Cd | 17,5 | 54,5 | 3,2 | 2,9 | | | | |
| Bi | 0,2 | 0,4 | 0,2 | 0,1 | | | | |

Potassium and rubidium binds with the same strength to both the perch tissue and pike tissue. Rubidium is very uncertain from the ICP-MS; this result should not be relied on. Regarding the metals associated to the metallothioneins; Cadmium binds the strongest to the perch and pike liver, while zinc binds the least strongest to the perch- and pike liver.

When examining these results, wide variations are found. The uptake of these elements seems to be a mixture of both the presence (diffusion dependent reactions) of the element and the availability of the element to biota. If, i.e. elements like Ga, Sb, Cd etc are examined it is found that there are large differences in the ratio between fish and water (in some cases it is zero, in some cases it is very large). These elements therefore seem to be present in a form that is available only in those organs to which the ratio is high. On the other hand there also are several elements that are incorporated at a fairly even rate to all organs. These elements however seems to be present in a form that are available through all channels, and therefore their uptake should be more based on diffusion type of reactions.

8.2.3.3.3. The elements analysed in the third way

Table 8l shows where the largest concentrations of specific metallic elements were found. There are only two elements that biomagnify in both liver and tissue (row three and four). These are mercury and lead. As is the case in Sandsjön, this result is unexpected. In this lake I expected an even clearer trend in biomagnification, because the pike feeds solely on perch in this lake. Also, as is the case in Sandsjön, antimony is only found in the perch liver and not in the pike liver, while antimony is 11 times higher in the pike tissue than in the perch tissue. Also it is interesting to see the similarities between pike tissue and perch tissue regarding the elements with the highest concentrations (row one and two). It seems as if essential elements with low valence prefer the tissue. Mercury seems to be the only toxic element that prefers both pike tissue and perch tissue. Also it seems possible that most bivalent metal ions prefer the liver, as well as the more toxic unessential metallic elements.

Table 8l: Differences in the metallic elements preferences for a metal binding site in pike and perch from the acidified lake.

| | |
|--|--|
| Following elements have higher concentrations in the perch liver than in the perch tissue | Following elements have higher concentrations in the perch tissue than in the perch liver |
| Na, Mn, Fe, Co, Ni, Cu, Zn, Ga, Y, Ag, Cd, Sn, Sb, Ba, La, Tl, Pb, Bi, <i>Ce, Hf, W, Th, U</i> , (Fe (236), Sb (15), La (6)) | Li, Mg, K, Ca, Ti, Rb, Sr, Nb, Rh, Cs, Hg, (Li (2,6), Cs (3), Hg (7)) |
| Following elements have higher concentrations in the pike liver than in the pike tissue | Following elements have higher concentrations in the pike tissue than in the pike liver |
| Li, Na, Mn, Fe, Co, Ni, Cu, Zn, Rb, Y, Cd, Sn, La, Tl, Pb, Bi, <i>Pd, Ag</i> , (Fe (47), Cu (9), Cd (19)) | Mg, K, Ca, Ti, Sr, Rh, Cs, Ba, Hg, <i>Nb, Sb, Pt</i> , (Ca (25), Sr (10), Ba (50)) |
| Following elements have higher concentrations in the perch liver than in the pike liver | Following elements have higher concentrations in the pike liver than in the perch liver |
| Tl, Na, Mg, Ca, Ti, Mn, Fe, Co, Zn, Sr, Y, Rh, Ag, Sn, Ba, La, <i>Ga, Ge, Nb, Sb, Ce, Nd</i> , (Ca (51), Sr (48), Ba (175)) | Hg, Pb, Bi, Li, K, Ni, Cu, Rb, Cd, Cs, <i>Pd, Hf, W</i> , (Li (2), Cd (3), Hg (33)) |
| Following elements have higher concentrations in the perch tissue than in the pike tissue | Following elements have higher concentrations in the pike tissue than in the perch tissue |
| Li, Mg, K, Ca, Ti, Mn, Ni, Cu, Zn, Ga, Rb, Sr, Y, Nb, Rh, Cd, Sn, Ba, La, Tl, Bi, (Ca (7), Ga (18), Sr(20)) | Na, Fe, Co, Sb, Cs, Hg, Pb, <i>Pt</i> , (Fe (5), Sb (11), Hg (6)) |

Regarding the metals associated to the metallothioneins; copper is the only element that biomagnify in the liver between pike and perch. Zinc and Cadmium has the highest concentrations in the perch liver. If row 1 and 2 in table 8l are examined, it is easy to see that the concentration of the metals associated to the metallothioneins are highest in the liver of both pike and perch, which is as expected.

8.2.4. Summary from tot quant analysis

From limed lake the following conclusions can be made:

- Metals are very available to biota. The metals that were present in the water were almost solely also present in the fish.
- Only two elements biomagnified in the liver and tissue; Zn and Zr.
- The perch were found to bind metals more efficiently than the pike; biomagnification does not seem as important as the surrounding medias content of metals when the elements are accumulated in the fish.

From neutral lake the following conclusions can be made:

- The metals are not very available to biota. Most of the metals found in this lake were only present in the water and did not bind to the fish strongly.
- Toxic elements were not accumulated to a high rate

From acidified lake the following conclusions can be made:

- The metals are available to biota. Many of the metals found in the water were also present in the fish.
- Toxic elements (Pb and Hg) biomagnify in the liver and tissue.
- The perch were found to bind metals more efficient than the pike; biomagnification does not seem as important as the surrounding medias content of metals when the elements are accumulated in the fish.

From this study it has been found that biomagnification is not a problem. This brings about some advantages for the forthcoming comparing study like:

- Metals that are present in other media to which the fish species are not exposed, i.e. sediment, will not affect the uptake of metals in the fish species selected
- Organisms present in other environments and exposed to metals under other conditions: If these organisms are available to the fish in the food chain they do not affect the rate of accumulation to the extent that it is more important than the content of metallic elements in the media the fish species are exposed to. This can i.e. be a water animal living in the sediments or flies on the surface that the fish feed on.

8.3. Results from Quantitative Analysis

8.3.0. Initial comments

The elements selected for the quantitative analysis are the following (table 8.3.0.):

Table 8.3.0: The elements selected for the quantitative analysis

| | | | | | |
|----|----|----|----|----|----|
| Li | Mn | Cu | Sb | Cd | Al |
| Na | Fe | Zn | Pb | Ni | Te |
| Mg | Co | Sr | Sn | Ag | Cr |

Potassium, rubidium, Rh and barium have been left out (see section 8.2.2.) because of analytical difficulties. Therefore some additional elements that was considered interesting have been added. The reasons for their selection are shown in table 8m. Mercury should have been analysed, but there was no standard available.

Table 8m: additional elements chosen for the quantitative analysis

| Element | Reason for selection |
|---------|--|
| Sn | This element was found in the water samples from the limed and acidified lake. The element is chosen because it differs in the way it is accumulated between these two lakes. The concentration is higher in the water samples from the limed lake, still the elements binds stronger to the fish in the acidified lake. |
| Cd | This element was found in the water samples from the limed and acidified lake. There are no intentions to make a comparison between the lakes, regarding cadmium. Since I have chosen Cu and Zn, decisions were made to select Cadmium too. Thereby all metals associated to the metallothioneins are included in the quantitative analysis. |
| Ni | This element was found in the water samples from the limed and neutral lake. The concentration is much higher in the water samples from the neutral lake, still it binds much stronger to the fish in the limed lake: Similar trends as found for Sn |
| Ag | This element was only found in the water samples from the acidified lake. Interesting is however the discovery that it was present in fish samples from both limed and neutral lake; Ag seems not to have an equally strong tendency to precipitate in acidified waters. The element should therefore be examined more carefully. |
| Al | This element was found in the neutral and acidified lake. It contained surprisingly high concentrations in the neutral lake. The two lakes that contained aluminum in the water samples did not contain aluminum in the fish, while the fish in the limed lake (the water concentrations of aluminum in the limed lake were zero) contained aluminum. It seems possible that aluminum toxicity is a larger problem in limed lakes than in acidified and neutral lakes. |
| Te | This element was found in the samples from the initial analysis. It was thereby found interesting. Te was however not present in any samples in the final analysis (exception for perch milt). Its presence is confirmed more carefully in this quantitative analysis. |
| Cr | This element was not found in any water samples from the neutral limed and acidified lake. It was however found in the fish samples from the limed lake. Maybe the bioavailability for chromium differs between limed and acidified/neutral waters in the same way as aluminum. |

8.3.1. Results from water measurements (quantitative analysis)

The elements are sorted in table 8n after the concentration of metallic elements found in the water samples. This table is based on the quantitative analysis. The table shows that the presence of metals is higher in the neutral unlimed lake than in the acidified/limed lake. The results found in the water measurements from the total quant measurement can therefore be confirmed.

Table 8n: The elements sorted after the concentration in the water samples (N=neutral lake, L=limed lake, A=Acidified lake)

| Element/ Isotope | Highest Conc. | Middle Conc. | Lowest conc. | Element/ Isotope | Highest Conc. | Middle Conc. | Lowest conc. |
|---------------------|------------------|-----------------|-----------------|---------------------|------------------|-----------------|-----------------|
| Cu | N | L | A | Co | N | A | L |
| Zn | N | A | L | Sb 123 | N | A | L |
| Sn 118 | - | - | - | Sb 121 | N | A | L |
| Sn 120 | L | - | - | Mn | N | A | L |
| Pb | A | N | - | Ni | N | A | L |
| Cd | N | A | L | Ag | A | - | - |
| Sr | N | L | A | Al | N | A | L |
| Na | N | L | A | Te 128 | - | - | - |
| Li | N | - | - | Te 130 | L | - | - |
| Mg | N | L | A | Cr | N | - | - |
| Fe | N | A | L | | | | |

From the total quant measurement elements with similar water concentration were chosen. Since the total quant is not as precise as the quantitative analysis a new investigation of the similarities found between the concentration of metals in the water samples have been made. The ratios between the concentration of metallic elements in the water samples (where it exists) are shown in table 8o. The ratio is regarding the maximum and minimum concentration.

Table 8o: The ratio between the concentration of metallic elements from the water samples in the limed, acidified and neutral lake (max conc./min conc.).

| Element/Isotope | ratio | Element/Isotope | ratio |
|-----------------|-------|-----------------|-------|
| Cu | 4,2 | Fe | 2,3 |
| Zn | 4,9 | Co | 8,4 |
| Sr | 7,2 | Sb 123 | 1,3 |
| Na | 2,3 | Sb 121 | 1,3 |
| Mg | 4,1 | Mn | 3,9 |

As can be seen in table 8o (Compare to table 8b), some elements were found not to have similar concentrations in the water samples. These elements, along with the other selected elements, have been investigated on their own. In table below (table 8p) specific hypothesizes about each of these element, based on what conclusions that can be made from their presence in the water samples have been made.

Table 8p: The element that did not have similar concentration in the water samples and the possibility of how to investigate them.

| Element /Isotope | Type of investigation |
|------------------|---|
| Sn | To achieve more certain results measurements are made for two isotopes regarding Sn (Sn 118 and Sn 120). The results should be the same since an isotope is basically just a fraction of the element. Both isotopes react the same in the environment and should therefore be regarded as the same. |
| Sn 118 | This fraction of Sn was not found in any of the water samples. If it is present in any of the fish samples, it is possible that the bioavailability of this element is higher in that specific lake. However, to conclude that this element for sure |

| | |
|--------|--|
| Sn 120 | <p>is more or less available cannot be done because of the chemical difference between the waters. It is possible that the element is present in i.e. the sediments where it is incorporated into biota and thereafter accumulated in the food chain.</p> <p>This fraction of Sn was found in the water samples from the limed lake. If it is incorporated in to the fish species in the limed lake it should be present in a bioavailable form. If it is not accumulated in the limed lake but in the acidified and neutral lake, it is possible that it binds stronger to the fish in the neutral or the acidified lake.</p> |
| Pb | <p>This element was originally intended to be included in the comparing analysis, but the quantitative analysis of the water samples dismissed those intentions since the water concentrations were found to differ too much between the lakes. However the accumulation of lead will be compared between the acidified and neutral lake where the concentrations were similar. If the concentrations of lead are higher in the fish from the limed lake than in the fish from the neutral and acidified lake, then it is possible that lead has a higher tendency to react with organic matter in limed lakes. If that is the case, lead is a toxicity problem when liming is performed</p> |
| Cd | The same as for lead |
| Li | <p>This element was originally intended to be included in the comparing analysis, but the quantitative analysis of the water samples dismissed those intentions. Regarding the water samples; it is only found in the neutral lake. There will be no comparing study between the lakes regarding lithium. However, it will be investigated if lithium is accumulated in the fish species in the limed and acidified lake. If lithium is accumulated in the limed and acidified lake but not in the neutral lake, then it is possible that lithium has a higher tendency to react with organic matter in limed and acidified lakes.</p> |
| Ni | The same as for lead and cadmium |
| Ag | <p>This element was only found in the acidified lake. There will be no comparing study between the lakes regarding silver. If Ag is accumulated in fish species in the limed and neutral lake, to an extent higher than in the acidified lake, then it is possible that acidification does not only bring harm to biological systems; acidification might successfully remove certain toxic elements from biota.</p> |
| Al | The same as for lead, cadmium and nickel with the exception of any comparing study; the concentrations of metallic elements differ too much in the water samples |
| Te | To achieve more certain results measurements are made for two isotopes regarding Te (Te 128 and Te 130). The results should be the same since an isotope is basically just a fraction of the element. Both isotopes react the same in the environment and should therefore be regarded as the same. |
| Te 128 | This fraction of Te was not found in any water samples and therefore it will be hard to investigate (if it is present in any fish sample) |
| Te 130 | This fraction of Te was found in the water samples from the limed lake. If the concentration of this isotope is higher in the fish samples from the acidified/neutral lake then liming should be a preventive caution for removing this isotope from biological systems |
| Cr | <p>This element was only found in the neutral lake. There will be no comparing study between the lakes regarding chromium. If the concentrations of this element are higher in the fish samples from the limed/acidified lake, then</p> |

| |
|--|
| liming will not support removal of chromium from acidified waters. |
|--|

8.3.2. About the following Analysis of the elements

The elements are analysed separately. It has not been taken into consideration that an elements uptake may be affected by the presence of other elements that in some way may put limitations to the incorporation rate into the fish. However, if or how much this interferes with the results is not understood. Also the neutral lake is situated in a completely different environment than the acidified/limed lake and it contains a more diverse composition of metallic elements in the water than the limed and acidified (according to the tot quant analysis). The ideal choice for selection of the lakes would be if there were several options available in advance in which the water concentrations could be investigated before the final choice of lakes were selected. However, no limitations can be found in the selection of the limed/acidified lake. The choice of these two lakes seems to be ideal for investigating what effects liming of acidified lakes has to the bioavailability of metallic elements. I might point out that I have considered the relation between the reference lake and the limed lake as reliable. If large deviations between these lakes in i.e. water concentration are found then it will be assumed that this is a consequence of the liming performed. No reasonable reason for why there otherwise should be a difference between these two lakes can be found.

In each investigation in the following part the concentration are included (in numbers) and some diagrams based on the concentration found in the water and fish samples. *One important note regarding the graphs are the units:* the unit in the water samples is in $\mu\text{g/l}$ and the unit in the fish samples is in mg/kg . This difference in units was kept to make the concentrations in the water samples more visible; the graphs are based on the concentration attached in the table in each investigation.

8.3.3. Analysis of the elements

8.3.3.1. Analysis of Copper

About the results

There are three samples in which the standard deviations were large enough to be considered a problem. These are: Tissue of the pike from the limed lake, tissue from the perch in acidified lake and tissue from the pike in the acidified lake. The results from these categories may be considered uncertain.

Table 8.3a: The concentration of Copper in fish species from the limed, acidified and neutral lake

| Element: Cu | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|----------------|---------------|---------------|
| Water conc. ($\mu\text{g/l}$) | $0,79\pm0,52$ | $1,2\pm0,90$ | $3,4\pm0,80$ |
| Perch liver (mg/kg) | $11\pm2,6$ | $9,2\pm2,2$ | $11\pm4,2$ |
| Pike liver (mg/kg) | $17\pm3,0$ | $37,0\pm11,0$ | |
| Perch tissue (mg/kg) | $1,3\pm1,3$ | $5,6\pm1,8$ | $0,79\pm0,57$ |
| Pike tissue (mg/kg) | $1,2\pm1,1$ | $2,0\pm4,5$ | |

Examination of the results

The concentrations of copper from the three lakes are shown in table 8.3a and figure 8.1-8.2. The main binding site for copper is the liver in all three lakes. The

concentrations in the water samples are similar in the three lakes, although it was unexpected that the concentration was found to be the lowest in the acidified lake. As can be seen in the graphs, copper has a strong tendency to bind to the fish in the limed lake. The concentrations of copper are found to be the highest in both the perch and pike from the limed lake. Liming of lakes seems to increase the bioavailability of copper to biota. If liming had not been performed, it is possible that the accumulation of copper had remained at a level similar to that in neutral/acidified lakes. The accumulation of copper is more or less similar in neutral and acidified lakes according to this result.

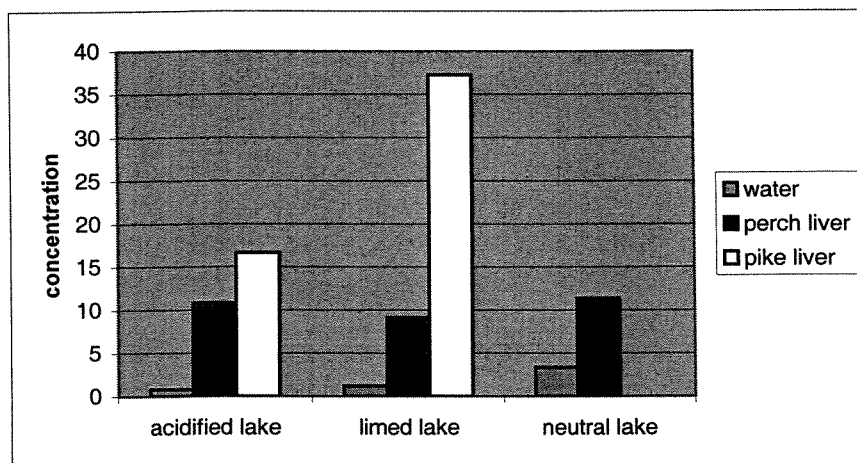


Figure 8.1.: Copper accumulation in the liver from the fish species in the limed, acidified and neutral lake

Also, copper seems to have a strong tendency to biomagnify in the liver. This is especially true for the limed lake, but also for the acidified where it biomagnifies to a smaller extent. The pike seems to be biomagnifying copper the strongest, while the perch contains approximately the same concentration of copper in all three lakes.

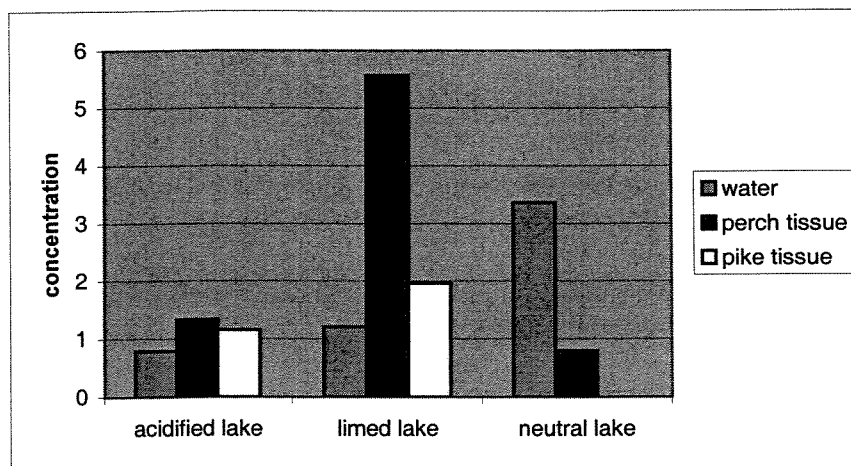


Figure 8.2.: Copper accumulation in the tissue from the fish species in the limed, acidified and neutral lake

Conclusion from analysis of Cu:

- Liming is a problem when performed; it increases the bioavailability of copper.

- Acidification does not increase the bioavailability of Cu; the accumulation of copper remains at a level similar to what was found in the neutral, unlimed lake.

8.3.3.2. Analysis of Zn

About the results

There are two samples that were found to have large uncertainties. These are the pike tissue from the limed lake and the perch liver from the acidified lake.

Table 8.3b: The concentration of Zinc in fish species from the limed, acidified and neutral lake

| Element: Zn | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|------------|--------------|
| Water conc. (µg/l) | 11±5,4 | 2,4±0,94 | 12±3,1 |
| Perch liver (mg/kg) | 140±72 | 98±12 | 120±28 |
| Pike liver (mg/kg) | 100±20 | 155±42 | |
| Perch tissue (mg/kg) | 30±10 | 37±8,2 | 29±4,5 |
| Pike tissue (mg/kg) | 30±9,2 | 100±49 | |

Examination of the results

The concentrations of zinc from the three lakes are shown in table 8.3b and figure 8.3-8.4. Firstly, there is a large difference found between the concentration of Zn in the water samples from the limed and acidified lake. The concentrations of Zn are much lower in the limed lake than in the acidified lake; it seems as if liming removes zinc from the water. In contradiction to that, zinc is found to be the biggest problem in the fish samples from the limed lake; it biomagnifies in both the liver and tissue. When biomagnification is compared between the limed and acidified lake one interesting thing is found: there seem to be a difference in the availability of zinc to the pike when the limed/acidified lake is compared. Instead of biomagnification in the acidified lake the concentrations of Zn decreases in the pike. Whether or not the difference in water concentrations is the reason for this difference remains unknown; it is possible that the high concentration of Zn in the water in the acidified lake saturate the rate at which zinc is biomagnified.

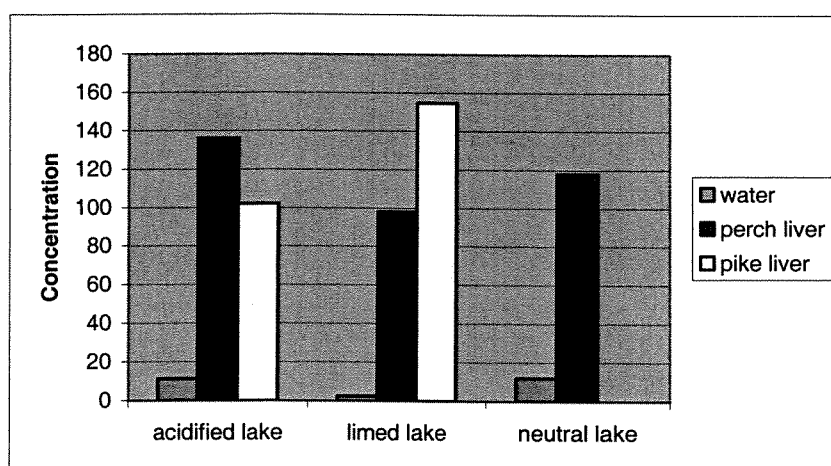


Figure 8.3.: Zinc accumulation in the liver from the fish species in the limed, acidified and neutral lake

There are no larger differences in the rate at which Zn is incorporated into the perch; acidification or liming seems not to affect the bioavailability of Zn compared to the neutral lake (regarding the perch).

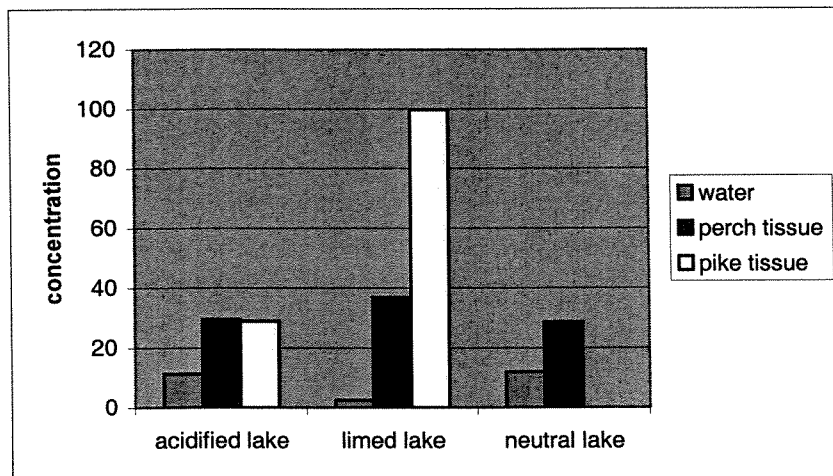


Figure 8.4.: Zinc accumulation in the tissue from the fish species in the limed, acidified and neutral lake

Conclusions from Zn:

- Liming increases and supports biomagnification compared to acidified lakes
- Acidification or liming does not increase the bioavailability of Zn

8.3.3.3. Analysis of Sn

About the results

There are two categories of samples that were found to have large uncertainties regarding Sn 118. These are the pike liver from the acidified lake and the water samples from the limed lake. For Sn 120 the following categories of samples had large standard deviations: the water samples from the limed lake and the pike liver from the acidified lake.

Table 8.3c: The concentration of Sn 118 in fish species from the limed, acidified and neutral lake

| Isotope: Sn 118 | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|-------------|--------------|
| Water conc. (µg/l) | 0,00±0 | 0,044±0,098 | 0,00±0 |
| Perch liver (mg/kg) | 0,091±0,079 | 0,10±0,09 | 0,24±0,31 |
| Pike liver (mg/kg) | 0,019±0,043 | 0,15±0,16 | |
| Perch tissue (mg/kg) | 0,025±0,039 | 0,21±0,25 | 0,00±0 |
| Pike tissue (mg/kg) | 0,00±0 | 0,00±0 | |

Table 8.3d: The concentration of Sn 120 in fish species from the limed, acidified and neutral lake

| Isotope: Sn 120 | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|------------|--------------|
| Water conc. (µg/l) | 0,00±0 | 0,047±0,11 | 0,00±0 |
| Perch liver (mg/kg) | 0,10±0,09 | 0,11±0,08 | 0,25±0,32 |
| Pike liver (mg/kg) | 0,030±0,06 | 0,15±0,16 | |
| Perch tissue (mg/kg) | 0,027±0,04 | 0,21±0,24 | 0,00±0 |
| Pike tissue (mg/kg) | 0,00±0 | 0,00±0 | |

Examination of the results

The concentrations of Sn from the three lakes are shown in table 8.3c-d. Sn was only found in the water samples from the limed lake. Sn was however present in all fish

samples. As could be expected from the water samples, the concentration of Sn in fish should be the largest in the limed lake. This was however not the case, the perch from the neutral lake contained even higher concentrations. In what form Sn is present in the neutral lake remains unknown, but it seems to have a strong tendency to bind to the perch liver in neutral lakes.

When comparing the neutral lake to the acidified lake, one good thing about acidification is found; Sn is not bioavailable to the same extent as in the neutral lake. If the limed and acidified lake are compared another effect of liming is found; Sn is more bioavailable in limed lakes (lakes which were acidified before liming was started). The trend found for Sn is not what was expected; it seems to be the most harmful, or accumulates the most, in neutral lakes, followed by limed lakes. The accumulation of Sn in fish was found to be the smallest in the fish from the acidified lake.

Different binding sites in fish are found for different lakes. In the acidified and neutral lake Sn binds the strongest to the liver, while in limed lakes it preferably binds to the tissue (for perch).

Sn is only biomagnified in the liver of the fish from the limed lake. Sn is not found in any of the pike tissue samples. Sn also seems to prefer to accumulate in the liver in fish from neutral waters; its concentrations in the tissue was zero (see also analysis of Mn for further investigation of this matter)

Conclusions from Analysis of Sn

- The bioavailability of Sn is the smallest in the acidified lake, while the concentrations were found to be the largest in the neutral lake; liming or acidification does not increase the bioavailability of Sn compared to neutral lakes
- Liming of acidified lakes increases the bioavailability of Sn compared to the acidified lake

8.3.3.4. Analysis of Pb

About the results

There are two categories of samples in which the standard deviation were large enough to be considered a problem. These are the pike tissue from the acidified lake and the water samples from the acidified lake.

Table 8.3e: The concentration of Lead in fish species from the limed, acidified and neutral lake

| Element: Pb | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|------------|--------------|
| Water conc. (µg/l) | 1,0±2,0 | 0,00±0 | 0,30±0,12 |
| Perch liver (mg/kg) | 0,64±0,57 | 1,4±0,85 | 1,2±0,74 |
| Pike liver (mg/kg) | 0,82±0,32 | 0,61±0,68 | |
| Perch tissue (mg/kg) | 0,50±0,32 | 1,1±0,92 | 0,00±0 |
| Pike tissue (mg/kg) | 0,23±0,31 | 0,10±0,12 | |

Examination of the results

The concentrations of lead from the three lakes are shown in table 8.3e and figure 8.5-8.6. Regarding the water samples, the concentrations were found to be the highest in the acidified lake. This is however as expected from acidified waters. Regarding the limed lake where the water concentrations were zero; the presence of lead in the

neutral and acidified lake bring about the possibility that liming removes lead from the water.

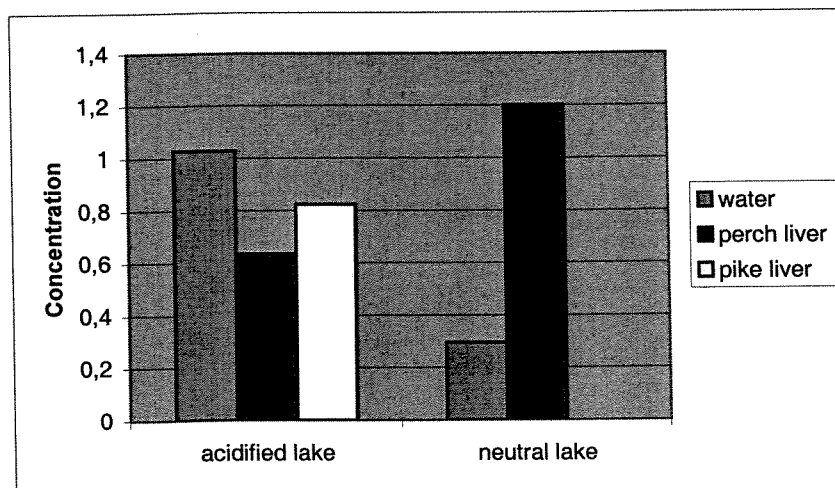


Figure 8.5.: Lead accumulation in the liver from the fish species in the acidified and neutral lake

Lead preferably binds to the perch in the three lakes investigated; the concentrations of lead are the highest in the perch. If i look more carefully at the fish samples, it is clear that lead does not accumulate as strong in the acidified lake as it does in the limed lake (where the highest ratios between water and fish are found) and the neutral lake. However, as for Sn, there seems to be a difference in where lead most preferably binds between limed/acidified and neutral lakes; In the neutral lake lead is not found in the tissue of the perch.

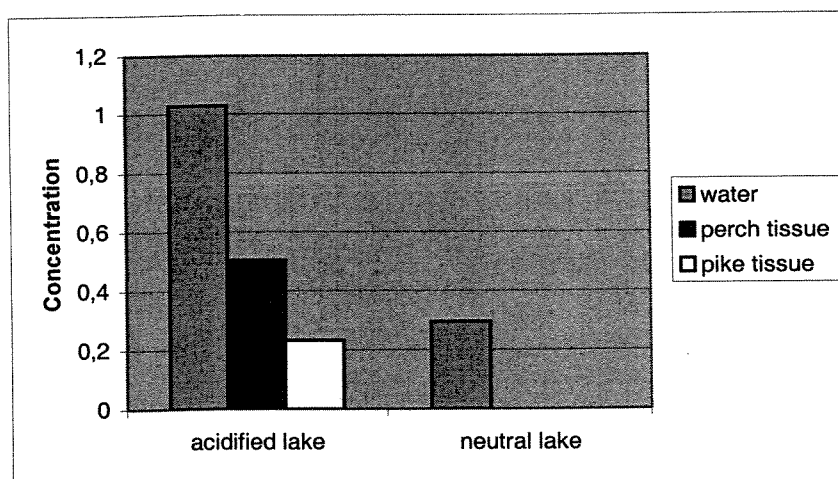


Figure 8.6.: Lead accumulation in the tissue from the fish species in the acidified and neutral lake

When biomagnification is examined it is found that lead biomagnifies in the liver in the acidified lake (to a small extent). When examining the uptake of lead in the limed lake; the liming seems to increase the uptake of lead in the perch, while the pike is actually favoured by liming; the concentrations of lead is less in the pike from the limed lake compared to the pike in the acidified lake.

Conclusions from analysis of lead

- Liming of acidified waters removes lead from the water
- Acidification do not support increased bioavailability of lead
- Liming increase the the bioavailability of lead to the perch

8.3.3.5. Analysis of Cd

About the results

There are three categories of samples in which the standard deviation were high. These are the pike liver from the limed lake and the water samples from the limed and acidified lake.

Table 8.3f: The concentration of Cadmium in fish species from the limed, acidified and neutral lake

| Element: Cd | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|--------------|--------------|
| Water conc. (µg/l) | 0,27±0,37 | 0,0060±0,013 | 0,39±0,25 |
| Perch liver (mg/kg) | 4,00±1,1 | 10,7±8,2 | 0,88±0,65 |
| Pike liver (mg/kg) | 13±4,4 | 1,4±1,8 | |
| Perch tissue (mg/kg) | 0,63±0,74 | 8,0±5,9 | 0,00±0 |
| Pike tissue (mg/kg) | 0,66±0,61 | 0,28±0,33 | |

Examination of the results

The concentrations of cadmium from the three lakes are shown in table 8.3f and figure 8.7-8.8. Cadmium belongs to the metallothioneins and therefore its concentrations are expected to be the highest in the liver, which they also were found to be. These Cd concentrations in fish are high. They may therefore show the importance of MT's.

Firstly, the concentrations of Cadmium in water samples are similar between the acidified and neutral lake; acidification seems not to release excessive Cd into the water. This result may however be due to naturally occurring differences as mentioned in section 8.3.2. In the limed lake however the same trend is found as for lead; the concentration of Cd is much less in the limed lake. It seems as if liming can make Cd precipitate from the water when liming is performed to acidified waters.

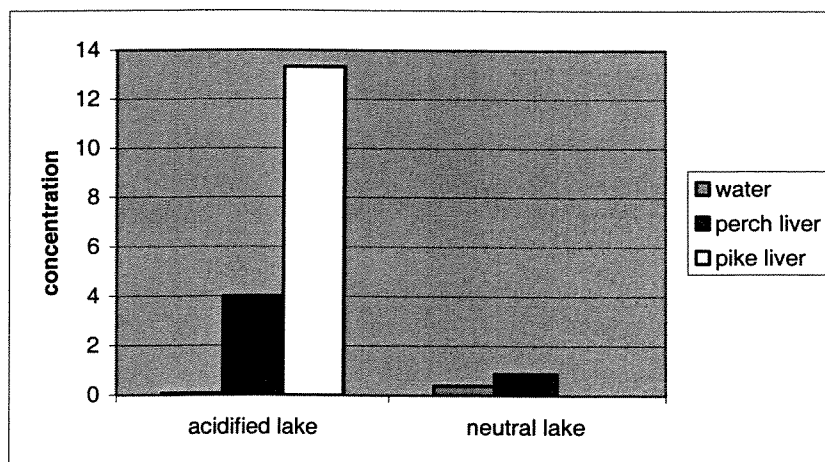


Figure 8.7.: Cadmium accumulation in the liver from the fish species in the acidified and neutral lake

Regarding Cd in the fish samples the trends are as could be expected; the highest concentration are found in the fish from the acidified lake. Cd biomagnifies in both

the liver and tissue in the acidified lake. In the neutral lake Cd does not seem to be bioavailable to the same extent; Cadmium binds weakly to the perch liver and not at all to the perch tissue.

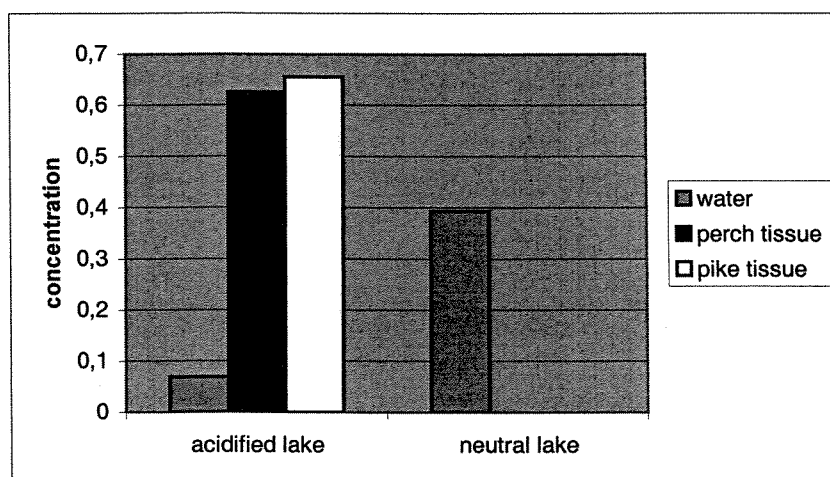


Figure 8.8.: Cadmium accumulation in the tissue from the fish species in the acidified and neutral lake

Liming however does not seem to decrease the bioavailability of Cadmium. The rate at which cadmium is incorporated into the fish in the limed lake is much larger than what was found in the acidified lake (in relation to the concentration in the water samples). Also, as was the case for lead, the perch is not favoured by liming when incorporation of Cd to this specie is examined; especially the perch tissue is a problem. In that context the pike seem to be favoured by liming; its concentrations are much less than in the pike from the acidified lake.

Conclusions from analysis of cadmium

- Acidification increases the bioavailability of Cd compared to neutral lakes
- Liming removes Cd from acidified waters, but at the same time increases the bioavailability of Cd, especially for perch.

8.3.3.6. Analysis of Sr

About the results

There are four samples in which the standard deviations were large enough to be considered a problem. These are the perch tissue from the limed lake, the pike tissue from the limed lake, the perch liver from the acidified lake and the pike tissue from the acidified lake

Table 8.3g: The concentration of Strontium in fish species from the limed, acidified and neutral lake

| Element: Sr | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|------------|--------------|
| Water conc. (µg/l) | 4,6±0,69 | 16±0,47 | 33±0,83 |
| Perch liver (mg/kg) | 13±22 | 0,73±0,8 | 0,75±0,71 |
| Pike liver (mg/kg) | 0,22±0,20 | 0,20±0,13 | |
| Perch tissue (mg/kg) | 19±15 | 6.0±8,1 | 0,66±0,80 |
| Pike tissue (mg/kg) | 2,6±3,0 | 1,3±2,4 | |

Examination of the results

The concentrations of strontium from the three lakes are shown in table 8.3g and figure 8.9-8.10. Regarding the water samples; the highest concentrations are found in the neutral lake. If the increased content of Sr in the limed lake is due to the lime or if the decreased concentration of Sr in the acidified lake is due to the acidification remains unknown. However, when liming is performed to acidified waters the concentrations of Sr increases after liming is performed.

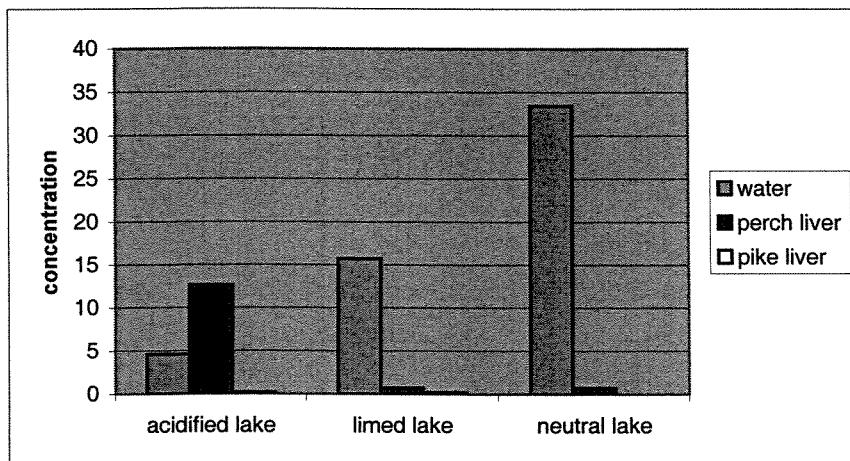


Figure 8.9.: Strontium accumulation in the liver from the fish species in the limed, acidified and neutral lake

Sr preferably binds to the tissue in the fish from the limed/acidified lakes while it prefers the liver in neutral lakes. When the rate at which Sr is incorporated in to biota is examined, the accumulation rate is found to follow an opposite trend as the water samples, which clearly shows where Sr is most bioavailable. The results are however as expected; Sr is mostly available to biota in the acidified lake and least available in neutral lakes. It is also found that, in the case of Sr, liming can restore an aquatic environment to the extent that it can be compared to the conditions in a neutral lake. As can be seen the accumulation of Sr is more similar to the accumulation in the neutral lake than the accumulation found in the acidified lake.

The strongest accumulation of Sr is found in the perch from the acidified lake. Sr does not biomagnify in any lake.

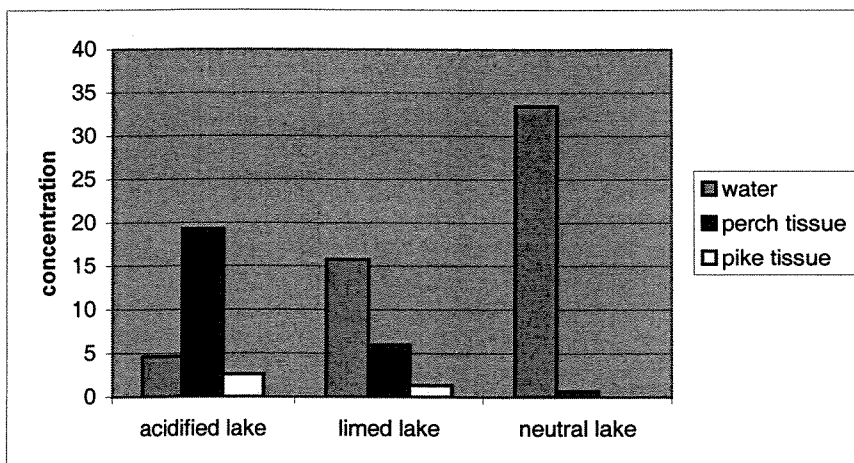


Figure 8.10.: Strontium accumulation in the tissue from the fish species in the limed, acidified and neutral lake

Conclusions from analysis of Sr

- Biomagnification of Sr is not a problem in limed and acidified lakes
- Acidification increases the bioavailability of Sr
- Liming makes accumulation of Sr in the fish more similar to the accumulation of Sr in fish found in neutral lakes than to the accumulation found in acidified lakes

8.3.3.7. Analysis of Na

About the results

There was no sample in which the standard deviation were large enough to be considered a problem.

Table 8.3h: The concentration of Sodium in fish species from the limed, acidified and neutral lake

| Element: Na | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|----------------|----------------|----------------|
| Water conc. ($\mu\text{g/l}$) | 1600 \pm 200 | 2100 \pm 110 | 3500 \pm 130 |
| Perch liver (mg/kg) | 2200 \pm 260 | 1700 \pm 340 | 2200 \pm 400 |
| Pike liver (mg/kg) | 1200 \pm 350 | 1100 \pm 140 | |
| Perch tissue (mg/kg) | 640 \pm 100 | 1200 \pm 300 | 900 \pm 140 |
| Pike tissue (mg/kg) | 810 \pm 80 | 1100 \pm 340 | |

Examination of the results

The concentrations of sodium from the three lakes are shown in table 8.3h and figure 8.11-8.12. Sodium preferably binds to the perch and pike liver. Sodium is the most abundant element in the quantitative analysis. Regarding the water samples the highest concentrations were found in the neutral lake and the lowest are found in the limed lake. Liming of acidified waters might possibly release more sodium to the water; the concentrations of sodium are higher in the limed lake compared to the acidified lake.

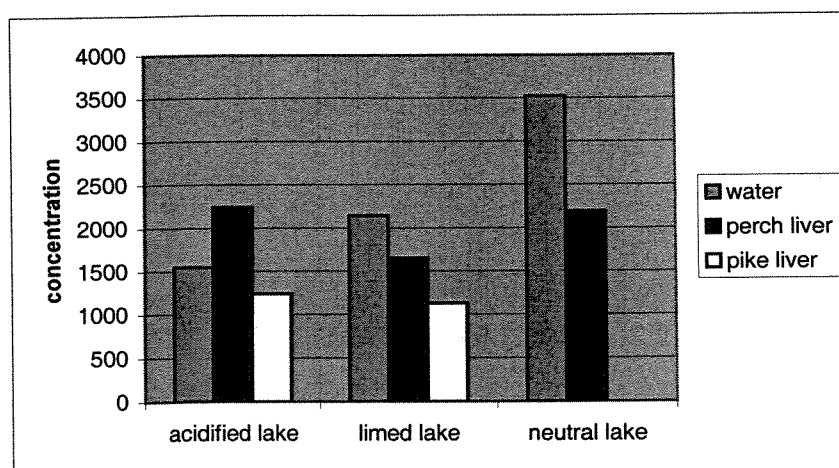


Figure 8.11.: Sodium accumulation in the liver from the fish species in the limed, acidified and neutral lake

The concentration of sodium in the fish samples is very similar; this is expected since the uptake of sodium should be controlled by homeostasis. The similarity is the most obvious between the acidified and neutral lake. For the limed lake it might be possible that liming affect (or disturbs) the distribution of sodium in the organism. This can be

seen in the graphs where it is found that the uptake of sodium is less in the liver and higher in the tissue of the fish from the limed lake compared to the neutral/acidified lake. The same trend is also found for the pike. The sum of the concentrations from each fish specie and lake however remains fairly similar.

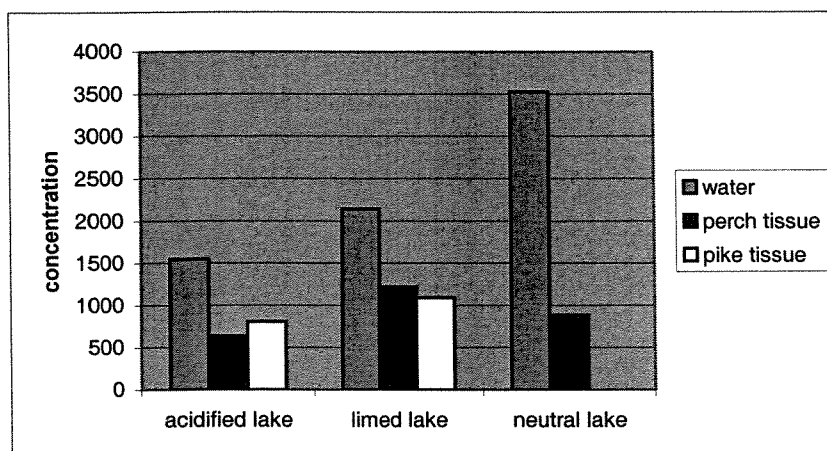


Figure 8.12.: Sodium accumulation in the tissue from the fish species in the limed, acidified and neutral lake

Sodium does not biomagnify in any lake; the concentration to which sodium is incorporated in the fish seems to be similar for pike and perch. This finding confirm the possibility of homeostasis even more.

Conclusions from analysis of Na

- Difference in bioavailability of sodium is small between limed, acidified and neutral lakes

8.3.3.8. Analysis of Lithium

About the results

There are two categories of samples in which the standard deviation were large enough to be considered a problem. These are the perch liver from the limed lake and the perch liver from the neutral lake.

Table 8.3i: The concentration of Lithium in fish species from the limed, acidified and neutral lake

| Element: Li | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|-----------------|--------------------|------------------|
| Water conc. ($\mu\text{g/l}$) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | 1,40 \pm |
| Perch liver (mg/kg) | 0,00 \pm 0,00 | 0,0064 \pm 0,017 | 0,04 \pm 0,088 |
| Pike liver (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | |
| Perch tissue (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | 0 \pm 0,00 |
| Pike tissue (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | |

Examination of the results

The concentrations of lithium from the three lakes are shown in table 8.3i. Lithium preferably binds to the perch liver in the limed and neutral lake. Lithium is found in water samples from the neutral lake; it is also accumulated to the perch liver in this lake. Lithium seems to be available to biota in neutral lakes.

Conclusion from analysis of Lithium

- Results are too inconclusive to confirm any trends

8.3.3.9. Analysis of Mg

About the results

There is no category of samples in which the standard deviation is large enough to be considered a problem.

Table 8.3j: The concentration of Magnesium in fish species from the limed, acidified and neutral lake

| Element: Mg | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|----------------|----------------|---------------|
| Water conc. ($\mu\text{g/l}$) | 470 \pm 58 | 880 \pm 35 | 2000 \pm 86 |
| Perch liver (mg/kg) | 560 \pm 86 | 580 \pm 95 | 650 \pm 100 |
| Pike liver (mg/kg) | 490 \pm 150 | 390 \pm 42 | |
| Perch tissue (mg/kg) | 1300 \pm 120 | 1300 \pm 150 | 1100 \pm 41 |
| Pike tissue (mg/kg) | 1200 \pm 47 | 1300 \pm 120 | |

Examination of the results

The concentrations of magnesium from the three lakes are shown in table 8.3j and figure 8.12-8.13. Magnesium is the second most abundant element in the quantitative analysis. Magnesium preferably binds to the pike and perch tissue in all three lakes. Regarding the water concentrations of magnesium the same difference between the limed and acidified lake is found as was found for sodium; liming of acidified waters seems to increase the abundance of magnesium in the water afterwards.

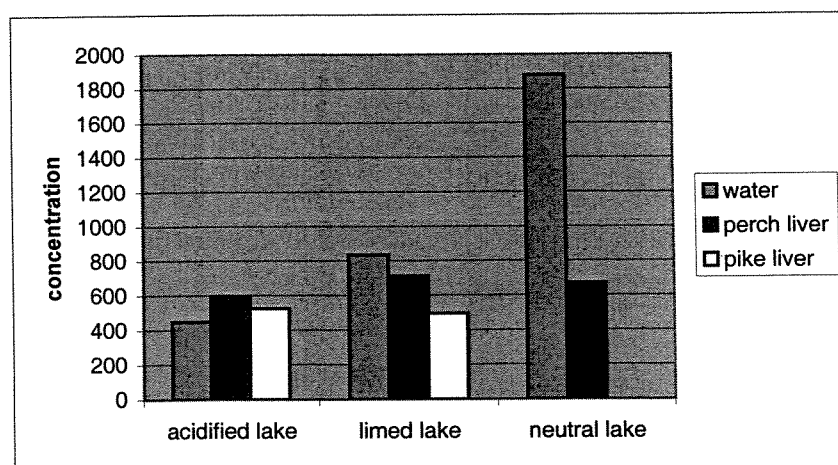


Figure 8.12.: Magnesium accumulation in the liver from the fish species in the limed, acidified and neutral lake

Regarding the fish samples; mg is found to be accumulated the strongest in the limed lake. The difference between the three lakes, regarding accumulation of mg in the fish, is however not very big. The rate at which magnesium is incorporated in the fish species is very similar between the acidified/neutral compared to the limed lake where the accumulation is somewhat higher.

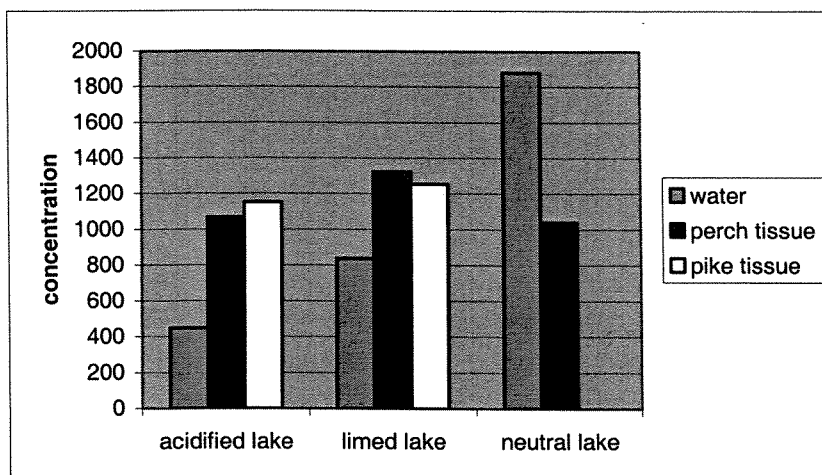


Figure 8.13.: Magnesium accumulation in the tissue from the fish species in the limed, acidified and neutral lake

Mg biomagnifies weakly in the acidified lake; the concentration of Mg remains fairly similar between the pike and the perch in the limed and acidified lake. This brings about the possibility that Mg uptake is controlled by homeostasis. This would mean that the water concentrations is of less importance when Mg is accumulated in the fish species; what really matters is a presence of Mg large enough in the water from which the organism can obtain quantities large enough to carry out its functions properly.

Conclusions from analysis of Mg

- Liming or Acidification does not increase the bioavailability of magnesium compared to neutral lakes

8.3.3.10. Analysis of Fe

About the results

There are three categories of samples in which the standard deviation were large enough to be considered a problem. These are the perch liver from the acidified lake, the pike tissue from the acidified lake and the water from the acidified lake.

Table 8.3k: The concentration of Iron in fish species from the limed, acidified and neutral lake

| Element: Fe | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|-----------------|----------------|---------------|
| Water conc. ($\mu\text{g/l}$) | 520 \pm 490 | 310 \pm 41 | 730 \pm 12 |
| Perch liver (mg/kg) | 2700 \pm 1200 | 460 \pm 160 | 290 \pm 95 |
| Pike liver (mg/kg) | 2400 \pm 930 | 1200 \pm 290 | |
| Perch tissue (mg/kg) | 50 \pm 18 | 100 \pm 72 | 9,0 \pm 3,0 |
| Pike tissue (mg/kg) | 140 \pm 270 | 26 \pm 17 | |

Examination of the results

The concentrations of iron from the three lakes are shown in table 8.3k and figure 8.14-8.15. Iron binds the strongest to the liver of both the perch and the pike. Regarding the water samples; the highest concentration were found in the acidified and neutral lake. If the limed lake is compared to the acidified it seems as if liming make Fe precipitate from the water. This is however as expected; liming should make iron precipitate from acidified waters.

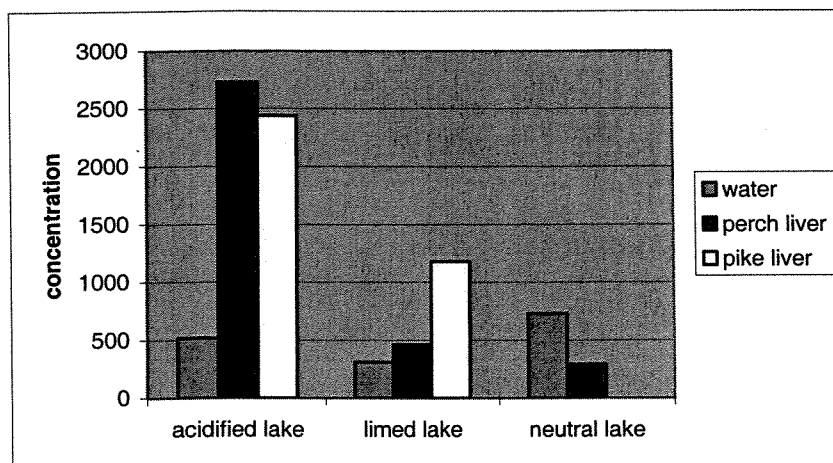


Figure 8.14.: Iron accumulation in the liver from the fish species in the limed, acidified and neutral lake.

For the fish samples the results are found to be as expected. The fish in the acidified lake accumulates Fe the strongest. One positive thing about liming can be found from this analysis; the bioavailability of iron in the limed lake is more similar to the bioavailability of iron found in the neutral lake (see i.e. the concentrations in the liver). For the tissue however liming seems to increase the bioavailability of iron, but these concentrations are small compared to the liver and therefore larger uncertainties might be present.

Also interesting is the high concentration of iron in the acidified lake. The concentrations in the tissue are however fairly similar in all three lakes; It seems as if excessive bioavailable iron are stored in the liver. The tissue might have a controlled uptake of iron to maintain its development, while excessive iron is stored in the liver.

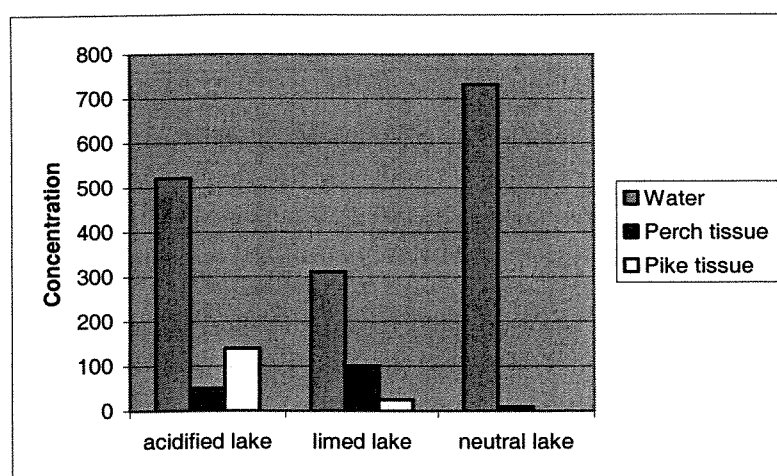


Figure 8.15: Iron accumulation in the tissue from the fish species in the limed, acidified and neutral lake.

Iron biomagnify in the liver in the limed lake and in the tissue of the acidified lake. The largest concentrations are however found in the perch from the acidified lake.

Conclusions from analysis of Fe

- Liming an acidified lake decreases the bioavailability of iron to a level more similar to that found in a neutral lake compared to an acidified lake
- Acidification increase the bioavailability of iron
- Liming of acidified waters decrease the concentration of iron in the water

8.3.3.11. Analysis of Cobalt

About the results

There are three categories of samples in which the standard deviation are large enough to be considered a problem. These are the perch tissues from the limed lake, the perch tissue from the acidified lake and pike tissue from the acidified lake.

Table 8.31: The concentration of Cobalt in fish species from the limed, acidified and neutral lake

| Element: Co | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|--------------|--------------|
| Water conc. (µg/l) | 0,083±0,046 | 0,023±0,0032 | 0,20±0,0060 |
| Perch liver (mg/kg) | 0,45±0,18 | 0,51±0,31 | 1,02±0,16 |
| Pike liver (mg/kg) | 0,10±0,034 | 0,12±0,083 | |
| Perch tissue (mg/kg) | 0,01±0,015 | 0,084±0,085 | 0,00±0,00 |
| Pike tissue (mg/kg) | 0,06±0,086 | 0,026±0,022 | |

Examination of the results

The concentrations of cobalt from the three lakes are shown in table 8.31 and figure 8.16-8.17. Cobalt binds the strongest to the liver of both pike and perch in limed, acidified and neutral lakes.

Liming might make Co precipitate from acidified waters; the concentrations are less in the water samples from the limed lake compared to the acidified lake. However, in the neutral lake the concentrations of Co are higher than in both the limed and acidified lake.

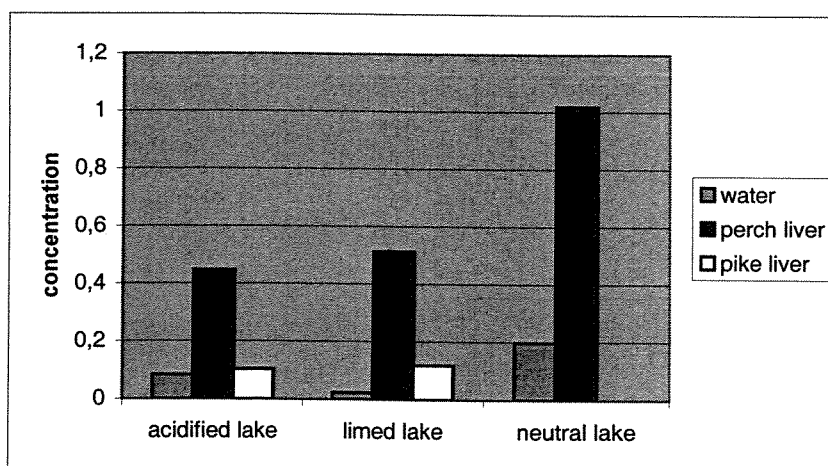


Figure 8.16.: Cobalt accumulation in the liver from the fish species in the limed, acidified and neutral lake.

For the fish samples the opposite to what was expected was found (before the water concentration were examined): Co binds the strongest to the fish in the neutral lake. The fish in the limed lake also contains even more Co compared to the fish in the acidified lake. It is however possible that the accumulation of Co in the neutral lake is

a reflection of the slightly higher water concentration of Co, compared to the other lakes.

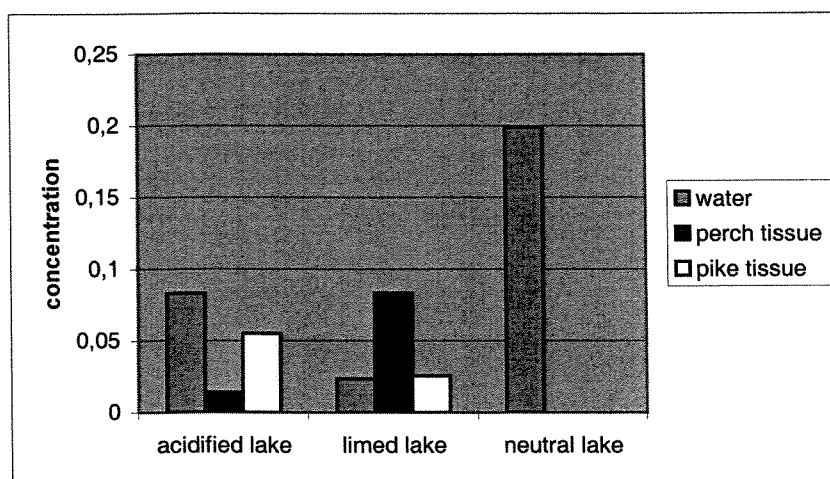


Figure 8.17.: Cobalt accumulation in the tissue from the fish species in the limed, acidified and neutral lake.

Co is biomagnified to a small extent in the tissue in the acidified lake. In all other categories the concentrations are higher in the perch than in the pike.

Conclusions from Co

- Acidification does not increase the bioavailability of Co
- Liming increase the bioavailability of Co to acidified lakes

8.3.3.12. Analysis of Sb

About the results

The concentrations of Sb were very small in the measurement, and therefore the small differences between the samples can generate a large standard deviation. The standard deviation is in many cases higher than the average value itself. However, the standard deviation never exceeded 2,5 times the average concentration of the samples.

Sb 123: the following category of samples had a standard deviation less than the average concentration: the perch tissue from the limed lake and the water samples from the acidified, neutral and limed lake.

Sb 121: the following category of samples had a standard deviation less than the average concentration: The perch tissue from the limed lake and the water samples from the limed, acidified and neutral lake.

Table 8.3m: The concentration of Sb fish species from the limed, acidified and neutral lake

| Isotope: Sb 123 | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|--------------|--------------|
| Water conc. (µg/l) | 0,079±0,071 | 0,077±0,0052 | 0,098±0,0095 |
| Perch liver (mg/kg) | 0,043±0,0080 | 0,15±0,30 | 0,018±0,037 |
| Pike liver (mg/kg) | 0,0028±0,0031 | 0,0058±0,013 | |
| Perch tissue (mg/kg) | 0,0059±0,0094 | 0,063±0,030 | 0,013±0,029 |
| Pike tissue (mg/kg) | 0,031±0,064 | 0,69±1,8 | |

Table 8.3n: The concentration of Sb fish species from the limed, acidified and neutral lake

| Isotope: Sb 121 | Acidified lake | Limed lake | Neutral lake |
|-----------------|----------------|------------|--------------|
|-----------------|----------------|------------|--------------|

| | | | |
|---------------------------------|-------------------|------------------|-----------------|
| Water conc. ($\mu\text{g/l}$) | $0,076\pm0,070$ | $0,076\pm0,0061$ | $0,10\pm0,0091$ |
| Perch liver (mg/kg) | $0,037\pm0,075$ | $0,15\pm0,30$ | $0,016\pm0,036$ |
| Pike liver (mg/kg) | $0,0028\pm0,0052$ | $0,0058\pm0,013$ | |
| Perch tissue (mg/kg) | $0,0038\pm0,0074$ | $0,064\pm0,030$ | $0,017\pm0,031$ |
| Pike tissue (mg/kg) | $0,028\pm0,062$ | $0,69\pm1,8$ | |

Examination of the results

The concentrations of antimony from the three lakes are shown in table 8.3m-n and figures 8.18-8.21. The concentration of Sb was very similar in the water samples. Liming of acidified waters does not seem to affect the concentration of sb in the water samples; the concentration of Sb in the limed lake is similar to the concentration found in the acidified lake.

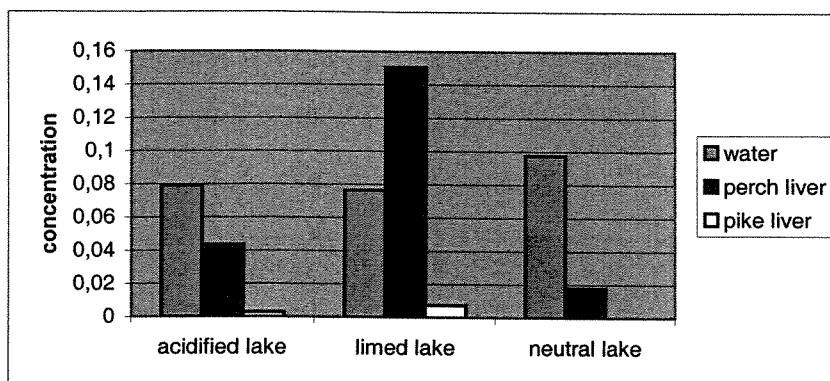


Figure 8.18.: Antimony accumulation in the liver from the fish species in the limed, acidified and neutral lake.

Accumulation of antimony in the fish was found to be the strongest in the limed lake. Both the liver and tissue from the limed lake contained the highest concentrations of antimony; liming seems to increase the bioavailability of antimony.

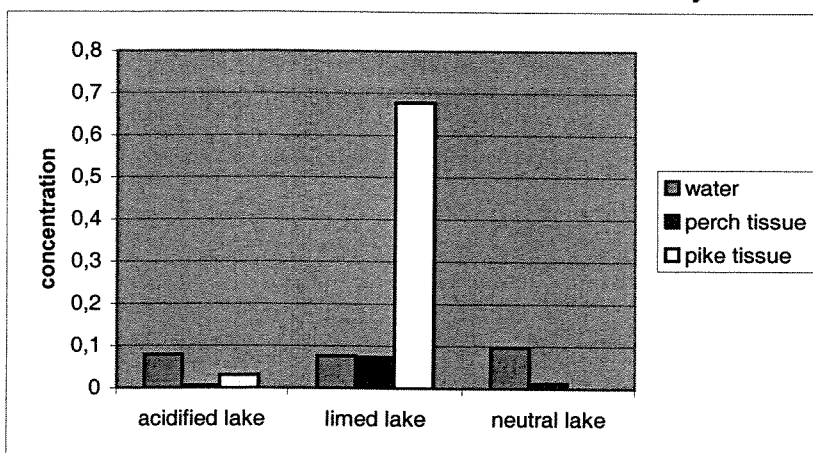


Figure 8.19.: Antimony accumulation in the tissue from the fish species in the limed, acidified and neutral lake.

When the acidified lake and its fish samples are compared to the fish from the neutral lake it is found that acidification does not increase the bioavailability of Sb. Antimony is biomagnified in the tissue in the limed and acidified lake. The selectivity regarding liver and tissue found in the total quant analysis still remains; Sb has a strong preference for binding to the liver in the perch while it binds stronger to the pike tissue than the pike liver.

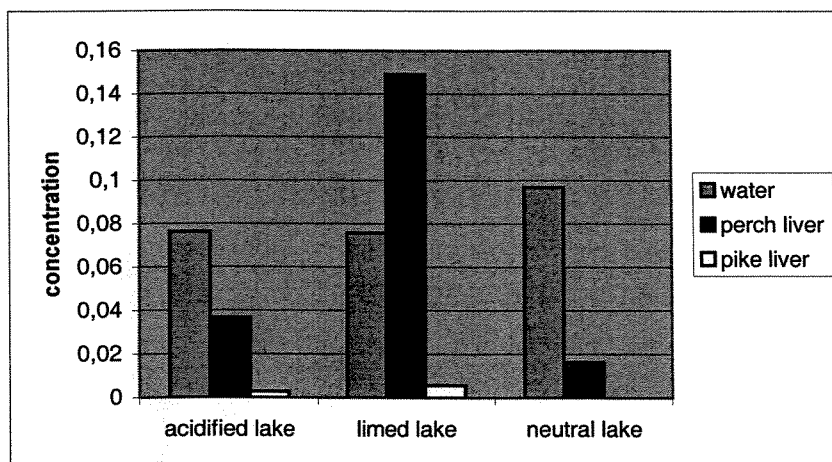


Figure 8.20.: Antimony accumulation in the liver from the fish species in the limed, acidified and neutral lake.

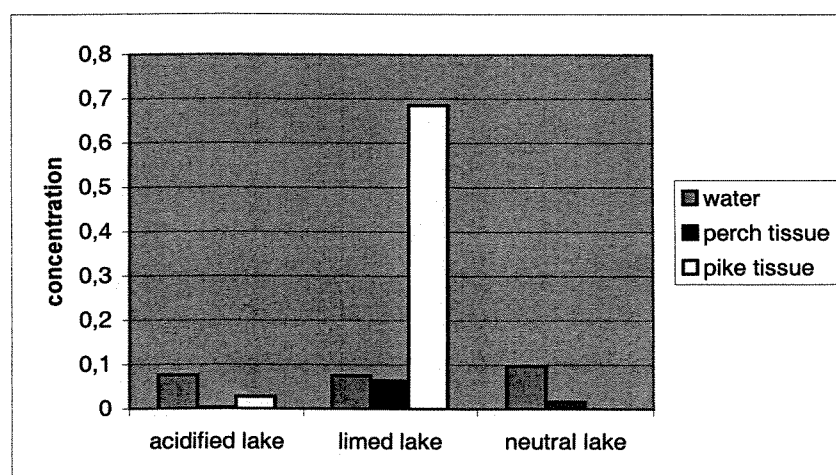


Figure 8.21.: Antimony accumulation in the tissue from the fish species in the limed, acidified and neutral lake.

Conclusions from analysis of Sb

- Liming increases the bioavailability of Sb
- Acidification does not increase the bioavailability of Sb; the accumulation of sb remains at a level similar to what was found in the neutral unlimed lake.

8.3.3.13. Analysis of Mn

About the results

There are three categories of samples that had a large standard deviation. These are the perch tissue from the limed lake, the pike tissue from the limed lake, the perch tissue from the acidified lake and the perch liver from the neutral lake.

Table 8.3o: The concentration of Manganese in fish species from the limed, acidified and neutral lake

| Element: Mn | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|----------------|----------------|---------------|
| Water conc. ($\mu\text{g/l}$) | $18 \pm 4,1$ | $7,2 \pm 0,76$ | $28 \pm 0,31$ |
| Perch liver (mg/kg) | $5,4 \pm 2,6$ | $4,9 \pm 2,4$ | $2,2 \pm 2,3$ |

| | | | |
|----------------------|-----------|-----------|-----------|
| Pike liver (mg/kg) | 3,9±3,8 | 2,3±0,74 | |
| Perch tissue (mg/kg) | 0,79±1,4 | 3,3±5,4 | 0,00±0,00 |
| Pike tissue (mg/kg) | 0,00±0,00 | 0,26±0,68 | |

Examination of the results

The concentrations of manganese from the three lakes are shown in table 8.3o and figure 8.22-8.23. Manganese binds the strongest to the liver of the fish samples.

The water samples from the acidified lake contains more manganese than the limed lake in the water samples; liming removes manganese from the waters.

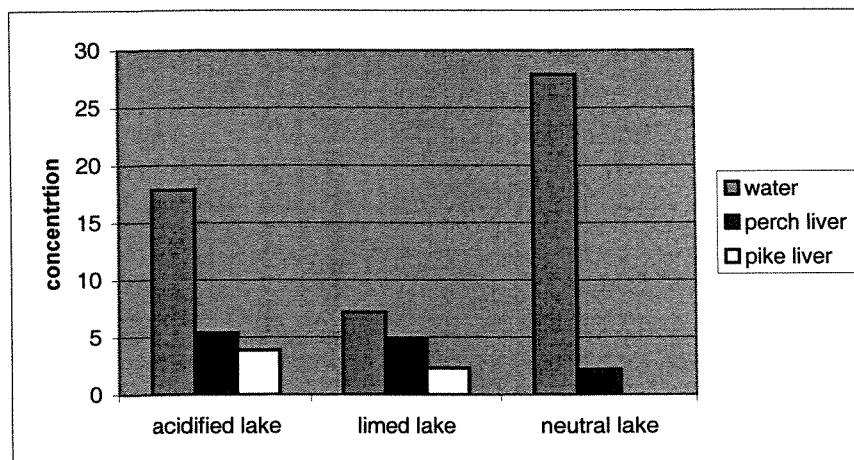


Figure 8.22.: Manganese accumulation in the liver from the fish species in the limed, acidified and neutral lake.

The accumulation of Mn is fairly similar in all three lakes; liming or acidification does not seem to increase the bioavailability of Mn. The concentration are however slightly higher in the limed lake but not to the extent that any conclusions can be confirmed.

One trend that is found for Mn and some other elements (Cd, Pb, Sn, Ni, Ag, Li, Co) is the way that these elements are distributed in the fish. In the fish samples from the neutral lake Mn is only found in the liver; it is not accumulated in the tissue. In the limed and acidified lake however, Mn and the other (mostly toxic) elements are found to be more evenly distributed between the liver and tissue. In the limed and acidified there seem to be less preference for where these elements most strongly binds. Whatever for this may be remains unknown. It is however possible that the affection liming and acidification has to waters disturbs the incorporation and selectivity of metals in the fish.

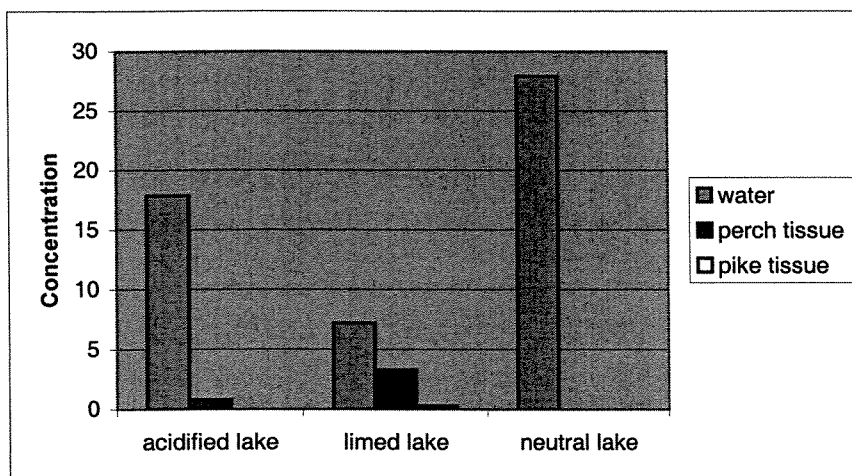


Figure 8.23.: Manganese accumulation in the tissue from the fish species in the limed, acidified and neutral lake.

Manganese does not biomagnify in either the limed or the acidified lake. The concentrations are however higher in the pike from the acidified compared to the pike from the limed lake.

Conclusion from Mn

- Manganese is not a problem in limed or acidified lakes when biomagnification is considered
- Results inconclusive regarding differences in bioavailability; Mn is however found to be the least available to biota in the neutral lake

8.3.3.14. Analysis of Ni

About the results

There are four categories of samples in which the standard deviation are large. These are the perch tissues from the acidified lake, the pike liver and tissue from the limed lake and the water samples from the acidified lake. The standard deviations are however fairly high in all these categories.

Table 8.3p: The concentration of Nickel in fish species from the limed, acidified and neutral lake

| Element: Ni | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|-----------------|-------------------|-----------------|
| Water conc. ($\mu\text{g/l}$) | $1,7 \pm 3,3$ | $0,030 \pm 0,059$ | $2,1 \pm 0,54$ |
| Perch liver (mg/kg) | $4,4 \pm 2,9$ | $3,9 \pm 4,2$ | $0,51 \pm 0,78$ |
| Pike liver (mg/kg) | $3,7 \pm 1,6$ | $2,2 \pm 2,5$ | |
| Perch tissue (mg/kg) | $0,31 \pm 0,43$ | $2,8 \pm 2,1$ | $0,00 \pm 0,00$ |
| Pike tissue (mg/kg) | $0,33 \pm 0,52$ | $0,27 \pm 0,57$ | |

Examination of the results

The concentrations of nickel from the three lakes are shown in table 8.3p and figure 8.24-8.25. Ni preferably binds to the liver of the fish samples in the limed, acidified and neutral lake.

Regarding the water samples; the concentration of Ni is much less in the limed lake compared to the acidified lake. When liming is performed to acidified waters, nickel is removed from the water.

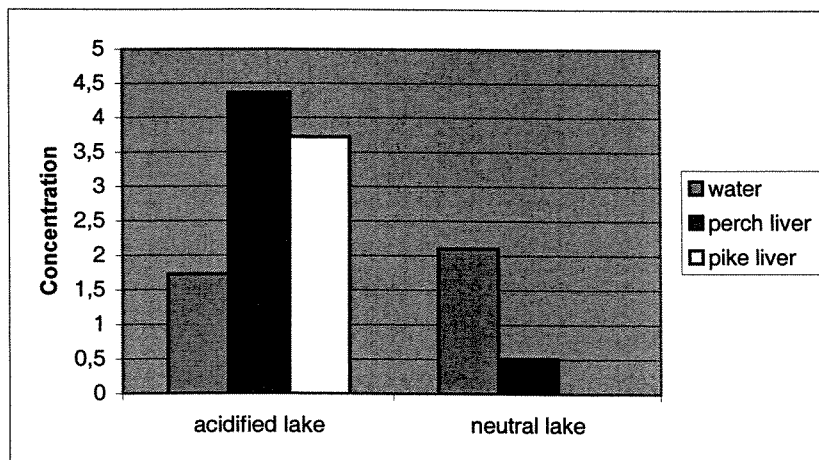


Figure 8.24.: Nickel accumulation in the liver from the fish species in the acidified and neutral lake.

For the fish samples the largest concentrations were found in the acidified lake; acidification increases the bioavailability of Ni compared to neutral lakes.

Regarding the limed lake where the water concentrations of Ni are very small, the fish was found to accumulate Ni strongly; liming increases the bioavailability of Ni

If the limed lake is compared to the neutral, and especially the ratios between water and fish, it is obvious that the affinity for Ni to bind to the fish increases when liming is performed.

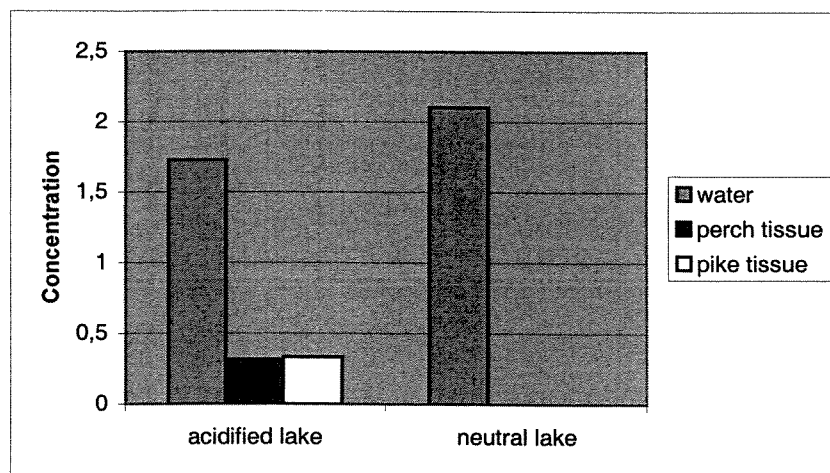


Figure 8.25.: Nickel accumulation in the tissue from the fish species in the acidified and neutral lake.

Ni biomagnifies to a small extent in the fish tissue in the acidified lake; the concentrations are however smaller in the tissue than the liver

Conclusions from Ni

- Acidification and liming, especially liming, increases the bioavailability of Ni compared to neutral lakes

8.3.3.15. Analysis of Ag

About the results

The following categories of samples have large standard deviations: the perch tissue from the acidified lake, the pike tissue from the acidified lake and the perch liver from the neutral lake.

Table 8.3q: The concentration of Silver in fish species from the limed, acidified and neutral lake

| Element: Ag | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|-------------|--------------|
| Water conc. (µg/l) | 0,15±0,34 | 0,00±0,00 | 0,00±0,00 |
| Perch liver (mg/kg) | 0,64±0,95 | 3,1±1,5 | 0,067±0,16 |
| Pike liver (mg/kg) | 0,28±0,35 | 0,91±0,37 | |
| Perch tissue (mg/kg) | 0,22±0,50 | 0,86±1,1 | 0,00±0,00 |
| Pike tissue (mg/kg) | 0,11±0,29 | 0,056±0,095 | |

Examination of the results

The concentrations of silver from the three lakes are shown in table 8.3q. Ag preferably binds to the liver and it is found to bind strongly to the fish also in the neutral lake.

Ag is only found in the water samples from the acidified lake. Compared to the limed lake, liming of acidified waters seems to decrease the concentration of silver in the water.

For the fish samples the highest concentrations were found in the limed lake; liming increases the bioavailability of silver, especially in the perch

Biomagnification of Ag is not a problem in limed or acidified lakes.

The bioaccumulation for Ag is very high in the lakes. Ag might possibly enter the fish through the sediments.

Conclusion from Ag

- Liming removes Ag from acidified waters but increases the bioavailability of Ag compared to acidified waters
- Biomagnification of Ag is not a problem in limed or acidified waters

8.3.3.16. Analysis of Aluminium

About the results

The following categories of samples have a large standard deviation: the perch liver and pike tissue from the limed lake and the water samples from the acidified lake. The standard deviations are however fairly high in all these categories. Also, during laboratory work, glass bottles were used which removes aluminium from the water and makes Al precipitate on the glass surface.

Table 8.3r: The concentration of Aluminium in fish species from the limed, acidified and neutral lake

| Element: Al | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|------------|--------------|
| Water conc. (µg/l) | 13±28 | 0,00±0,00 | 510±23 |
| Perch liver (mg/kg) | 0,00±0,00 | 45±58 | 0,00±0,00 |
| Pike liver (mg/kg) | 0,00±0,00 | 11±10 | |
| Perch tissue (mg/kg) | 0,00±0,00 | 29±27 | 0,00±0,00 |
| Pike tissue (mg/kg) | 0,00±0,00 | 6±7 | |

Examination of the results

The concentrations of aluminium from the three lakes are shown in table 8.3r. Aluminium solely binds to the perch and pike from the limed lake.

Regarding the water samples: the neutral lake contained the highest concentrations, which was unexpected. The result from the neutral lake is not reasonable for this lake. If the limed lake is compared to the neutral lake, the results are as expected; higher concentrations of Aluminium are found in the acidified lake. Liming seems to make aluminium precipitate from the water.

Regarding the fish samples it is found that liming can cause large aluminium toxicity; liming of acidified waters increases the bioavailability of aluminium to the fish species selected.

If the acidified and neutral lake are examined it is found that acidification does not seem to increase the bioavailability of aluminium to the fish.

Biomagnification is not a problem in the limed lake; the highest concentrations are found in the perch samples

Conclusions from analysis of Aluminium

- Biomagnification of aluminium is not a problem in limed lakes
- Liming increases the bioavailability of Aluminium. Liming also makes aluminium precipitate from the water
- Acidification does not increase the bioavailability of aluminium compared to neutral lakes

8.3.3.17. Analysis of Te

About the results

The sample in which Te was found has a standard deviation of ± 0.0085 .

Table 8.3s: The concentration of Tellurium in fish species from the limed, acidified and neutral lake

| Isotope: Te 128 | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|-----------------|-----------------|-----------------|
| Water conc. ($\mu\text{g/l}$) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | 0,00 \pm 0,00 |
| Perch liver (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | 0,00 \pm 0,00 |
| Pike liver (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | |
| Perch tissue (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | 0,00 \pm 0,00 |
| Pike tissue (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | |

Table 8.3t: The concentration of Tellurium in fish species from the limed, acidified and neutral lake

| Isotope: Te 130 | Acidified lake | Limed lake | Neutral lake |
|---------------------------------|-----------------|---------------------|-----------------|
| Water conc. ($\mu\text{g/l}$) | 0,00 \pm 0,00 | 0,0083 \pm 0,0085 | 0,00 \pm 0,00 |
| Perch liver (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | 0,00 \pm 0,00 |
| Pike liver (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | |
| Perch tissue (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | 0,00 \pm 0,00 |
| Pike tissue (mg/kg) | 0,00 \pm 0,00 | 0,00 \pm 0,00 | |

Examination of the results

The concentrations of tellurium from the three lakes are shown in table 8.3s-t. Not much can be concluded about this result; Te does not seem to bind to the fish in limed lakes

Regarding the difference between the water samples in the limed and acidified lake; it is possible that liming dissolves Te into the water from i.e. the sediments.

Conclusion from analysis of Te

- Results are not sufficient to make any conclusions

8.3.3.18. Analysis of Chromium

About the results

The standard deviations are large in both the perch liver from the limed lake and the water samples from the neutral lake.

Table 8.3u: The concentration of Chromium in fish species from the limed, acidified and neutral lake

| Element: Cr | Acidified lake | Limed lake | Neutral lake |
|----------------------|----------------|------------|--------------|
| Water conc. (µg/l) | 0,00±0,00 | 0,00±0,00 | 1,5±2,6 |
| Perch liver (mg/kg) | 0,00±0,00 | 0,60±1,6 | 0,00±0,00 |
| Pike liver (mg/kg) | 0,00±0,00 | 0,00±0,00 | |
| Perch tissue (mg/kg) | 0,00±0,00 | 0,00±0,00 | 0,00±0,00 |
| Pike tissue (mg/kg) | 0,00±0,00 | 0,00±0,00 | |

Examination of the results

The concentrations of chromium from the three lakes are shown in table 8.3u. The concentrations are low. Cr is only found in the water samples from the neutral lake; chromium does not accumulate in the fish from the neutral lake. Regarding the limed and acidified the results are inconclusive; it is possible that liming increase the bioavailability of chromium when acidified waters are being limed. Anyway, the bioavailability of chromium is larger in the limed lake compared to the neutral lake. Chromium does not biomagnify in any lake; the concentrations are the largest in the perch from the limed lake.

Conclusions from analysis of Cr

- Liming increases the bioavailability of Cr compared to neutral lakes

8.3.4. Summary from quantitative analysis

If the limed/acidified lake is compared to the neutral lake the following conclusions can be made:

- Liming increases the bioavailability of Cu, Pb, Cd, Sb, Ni, Al and Cr
- Liming decrease/maintain the bioavailability of Zn, Sn and Mg
- Acidification increases the bioavailability of Cd, Sr, Fe and Ni
- Acidification decrease/maintain the bioavailability of Cu, Zn, Sn, Pb, Mg, Co, Sb and Al

If the neutral lake is compared to the limed/acidified lake the following conclusion can be made:

- Liming/acidification decreases the bioavailability of Sn

If the limed lake is compared to the acidified lake the following conclusions can be made:

- Liming increases the bioavailability of Cu, Sn, Pb, Sb, Ag and Al
- Liming decreases the bioavailability of Sr and Fe

The above found conclusions suggest that liming actually cause harm to the fish species selected in the sense of metal accumulation. Liming more or less removes many of the metals present in the water in the reference acidified lake. In this way liming makes the water cleaner and thereby set the preconditions for the fry to survive the procedure of spawning; the exposure of metallic elements decreases for the fry in their early stages of life due to the liming. The adult species (which are the ones examined in this project) are however found to accumulate metals to a large extent, in many cases the accumulation is larger than in the acidified lake. This brings about the question of how the fish can maintain reproduction in limed lakes when the fish (adult species) actually are accumulating more metals than what has been found in the fish from the acidified lake. One of the main reasons for why the fish cannot maintain reproduction in acidified waters is because of the roes large exposure of metallic elements, which causes the roe to suffocate. It is possible that the different biological channels present in adult species, via which metals are accumulated, are not available in fish roe and milt and thereby they are protected from metal toxicity. However in adult species these channels are fully developed and the metal complexes formed due to liming may have a larger affinity to enter the organism via these channels. In acidified lakes the scene might be the opposite; the type of metal complex (or ion) present in acidified waters may have a larger affinity to precipitate on the roe than to enter the adult species via their channels. As stated in chapter 8.2.3.1.2 and 8.2.3.3.2 diffusion actually seems to be of more concern when regarding the uptake of metals in the limed lake; most metals found in the water were also present in the fish. This means that the metals are transformed, possibly when liming was performed, to a complex that has a larger affinity to enter the adult species. In the acidified lake the same reason for accumulation cannot be confirmed; the accumulation seems to be a mixture of both the presence of the metallic elements and its availability to biota. This means that metals are not available to the same extent to adult species since the metals that are present in the water was not bioavailable to the same extent as in the limed lake (which was actually found from the measurements). This was however not the case in the limed lake. Acidification increases the metal concentration in the water compared to limed lakes (based on the results from the quantitative analysis) and thereby the exposure of metallic elements increases for the fish.

It is also possible that there is a certain metal ion that is more toxic than others, in the early stages of life for aquatic organisms, which causes the fry to suffocate. If that is the case then it might be useless to lime if you are basically only after one or several toxic metals. If the goal is to maintain aquatic life then it might be possible to focus on the removal of toxic elements instead of raising the pH. Maybe one can add something else than lime that possesses those properties.

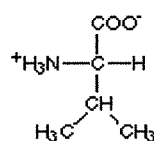
However the overall conclusions from this project is that liming of acidified lakes make metallic elements precipitate from the water. Liming also increases the bioavailability of metallic elements. Acidification on the other hand makes metallic elements dissolve into the water but the bioavailability to adult fish species is in many cases maintained at a level similar to the neutral lake.

9. Reference list

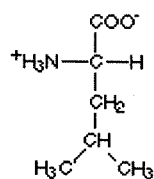
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Appendix 1

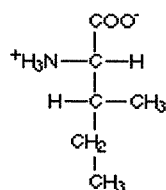
Amino acids with hydrophobic side groups



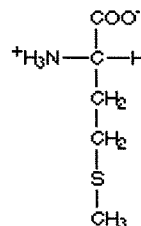
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(val)



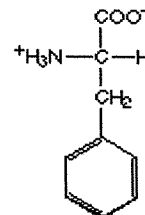
Leucine
(leu)



Isoleucine
(ile)

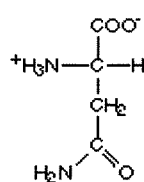


Methionine
(met)

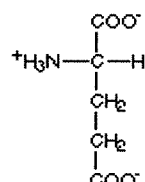


Phenylalanine
(phe)

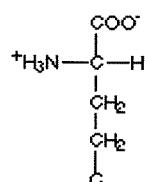
Amino acids with hydrophilic side groups



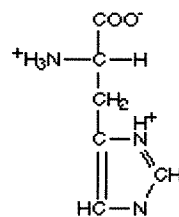
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(asn)



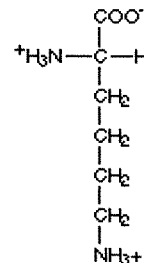
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(glu)



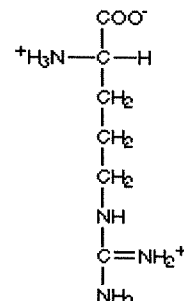
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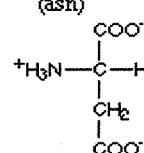
Histidine
(his)



Lysine
(lys)

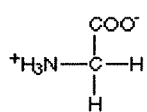


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(arg)

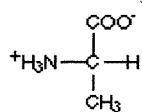


Aspartic acid
(asp)

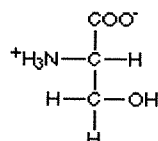
Amino acids that are in between



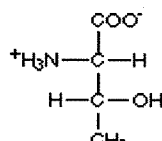
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(gly)



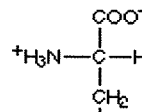
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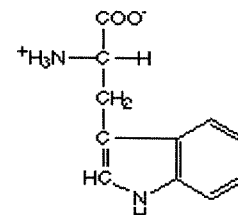
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(ser)



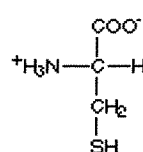
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(thr)



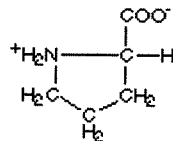
Tyrosine
(tyr)



Tryptophan
(trp)



Cysteine
(cys)



Proline
(pro)

Appendix 2

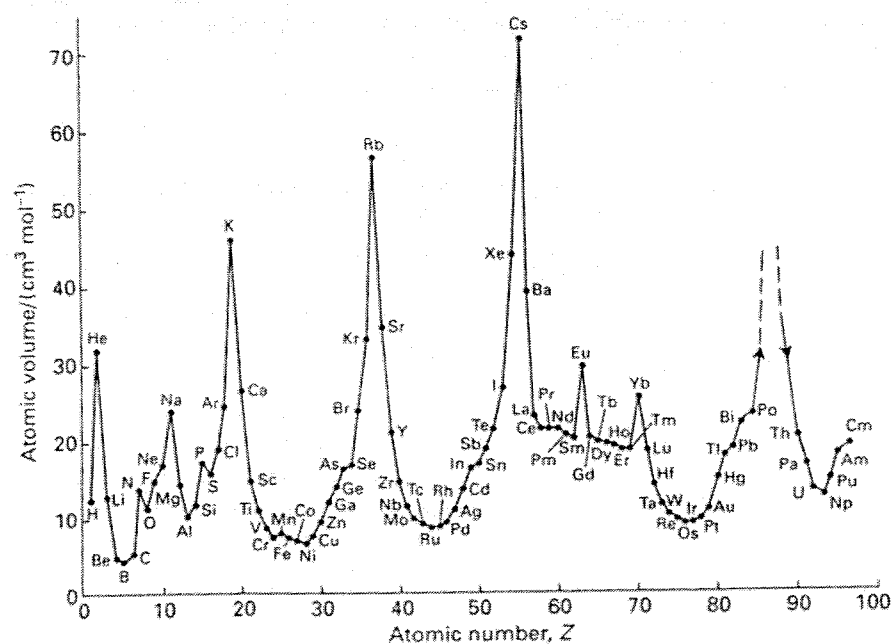


Figure 5: The variation of atomic volume with atomic number (Williams, Frausto da Silva 1996)

Table 1: The radius of the metallic elements (Williams, Frausto da Silva 1996)

[illegible]

Appendix 2 (cont.)

| | |
|--------|---------|
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Figure 6: The most common valences of the elements (Jackson 1996)

Appendix 2. (Cont.)

Table 2: the ratio between valence and radius (in the order they are found in the periodic table of the elements).

| | Valens | Radius (Å) | valens/radius | | Valens | Radius (Å) | valens/radius | | Valens | Radius (Å) | Valens/radii |
|---------|--------|------------|---------------|---------|--------|------------|---------------|---------|--------|------------|--------------|
| Li | 1 | 1,57 | 0,636943 | Ru | 3 | 1,34 | 2,238806 | Yb | 2 | 1,933 | 1,034661 |
| Be | 2 | 1,12 | 1,785714 | Ru | 4 | 1,34 | 2,985075 | Yb | 3 | 1,933 | 1,551992 |
| Na | 1 | 1,91 | 0,52356 | Ru | 5 | 1,34 | 3,731343 | Average | | | 1,293326 |
| Mg | 2 | 1,6 | 1,25 | Ru | 6 | 1,34 | 4,477612 | Lu | 3 | 1,738 | 1,726122 |
| Al | 3 | 1,43 | 2,097902 | Ru | 8 | 1,34 | 5,970149 | Lu | 3 | 1,72 | 1,744186 |
| K | 1 | 2,35 | 0,425532 | average | | | 3,88 | Hf | 4 | 1,59 | 2,515723 |
| Ca | 2 | 1,97 | 1,015228 | Rh | 2 | 1,34 | 1,492537 | Ta | 5 | 1,47 | 3,401361 |
| Sc | 3 | 1,64 | 1,829268 | Rh | 3 | 1,34 | 2,238806 | W | 2 | 1,41 | 1,41844 |
| Ti | 2 | 1,47 | 1,360544 | Rh | 4 | 1,34 | 2,985075 | W | 4 | 1,41 | 2,836879 |
| Ti | 3 | 1,47 | 2,040816 | average | | | 2,2 | W | 5 | 1,41 | 3,546099 |
| Ti | 4 | 1,47 | 2,721088 | Pd | 2 | 1,37 | 1,459854 | W | 6 | 1,41 | 4,255319 |
| average | | | 2,04 | Pd | 4 | 1,37 | 2,919708 | average | | | 3 |
| V | 2 | 1,35 | 1,481481 | average | | | 2,2 | Re | 2 | 1,37 | 1,459854 |
| V | 3 | 1,35 | 2,222222 | Ag | 1 | 1,44 | 0,694444 | Re | 4 | 1,37 | 2,919708 |
| V | 4 | 1,35 | 2,962963 | Cd | 2 | 1,52 | 1,315789 | Re | 5 | 1,37 | 3,649635 |
| V | 5 | 1,35 | 3,703704 | In | 1 | 1,67 | 0,598802 | Re | 6 | 1,37 | 4,379562 |
| average | | | 2,6 | In | 3 | 1,67 | 1,796407 | Re | 7 | 1,37 | 5,109489 |
| Cr | 2 | 1,29 | 1,550388 | average | | | 1,2 | average | | | 3,5 |
| Cr | 3 | 1,29 | 2,325581 | Sn | 2 | 1,58 | 1,265823 | Os | 2 | 1,35 | 1,481481 |
| Cr | 6 | 1,29 | 4,651163 | Sn | 4 | 1,58 | 2,531646 | Os | 3 | 1,35 | 2,222222 |
| average | | | 2,84 | average | | | 1,9 | Os | 4 | 1,35 | 2,962963 |
| Mn | 2 | 1,27 | 1,574803 | Sb | 1 | 1,45 | 0,689655 | Os | 6 | 1,35 | 4,444444 |
| Mn | 3 | 1,27 | 2,362205 | Sb | 3 | 1,45 | 2,068966 | Os | 8 | 1,35 | 5,925926 |
| Mn | 4 | 1,27 | 3,149606 | Sb | 5 | 1,45 | 3,448276 | average | | | 3,4 |
| Mn | 6 | 1,27 | 4,724409 | Average | | | 2,068966 | Ir | 2 | 1,36 | 1,470588 |
| Mn | 7 | 1,27 | 5,511811 | Cs | 1 | 2,72 | 0,367647 | Ir | 4 | 1,36 | 2,941176 |
| average | | | 3,46 | Ba | 2 | 2,24 | 0,892857 | Ir | 6 | 1,36 | 4,411765 |
| Fe | 2 | 1,26 | 1,587302 | La | 3 | 1,83 | 1,639344 | Ir | 3 | 1,36 | 2,205882 |
| Fe | 3 | 1,26 | 2,380952 | Average | | | 1,639344 | average | | | 2,8 |
| Fe | 6 | 1,26 | 4,761905 | Ce | 3 | 1,818 | 1,650165 | Pt | 2 | 1,39 | 1,438849 |
| average | | | 2,91 | Ce | 4 | 1,818 | 2,20022 | Pt | 4 | 1,39 | 2,877698 |
| Co | 2 | 1,25 | 1,6 | Average | | | 1,925193 | Pt | 6 | 1,39 | 4,316547 |
| Co | 3 | 1,25 | 2,4 | Pr | 3 | 1,824 | 1,644737 | average | | | 2,9 |
| average | | | 2 | Pr | 4 | 1,824 | 2,192982 | Au | 1 | 1,44 | 0,694444 |
| Ni | 2 | 1,25 | 1,6 | Average | | | 1,91886 | Au | 3 | 1,44 | 2,083333 |
| Ni | 3 | 1,25 | 2,4 | Nd | 2 | 1,814 | 1,102536 | average | | | 1,4 |
| average | | | 2 | Nd | 3 | 1,814 | 1,653804 | Hg | 1 | 1,55 | 0,645161 |
| Cu | 1 | 1,28 | 0,78125 | Average | | | 1,37817 | Hg | 2 | 1,55 | 1,290323 |
| Cu | 2 | 1,28 | 1,5625 | Pm | 3 | 1,834 | 1,635769 | average | | | 0,97 |
| average | | | 1,17 | Sm | 2 | 1,834 | 1,090513 | Tl | 1 | 1,71 | 0,584795 |
| Zn | 2 | 1,37 | 1,459854 | Sm | 3 | 1,834 | 1,635769 | Tl | 3 | 1,71 | 1,754386 |
| Ga | 3 | 1,53 | 1,960784 | Average | | | 1,363141 | average | | | 1,2 |
| Rb | 1 | 2,5 | 0,4 | Eu | 2 | 2,084 | 0,959693 | Pb | 2 | 1,75 | 1,142857 |
| Sr | 2 | 2,15 | 0,930233 | Eu | 3 | 2,084 | 1,439539 | Pb | 4 | 1,75 | 2,285714 |
| Y | 3 | 1,82 | 1,648352 | Average | | | 1,199616 | average | | | 1,71 |
| Zr | 2 | 1,6 | 1,25 | Gd | 3 | 1,804 | 1,662971 | Bi | 3 | 1,82 | 1,648352 |
| Zr | 3 | 1,6 | 1,875 | Average | | | 1,662971 | Bi | 5 | 1,82 | 2,747253 |
| Zr | 4 | 1,6 | 2,5 | Tb | 3 | 1,773 | 1,692047 | average | | | 2,2 |
| average | | | 1,875 | Tb | 4 | 1,773 | 2,256063 | Po | 4 | 1,64 | 2,439024 |
| Nb | 3 | 1,47 | 2,040816 | Average | | | 1,974055 | Po | 6 | 1,64 | 3,658537 |
| Nb | 5 | 1,47 | 3,401361 | Dy | 2 | 1,781 | 1,122965 | Average | | | 3,04878 |
| average | | | 2,72 | Dy | 3 | 1,781 | 1,684447 | Th | 4 | 1,79 | 2,234637 |
| Mo | 2 | 1,4 | 1,428571 | Average | | | 1,403706 | U | 3 | 1,56 | 1,923077 |
| Mo | 3 | 1,4 | 2,142857 | Ho | 3 | 1,762 | 1,702611 | U | 4 | 1,56 | 2,564103 |
| Mo | 4 | 1,4 | 2,857143 | Er | 3 | 1,761 | 1,703578 | U | 5 | 1,56 | 3,205128 |
| Mo | 5 | 1,4 | 3,571429 | Tm | 2 | 1,759 | 1,13701 | U | 6 | 1,56 | 3,846154 |
| Mo | 6 | 1,4 | 4,285714 | Tm | 3 | 1,759 | 1,705514 | Average | | | 2,884615 |
| average | | | 2,85 | Average | | | 1,421262 | | | | |
| Tc | 7 | 1,35 | 5,185185 | | | | | | | | |

Appendix 2 (cont.)

Table 3: The elements divided by increasing ratio between valence/radius.

| Ratio: valence/radius | Elements | Ratio: valence/radius | Elements |
|-----------------------|---|-----------------------|--|
| 0,3 | Cs ⁺ | 2,95 | V ⁴⁺ , Ru ⁴⁺ , Rh ⁴⁺ , Os ⁴⁺ |
| 0,4 | K ⁺ , Rb ⁺ | 3,0 | W ^{average} , Po ^{average} , Pa ⁵⁺ |
| 0,5 | Na ⁺ , Tl ⁺ , In ⁺ | 3,1 | Mn ⁴⁺ |
| 0,6 | Li ⁺ , Ag ⁺ , Au ⁺ , Hg ⁺ , Sb ⁺ | 3,2 | U ⁵⁺ |
| 0,7 | Cu ⁺ | 3,3 | |
| 0,8 | Ba ²⁺ | 3,4 | Mn ^{average} , Nb ⁵⁺ , Ta ⁵⁺ , Os ^{average} , Sb ⁵⁺ , Am ⁶⁺ |
| 0,9 | Sr ²⁺ , Hg ^{average} , Eu ²⁺ | 3,5 | Mo ⁵⁺ , W ³⁺ , Re ^{average} |
| 1,0 | Ca ²⁺ , Sm ²⁺ , Yb ²⁺ | 3,6 | Re ⁵⁺ , Po ⁶⁺ |
| 1,1 | Cu ^{average} , Pb ²⁺ , Nd ²⁺ , Eu ^{average} , Dy ²⁺ , Tm ²⁺ , Am ²⁺ | 3,7 | V ⁵⁺ , Ru ⁵⁺ |
| 1,2 | Mg ²⁺ , Zr ²⁺ , Hg ²⁺ , Tl ^{average} , Yb ^{average} , In ^{average} | 3,8 | Ru ^{average} , U ⁶⁺ |
| 1,3 | Sn ²⁺ , Ti ²⁺ , Au ²⁺ , Nd ^{average} , Sm ^{average} | 3,9 | |
| 1,4 | Mo ²⁺ , W ²⁺ , Pt ²⁺ , Au ^{average} , Eu ³⁺ , Dy ^{average} , Tm ^{average} | 4,0 | |
| 1,45 | V ²⁺ , Zn ²⁺ , Rh ²⁺ , Pd ²⁺ , Re ²⁺ , Os ²⁺ , Ir ²⁺ | 4,1 | |
| 1,5 | Cr ²⁺ , Mn ²⁺ , Fe ²⁺ , Cu ²⁺ , Yb ³⁺ | 4,2 | Mo ⁶⁺ , W ⁶⁺ |
| 1,6 | Co ²⁺ , Ni ²⁺ , Y ³⁺ , Bi ³⁺ , La ³⁺ , Pr ³⁺ , Pm ³⁺ , Sm ³⁺ | 4,3 | Re ⁶⁺ , Pt ⁶⁺ |
| 1,65 | Ce ³⁺ , Nd ³⁺ , Gd ³⁺ , Tb ³⁺ , Dy ³⁺ | 4,4 | Ru ⁶⁺ , Os ⁶⁺ , Ir ⁶⁺ |
| 1,7 | Lu ³⁺ , Pb ^{average} , Ho ³⁺ , Er ³⁺ , Tm ³⁺ , Lu ³⁺ , Am ³⁺ | 4,5 | |
| 1,75 | Be ²⁺ , In ³⁺ , Tl ³⁺ | 4,6 | Cr ⁶⁺ |
| 1,8 | Sc ³⁺ , Zr ³⁺ , Zr ^{average} , Pa ³⁺ | 4,7 | Mn ⁶⁺ , Fe ⁶⁺ |
| 1,9 | Ga, Sn ^{average} , Ce ^{average} , Pr ^{average} , Tb ^{average} , U | 4,8 | |
| 2,0 | Ti ³⁺ , Ti ^{average} , Co ^{average} , Ni ^{average} , Nb ³⁺ | 4,9 | |
| 2,05 | Al ³⁺ , Au ³⁺ , Sb ³⁺ , Sb ^{average} | 5,0 | |
| 2,1 | Mo ³⁺ , Pr ⁴⁺ | 5,1 | Tc ⁷⁺ , Re ⁷⁺ |
| 2,2 | V ³⁺ , Ru ³⁺ , Rh ^{average} , Rh ³⁺ , Pd ^{average} , Os ³⁺ , Ir ³⁺ , Bi ^{average} , Ce ⁴⁺ , Th ⁴⁺ | 5,2 | |
| 2,25 | Pb ⁴⁺ , Tb ⁴⁺ | 5,3 | |
| 2,3 | Mn ³⁺ , Fe ³⁺ , Am ^{average} , Am ⁴⁺ , Cr ³⁺ | 5,4 | |
| 2,4 | Co ³⁺ , Ni ³⁺ , Hf ⁴⁺ , Po ⁴⁺ , Pa ^{average} , Pa ⁴⁺ | 5,5 | Mn ⁷⁺ |
| 2,5 | Zr ⁴⁺ , Sn ⁴⁺ , U ⁴⁺ | 5,6 | |
| 2,6 | V ^{average} | 5,7 | |
| 2,7 | Ti ⁴⁺ , Nb ^{average} , Bi ⁵⁺ | 5,8 | |
| 2,8 | Cr ^{average} , Mo ⁴⁺ , Mo ^{average} , W ⁴⁺ , Ir ^{average} , Pt ⁴⁺ , U ^{average} , Am ⁵⁺ | 5,9 | Os ⁸⁺ , Ru ⁸⁺ |
| 2,9 | Fe ^{average} , Pd ⁴⁺ , Re ⁴⁺ , Ir ⁴⁺ , Pt ^{average} | 6,0 | |

Appendix 2 (cont.)

Table 4: The elements divided by increasing radius

| Radius (Å) | Element | Radius (Å) | Element |
|------------|-----------------------------------|------------|------------|
| 1,1 | Be | 1,9 | Na, Ca, Yb |
| 1,2 | Cr, Mn, Fe, Co, Ni, Cu | 2,0 | Eu |
| 1,3 | Ru, Rh, Re | 2,1 | Sr |
| 1,35 | V, Zn, Tc, Pd, Os, Ir, Pt | 2,2 | Ba |
| 1,4 | Al, Mo, Ag, W, Au | 2,3 | K |
| 1,45 | Ti, Nb, Ta, Sb | 2,4 | |
| 1,5 | Ga, Cd, Sn | 2,5 | Rb |
| 1,55 | Li, Sn, Hf, Hg, U | 2,6 | |
| 1,6 | Mg, Sc, Zr, In, Po, Pa | 2,7 | Cs |
| 1,7 | Lu, Tl, Lu, Am | 2,8 | |
| 1,75 | Pb, Tb, Dy, Ho, Er, Tm, Th | 2,9 | |
| 1,8 | Y, Bi, La, Ce, Pr, Nd, Pm, Sm, Gd | 3,0 | |
| 1,85 | | | |

Appendix 3

Results from total quantitative analysis

Description of the samples:

Samples 1-7: Perch liver from limed lake (mg/kg of dry weight)

Samples 8-14: Perch tissue from limed lake (mg/kg of dry weight)

Samples 15-21: Pike liver from limed lake (mg/kg of dry weight)

Samples 21-28: Pike tissue from limed lake (mg/kg of dry weight)

Samples 29-34: Perch liver from acidified lake (mg/kg of dry weight)

Samples 35-40: Perch tissue from acidified lake (mg/kg of dry weight)

Samples 43-49: Pike liver from acidified lake (mg/kg of dry weight)

Samples 50-56: Pike tissue from acidified lake (mg/kg of dry weight)

Samples 57-61: Water samples from acidified lake (µg/l)

Samples 62-66: Water samples from limed lake (µg/l)

Samples 67-72: Perch liver from neutral lake (mg/kg of dry weight)

Samples 73-74: Perch milt from neutral lake (mg/kg of dry weight)

Samples 75-80: Perch tissue from neutral lake (mg/kg of dry weight)

Samples 81-83: Water samples from neutral lake (µg/l)

[illegible]

| 0 Au | 0 Au | 0 Au | 0 Au | 0 Au | 0 Au | 0 Au | 0 Au |
|-----------|------------|-----------|-----------|-----------|------------|------------|------------|
| 0,7006 Hg | 0,64792 Hg | 0,3664 Hg | 0,892 Hg | 0,0409 Hg | 0,30082 Hg | 1,11023 Hg | 1,81104 Hg |
| 0,2049 Tl | 0,09835 Tl | 0,0602 Tl | 0,0282 Tl | 0,5511 Tl | 0,24518 Tl | 0,23221 Tl | 0,16727 Tl |
| 1,1737 Pb | 0,80449 Pb | 0,4752 Pb | 0,0627 Pb | 0,7756 Pb | 0,70516 Pb | 1,10598 Pb | 0,8174 Pb |
| 0,0231 Bi | 0,00244 Bi | 0,0267 Bi | 0 Bi | 0,0296 Bi | 0,02502 Bi | 0,04563 Bi | 0,0157 Bi |
| 0,015 Th | 0 Th | 0 Th | 0 Th | 0 Th | 0 Th | 0 Th | 0 Th |
| 0,003 U | 0,00868 U | 0 U | 0 U | 0 U | 0 U | 0 U | 0 U |

| average 57-61 | average 62-66 | average 67-72 | average 73-74 | average 75-80 | average 81-83 |
|---------------|---------------|---------------|---------------|---------------|---------------|
| 0,024 Li | 0,034 Li | 0,0784994 Li | 0,0508492 Li | 0,027479 Li | 0,2133333 Li |
| 0 Be | 0 Be | 0 Be | 0 Be | 0 Be | 0,01 Be |
| 996,322 Na | 1406,176 Na | 1514,2825 Na | 1943,1955 Na | 721,5863 Na | 2355,4967 Na |
| 472,845 Mg | 881,789 Mg | 651,38462 Mg | 767,67639 Mg | 1100,785 Mg | 2000,2417 Mg |
| 5,13 Al | 0 Al | 0 Al | 0 Al | 0 Al | 360,56333 Al |
| 102,189 K | 114,583 K | 3665,3839 K | 5505,7034 K | 6184,453 K | 726,19833 K |
| 196,029 Ca | 1960,241 Ca | 156,73739 Ca | 16,155412 Ca | 149,3191 Ca | 2249,0017 Ca |
| 0,002 Sc | 0,174 Sc | 0 Sc | 0 Sc | 0 Sc | 0,64 Sc |
| 0,401 Ti | 3,477 Ti | 1,5266268 Ti | 0,856149 Ti | 1,847278 Ti | 24,165 Ti |
| 0 V | 0 V | 0 V | 0 V | 0 V | 0,04 V |
| 0 Cr | 0 Cr | 0 Cr | 0 Cr | 0 Cr | 0 Cr |
| 25,15 Mn | 11,978 Mn | 9,9685967 Mn | 0,5439943 Mn | 0,721764 Mn | 37,083333 Mn |
| 517,716 Fe | 271,018 Fe | 255,04028 Fe | 0 Fe | 0 Fe | 829,14 Fe |
| 0,095 Co | 0,033 Co | 1,2345544 Co | 0 Co | 0,005071 Co | 0,245 Co |
| 0 Ni | 0,013 Ni | 0,2274135 Ni | 0 Ni | 0,140297 Ni | 0,4816667 Ni |
| 0,633 Cu | 0,885 Cu | 7,3278046 Cu | 3,2366483 Cu | 2,285638 Cu | 1,635 Cu |
| 4,916 Zn | 0,93 Zn | 55,695208 Zn | 26,078678 Zn | 16,49424 Zn | 3,9 Zn |
| 0,016 Ga | 0 Ga | 0,0041876 Ga | 0 Ga | 0 Ga | 0,18 Ga |
| 0,538 Rb | 0,884 Rb | 26,960869 Rb | 36,974013 Rb | 32,52241 Rb | 3,6466667 Rb |
| 4,992 Sr | 17,136 Sr | 0,8971258 Sr | 0,059955 Sr | 0,799063 Sr | 36,826667 Sr |
| 0,04 Y | 0,146 Y | 0,0221606 Y | 0 Y | 0,003381 Y | 1,3233333 Y |
| 0 Zr | 0 Zr | 0 Zr | 0 Zr | 0 Zr | 1,8733333 Zr |
| 0,004 Nb | 0,002 Nb | 0 Nb | 0 Nb | 0 Nb | 0,29 Nb |
| 0 Mo | 0 Mo | 0 Mo | 0 Mo | 0 Mo | 0,0866667 Mo |
| 0 Ru | 0 Ru | 0 Ru | 0 Ru | 0 Ru | 0 Ru |
| 0,612 Rh | 2,108 Rh | 2,9886051 Rh | 0,6296296 Rh | 0 Rh | 2,1533333 Rh |
| 0 Pd | 0 Pd | 0 Pd | 0 Pd | 0 Pd | 0,0566667 Pd |
| 0,053 Ag | 0 Ag | 0,1073474 Ag | 0 Ag | 0 Ag | 0 Ag |
| 0,242 Cd | 0,021 Cd | 0,8215055 Cd | 0 Cd | 0 Cd | 0 Cd |
| 0 In | 0 In | 0 In | 0 In | 0,003381 In | 0,0066667 In |
| 0,002 Sn | 0,044 Sn | 0,2783006 Sn | 0 Sn | 0,003779 Sn | 0 Sn |
| 0,032 Sb | 0,03 Sb | 0,0072622 Sb | 0 Sb | 0 Sb | 0,0366667 Sb |
| 0,01 Cs | 0 Cs | 0,0347515 Cs | 0,0646613 Cs | 0,051375 Cs | 0,02 Cs |
| 1,85 Ba | 7,162 Ba | 0,1465662 Ba | 0 Ba | 0 Ba | 3,76 Ba |
| 0,004 La | 0,026 La | 0,0137854 La | 0 La | 0 La | 0,2366667 La |
| 0,008 Ce | 0,026 Ce | 0 Ce | 0 Ce | 0 Ce | 0,2966667 Ce |
| 0 Pr | 0,002 Pr | 0 Pr | 0 Pr | 0 Pr | 0,05 Pr |
| 0,002 Nd | 0,012 Nd | 0 Nd | 0 Nd | 0 Nd | 0,1533333 Nd |
| 0 Sm | 0 Sm | 0 Sm | 0 Sm | 0 Sm | 0,02 Sm |
| 0 Eu | 0 Eu | 0 Eu | 0 Eu | 0 Eu | 0 Eu |
| 0 Gd | 0 Gd | 0 Gd | 0 Gd | 0 Gd | 0,02 Gd |
| 0 Tb | 0 Tb | 0 Tb | 0 Tb | 0 Tb | 0 Tb |
| 0 Dy | 0 Dy | 0 Dy | 0 Dy | 0 Dy | 0,01 Dy |
| 0 Ho | 0 Ho | 0 Ho | 0 Ho | 0 Ho | 0 Ho |
| 0 Er | 0 Er | 0 Er | 0 Er | 0 Er | 0,01 Er |
| 0 Tm | 0 Tm | 0 Tm | 0 Tm | 0 Tm | 0 Tm |
| 0 Yb | 0 Yb | 0 Yb | 0 Yb | 0 Yb | 0,01 Yb |
| 0 Lu | 0 Lu | 0 Lu | 0 Lu | 0 Lu | 0 Lu |
| 0 Hf | 0 Hf | 0 Hf | 0 Hf | 0 Hf | 0,0033333 Hf |
| 0 Ta | 0 Ta | 0 Ta | 0 Ta | 0 Ta | 0 Ta |

| | | | | | |
|----------|----------|--------------|--------------|-------------|--------------|
| 0 W | 0 W | 0 W | 0 W | 0 W | 0 W |
| 0 Re | 0 Re | 0 Re | 0 Re | 0 Re | 0 Re |
| 0 Os | 0 Os | 0 Os | 0 Os | 0 Os | 0 Os |
| 0 Ir | 0 Ir | 0 Ir | 0 Ir | 0 Ir | 0 Ir |
| 0 Pt | 0 Pt | 0 Pt | 0 Pt | 0 Pt | 0,0033333 Pt |
| 0 Au | 0 Au | 0,0072179 Au | 0 Au | 0 Au | 0 Au |
| 0 Hg | 0 Hg | 0 Hg | 0 Hg | 0,173048 Hg | 0 Hg |
| 0 Tl | 0,002 Tl | 0,0738475 Tl | 0,0508492 Tl | 0,013998 Tl | 0,0033333 Tl |
| 1,544 Pb | 0,206 Pb | 2,239824 Pb | 3,7979333 Pb | 0,712893 Pb | 0,5033333 Pb |
| 0,128 Bi | 0,23 Bi | 0,0452759 Bi | 0,059955 Bi | 0 Bi | 0 Bi |
| 0,004 Th | 0,002 Th | 0 Th | 0 Th | 0 Th | 0,84 Th |
| 0,002 U | 0,026 U | 0,0071241 U | 0 U | 0 U | 0,18 U |

Appendix 4: Correlation Graphs

The following graphs are included:

From Sandsjön

- Correlation between water and perch liver
- Correlation between water and perch tissue
- Correlation between water and pike liver
- Correlation between water and pike tissue
- Correlation between perch liver and pike liver
- Correlation between perch tissue and pike tissue

From Rammsjön

- Correlation between water and perch liver
- Correlation between water and perch tissue
- Correlation between water and pike liver
- Correlation between water and pike tissue
- Correlation between perch liver and pike liver
- Correlation between perch tissue and pike tissue

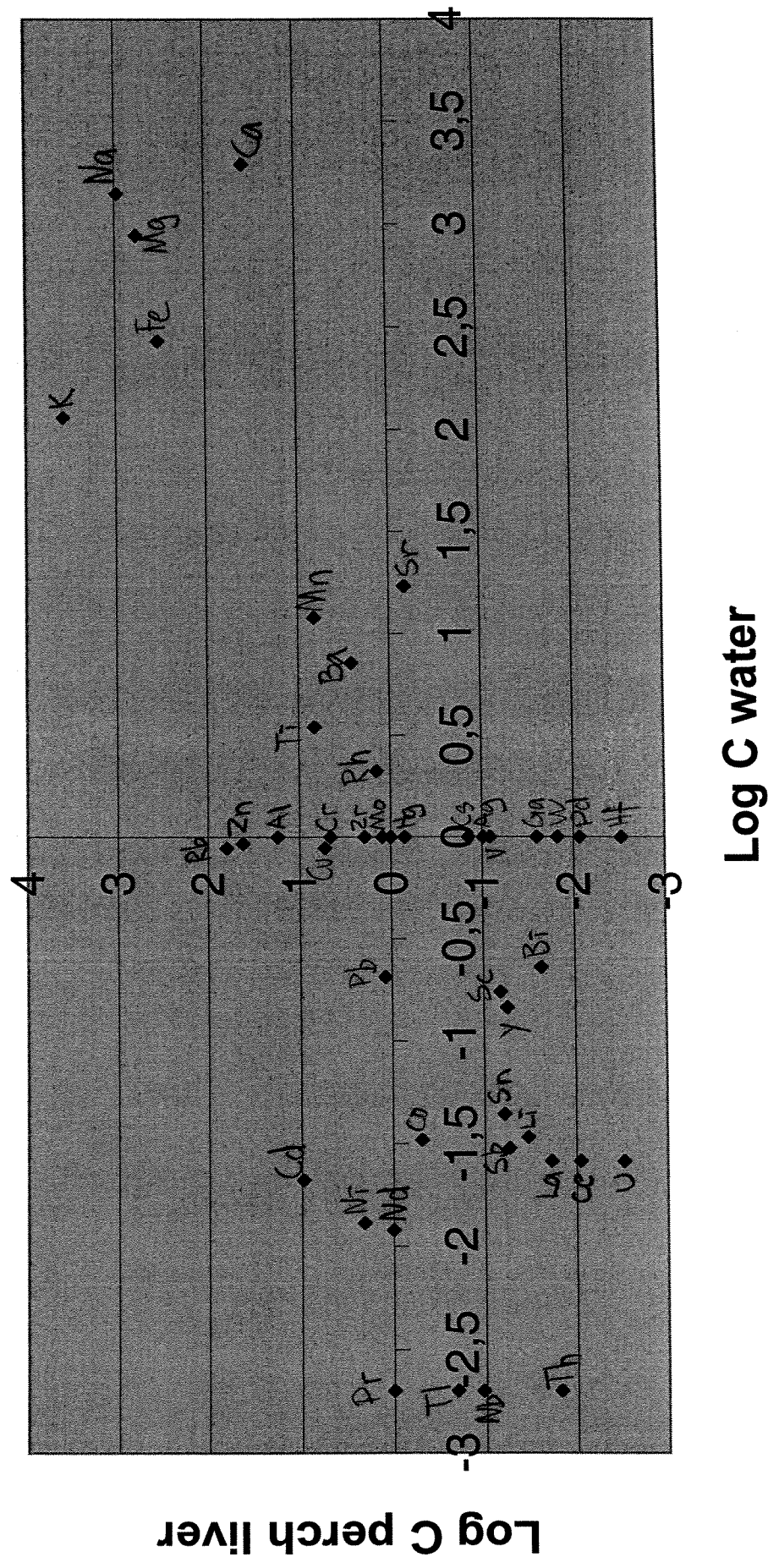
From Anten

- Correlation between water and perch liver
- Correlation between water and perch tissue
- Correlation between perch liver and perch tissue

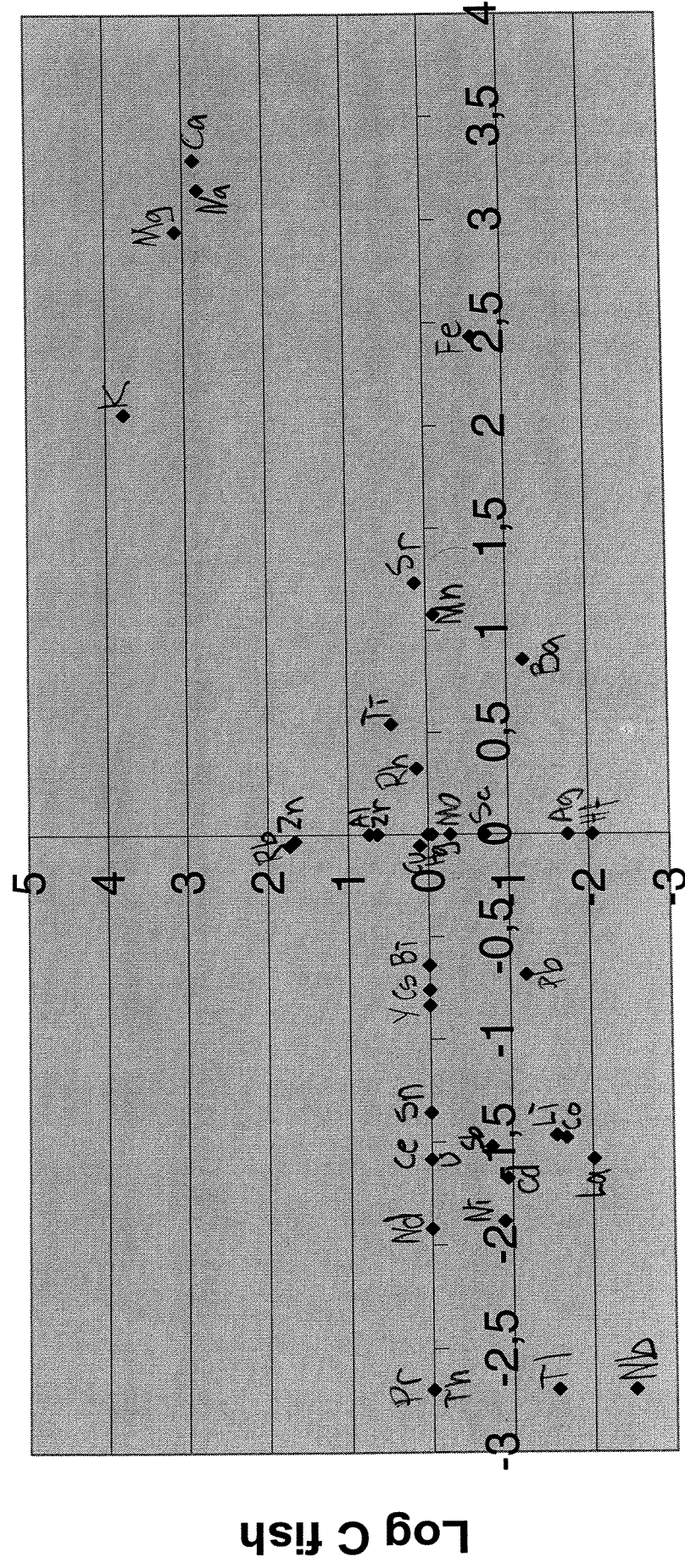
Comments to the correlation graphs:

There are some trends that are shown all way through in these graphs. The bulk elements (K, Na, Mg etc) are found to have the highest concentrations in both the water and fish. These elements are followed by essential elements (Zn, Ca, Cd etc.). The remaining elements are found in the third square with negative values on both axes. The main part of the elements are found in the first and third square.

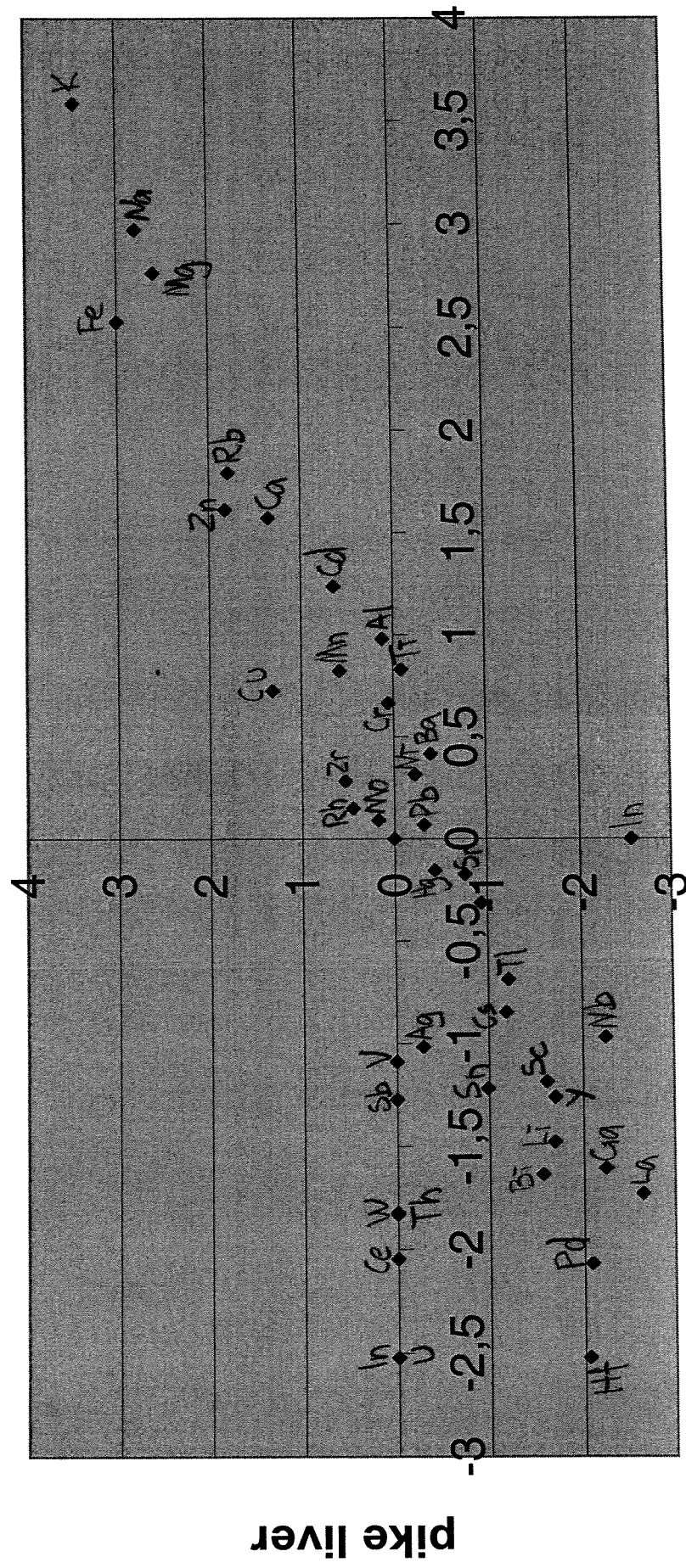
correlation between water and perch liver. Sandsjön



correlation between water and pike tissue. Sandsjön



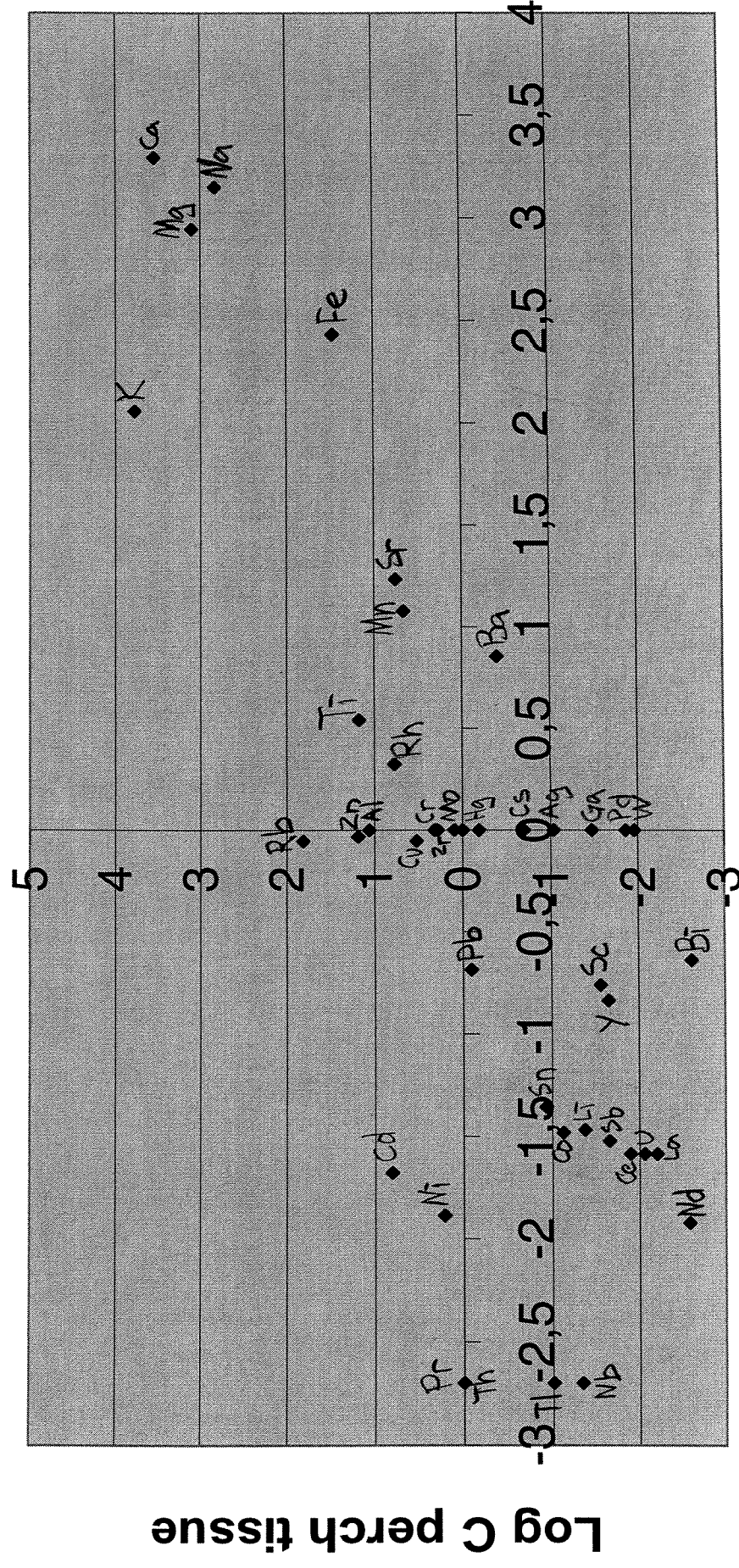
Correlation between perch liver and pike liver. sandsjön



perch liver

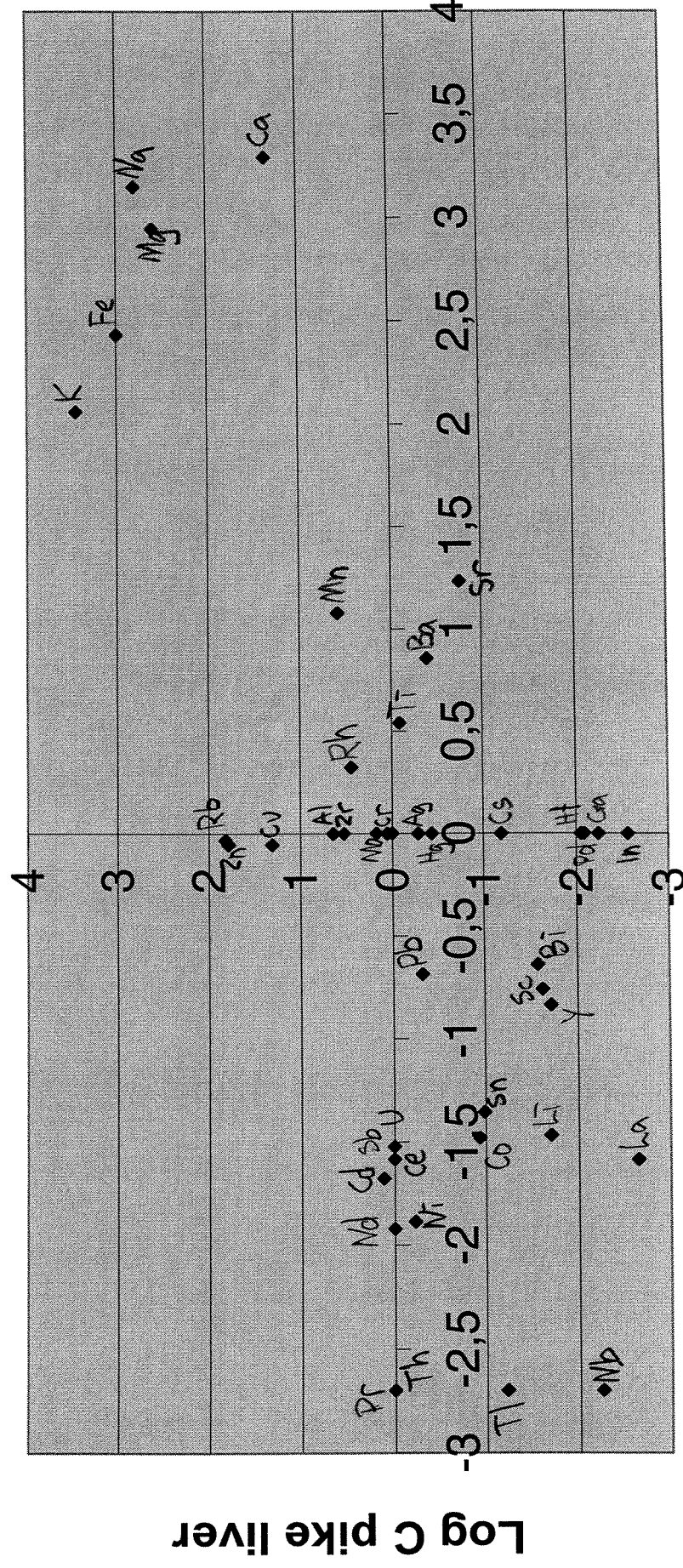
Correlation between water and perch tissue.

Sandsjön

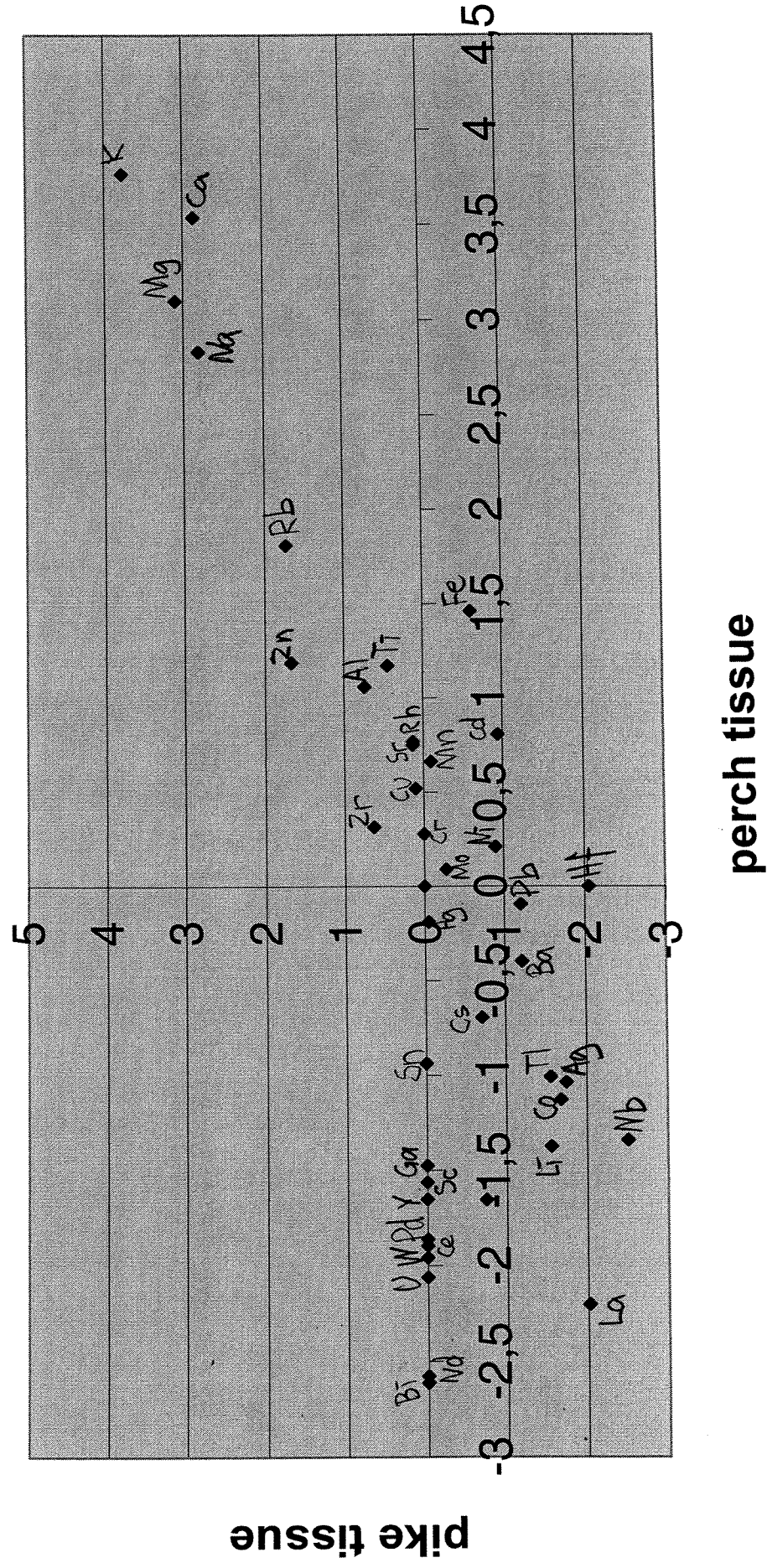


Log C water

Correlation between water and pike liver. Sandsjön

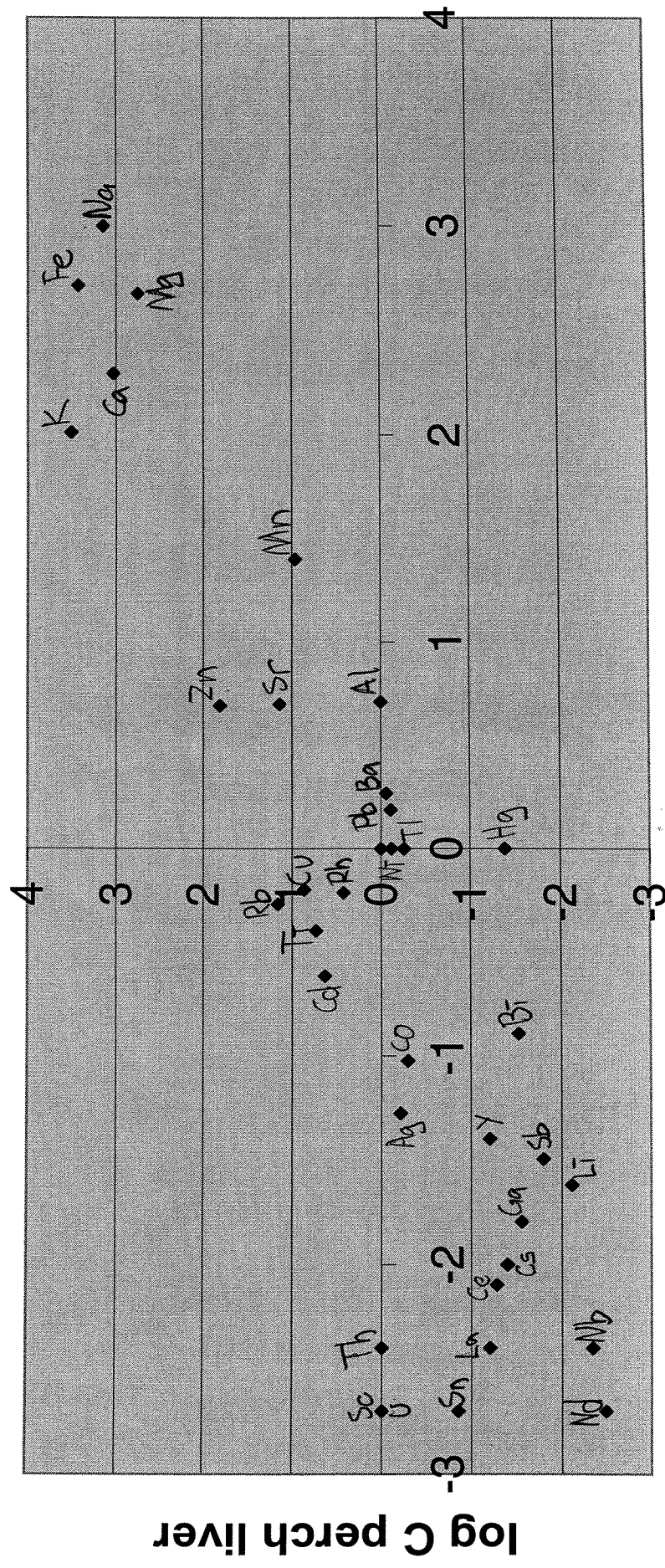


Correlation between perch tissue and pike tissue. sandsjön



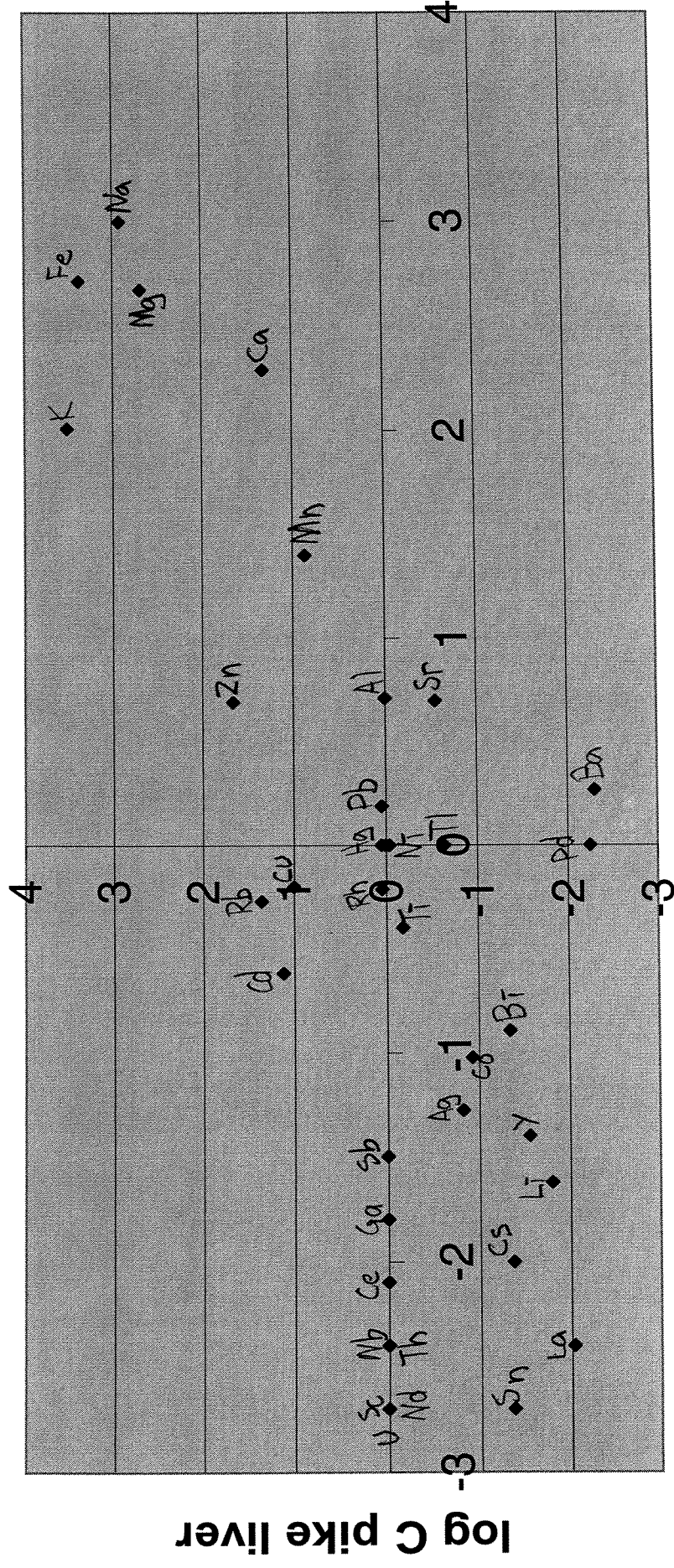
correlation between water and perch liver.

Rammsjön



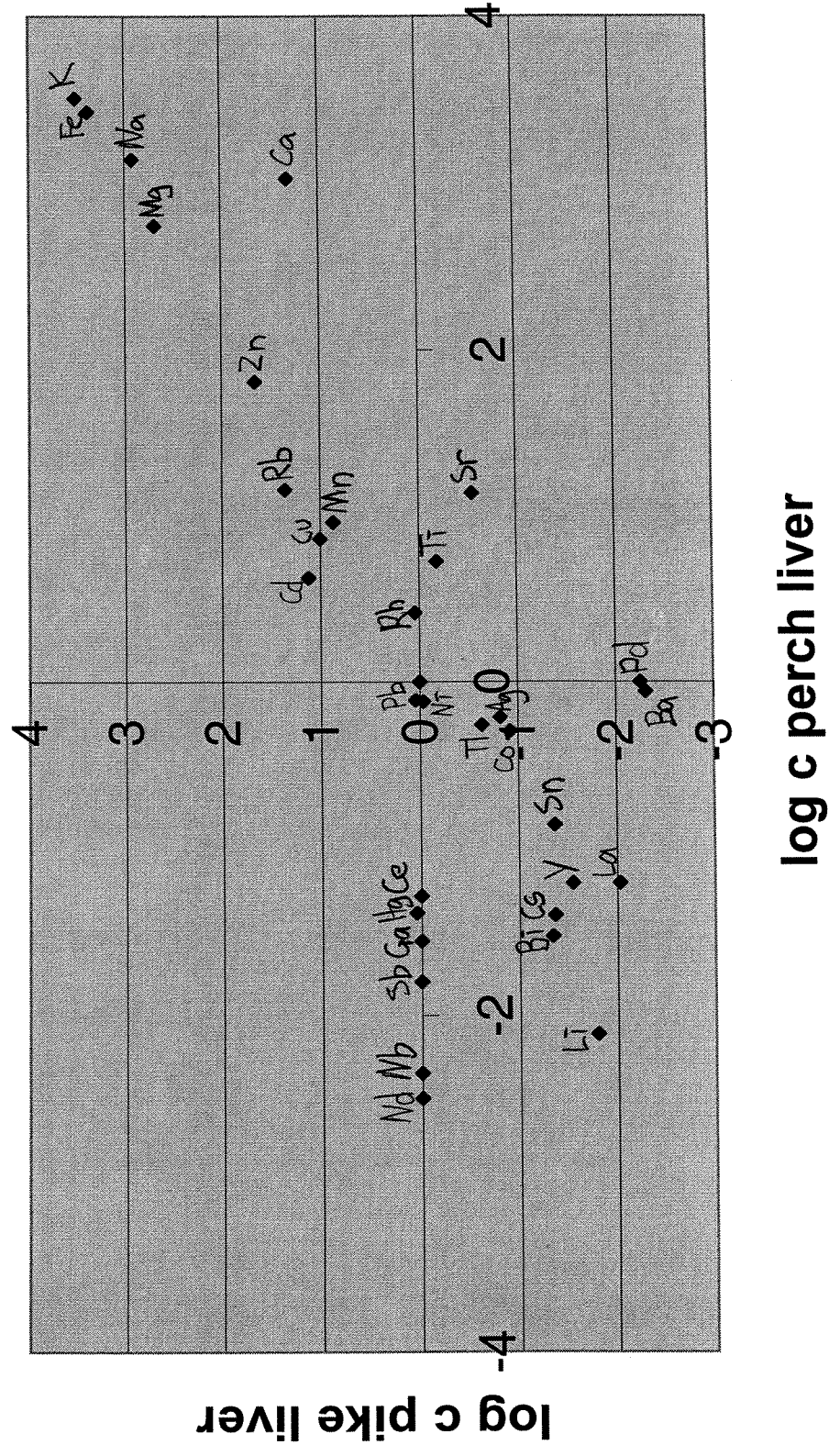
log C water

Correlation between water and pike liver. rammsjön

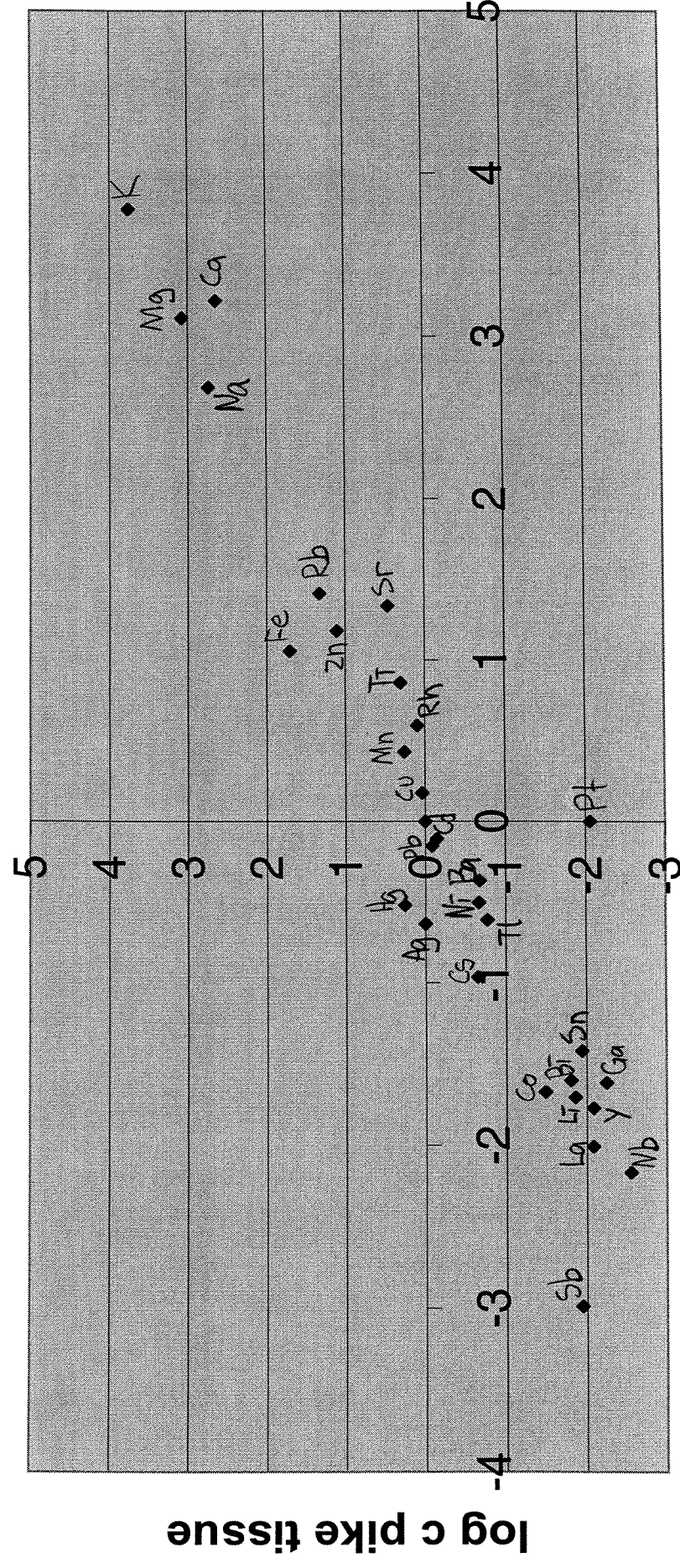


log C water

correlation between perch liver and pike liver.



Correlation between perch tissue and pike tissue. Rammsjön

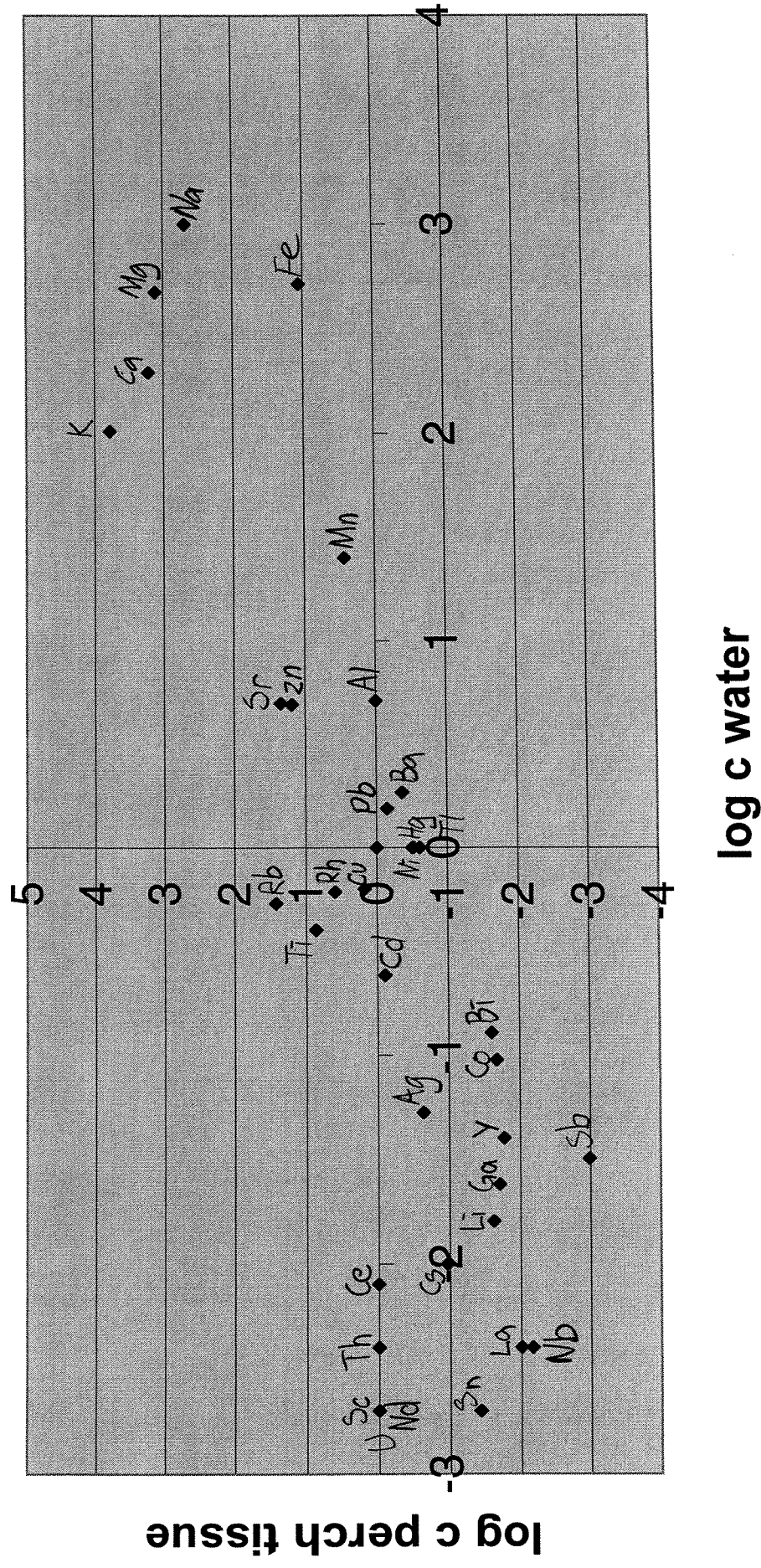


log C perch tissue

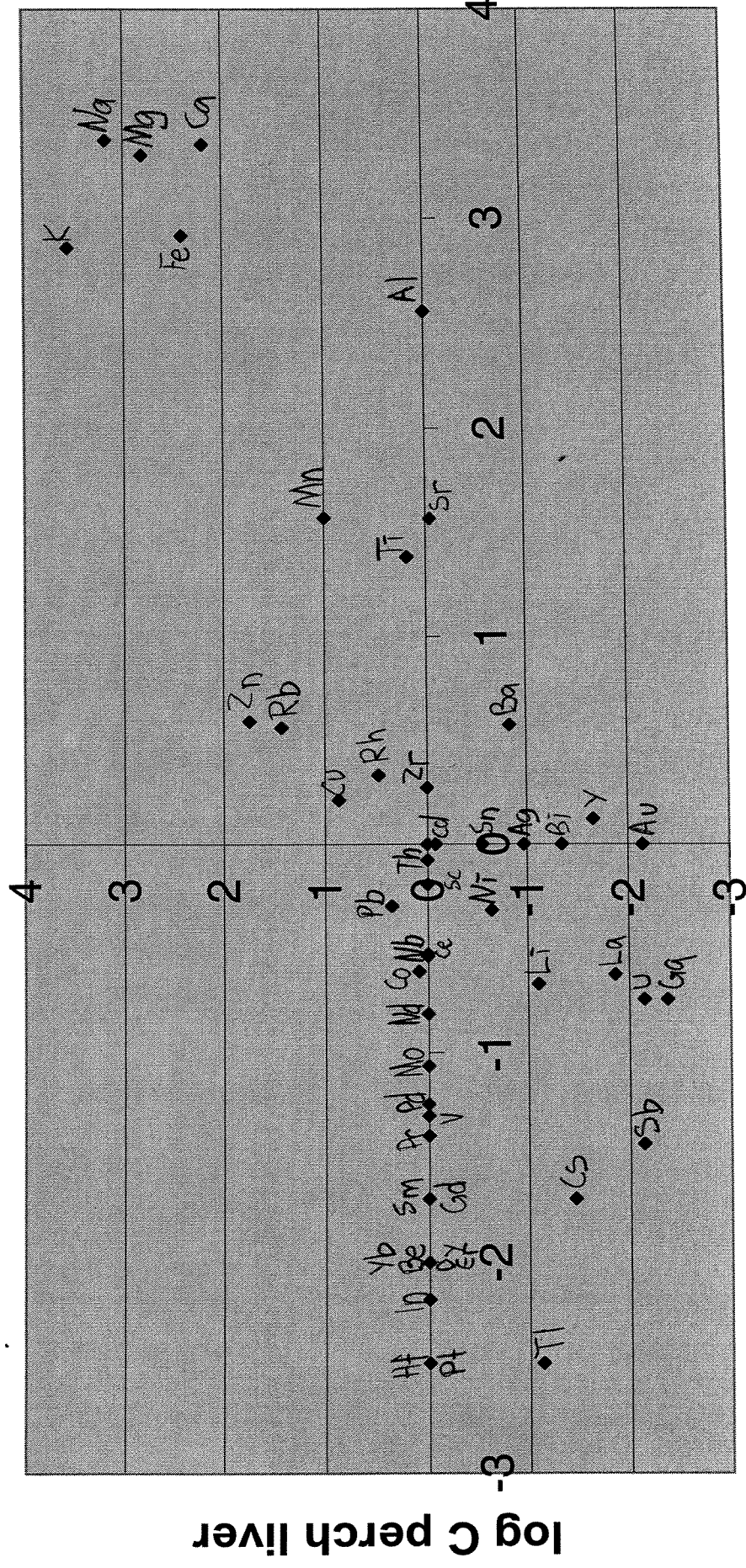
Correlation between water and pike tissue.



Correlation between water and perch tissue. Rammsjön



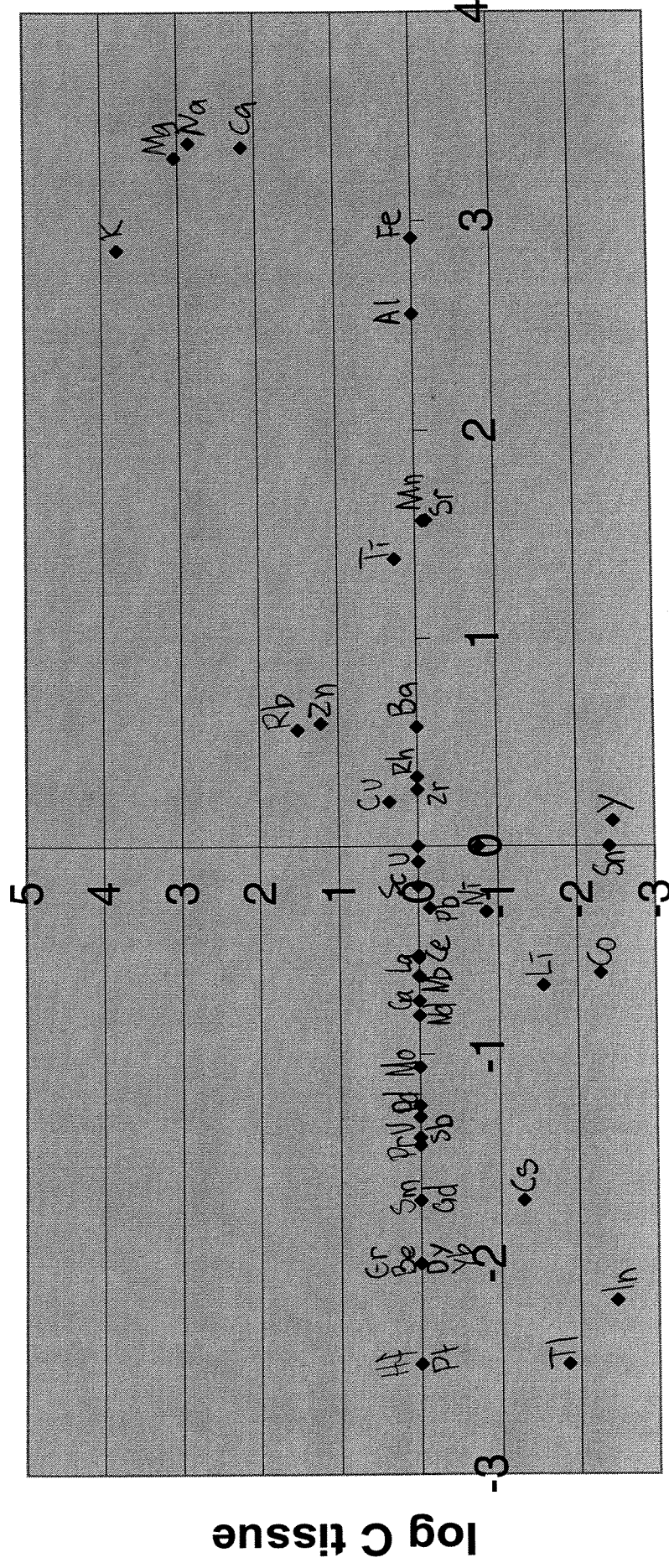
correlation between water and perch liver. Anten



Log C water

correlation between water and perch tissue.

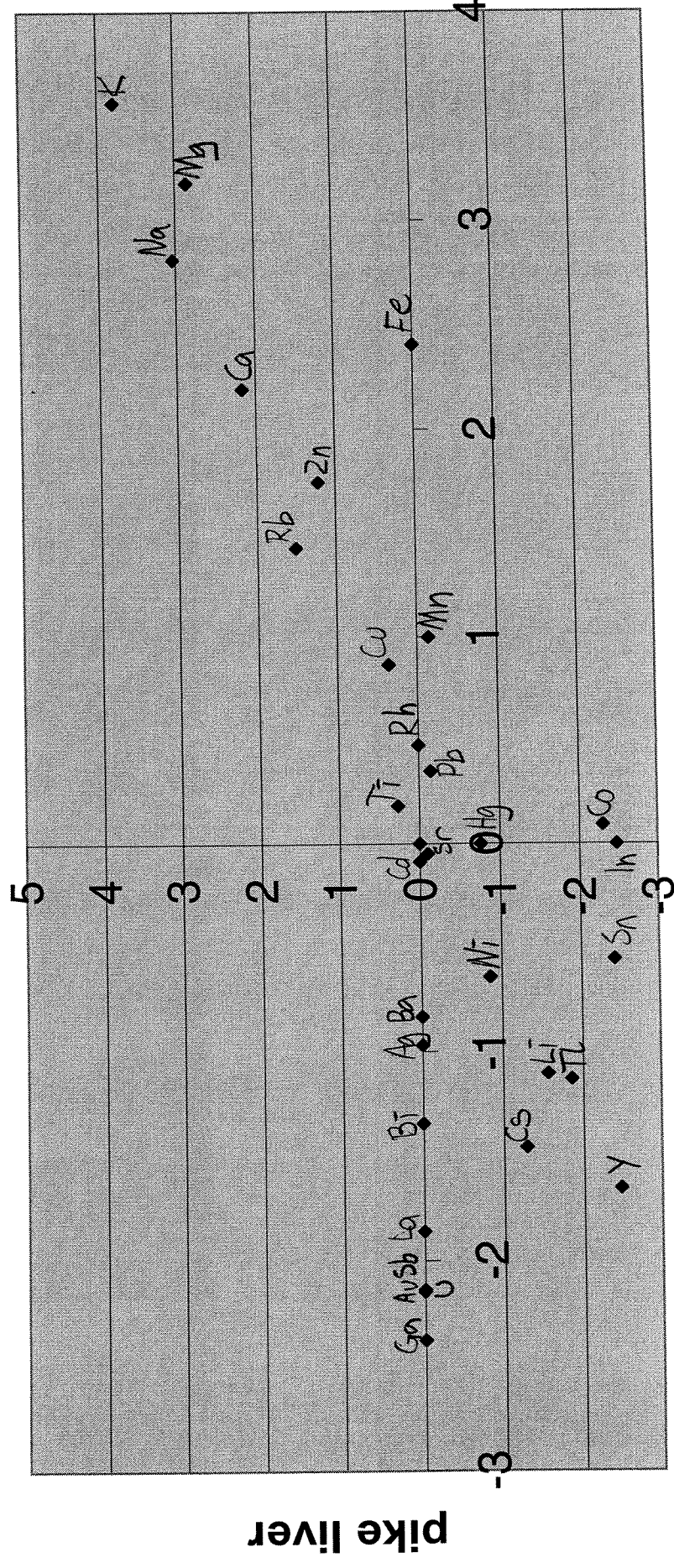
Anten



log C water

correlation between perch liver and perch tissue.

Anten



perch liver

