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Study of Water Quality of Recirculated Water in Aquaponic Systems

Study of speciation of selected metals and characterization of the properties of natural organic matter

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Chemistry
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Summary

This thesis describes an extensive study on how water quality changes over time in a small scale recirculating system where waste water from smolt production was used to grow lettuce for commercial use. The treatment effect of lettuce on different solutions was tested and corresponding lettuce yield was evaluated. In order to enhance the treatment effect a rock wool filter was used on certain solutions. Important water quality parameters were measured every day, and the element concentration in water, lettuce and soil was analyzed. Natural organic matter in the solutions was also analyzed.

The filter had no influence on either element concentration of organic matter, and observed changes were therefore attributed lettuce or soil. The organic matter increased during the experiment period, but the total concentration was too low to evaluate the character and the ability to form complexes. Phosphorus, potassium, manganese, zinc and copper decreased significantly in most of the waste water solutions, these elements are all nutrients for plants, hence they are most likely taken up by the lettuce. Despite the uptake of essential nutrients the lettuce did not grow optimally and had several signs of distress symptoms both during and at the end of the experiment. Magnesium and chloride increased significantly due to evaporation from the reservoirs. Together with the high concentration found for sodium in all the waste water solutions it was believed that the lettuce was exposed to toxic levels of salt. This was a possible explanation to why the lettuce did not grow sufficiently. The lettuce analysis showed that the concentration of most of the nutrients were not sufficient for growth. The lettuce had clear signs of nutrient deficiency such as discolored leaves and stunted growth. These symptoms were thought to be a result of both salinity and nutrient deficiency. The content of toxic metals was higher in lettuce cultivated with waste water than lettuce cultivated with a commercial nutrient solution, but lower than what is considered as limiting for growth. Considering optimal conditions for lettuce growth the pH of the waste water solutions was too high, and the electrical conductivity was higher than recommended.

The rock wool filter appeared to release metals such as aluminum and iron. Both these metals are toxic to fish and it was concluded that rock wool filters should not be used in with aquaponics. One of the challenges with integrated production of salmon smolt and plants is the high content of salt in the waste water. The salt is necessary in most cases for production of salmon smolt, but inhibits plant growth. A possible solution to this is to use a more salt tolerant plant than the one used in this experiment. If the plant is able to treat the water for nutrients and other waste products, without being depressed by the salt, re-use of the water is possible in addition to getting a marketable product.

Sammendrag

Denne masteroppgaven var en utvidet studie av hvordan vannkvalitet endres over tid i et småskala resirkulerings anlegg der avfallsvann og slam (fast avfall) fra produksjon av smolt ble brukt til å dyrke salat for kommersielt bruk. Salats renseeffekt av ulike løsninger ble testet og tilhørende salatvekst vurdert. For å undersøke om renseeffekten kunne forbedres ved hjelp av filtrering ble noen utvalgte løsninger knyttet til et steinullsfilter. Viktige vannkvalitetsparametere ble målt hver dag, og element konsentrasjonen i vann, salat og jord ble analysert. Naturlig organisk materiale i løsningene ble også analysert.

Filteret hadde ingen innvirkning på verken elementkonsentrasjon eller organisk materiale, og observerte endringer ble derfor antatt å skyldes salat eller jord. Det organiske materialet viste seg å øke gjennom perioden, men total konsentrasjonen var for lav til at det var mulig å si noe om karakter og komplekserings evne. Fosfor, kalium, mangan, sink og kobber minket signifikant i de fleste avfallsløsningene. Siden disse elementene alle er næringsstoffer for planter, ble de regnet for å være tatt opp av salaten. På tross av opptaket av essensielle næringsstoffer vokste ikke salaten normal, og viste flere sykdomssymptomer underveis og ved forsøkets slutt. Magnesium og klorid økte signifikant som følge av at vann fordamper fra reservoarene. Sammen med den høye konsentrasjonen av natrium som ble funnet i alle avfallsløsninger ble det antatt at salaten ble utsatt for toksiske nivåer av salt. Dette var en mulig forklaring på hvorfor salaten ikke vokste tilstrekkelig. Salatanalysen viste at konsentrasjonen av de fleste næringsstoffene var sub-optimal for plantevekst. Salaten hadde synlige tegn på mangelsymptomer i form av misfargede blader og stagnert vekst. Disse symptomene syntes å være et resultat av både høyt saltinnhold i løsningen og mangel på essensielle næringsstoffer. Innholdet av toksiske metaller var høyere i salat dyrket med avløpsvann enn i salat dyrket med kommersiell næringsløsning, men lavere enn det som anses å begrense vekst. I forhold til optimale forhold for plantevekst var pH i avfallsløsningene for høy, og den elektriske ledningsevne var høyere enn anbefalt.

Steinullsfilteret viste seg å avgi metaller som aluminium og jern til løsningene. Både aluminium og jern er toksiske for fisk og det kan konkluderes med at steinullsfiltre er uegnet i forbindelse med akvakultur. En av utfordringene i integrert produksjon av laksesmolt og

planter er det høye innholdet av salt i avfallsvannet. Saltet er nødvendig i produksjon av smolt, men hemmer plantevekst. En mulig løsning på dette er å benytte en mer salttolerant plante enn den som ble brukt i dette forsøket. Dersom planten kan rense vannet for næringsstoffer og andre avfallsprodukter, uten å ta skade av saltet, vil gjenbruk av vannet være mulig samtidig som en får et salgbart produkt.

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[1] Figure 2.1 is retrieved from (Cermaq, 2010), Figure 2.2 is retrieved from, (Aquaponics, 2012) and Figure 2.3 is retrieved from (Hydroponics, 2008)

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Abbreviations

Al – Aluminum
B – Boron
Ca – Calcium
Cl – Chloride
Cr – Chromium
Cu – Copper
DO – Dissolved Oxygen
DOC – Dissolved Organic Carbon
EC – Electrical conductivity
E_h – Redox potential
FCR – Feed Conversion Ration
Fe – Iron
FIM – Free Ion Model
ICP-MS – Inductively Coupled Plasma Mass Spectrometry
K – Potassium
Mg – Magnesium
Mn – Manganese
Mo – Molybdenum
N – Nitrogen
Na – Sodium
NF – Nutrient solution filtered
NFT – Nutrient Film Technique
Ni – Nickel
NIVA – Norwegian Institute of water research
NOM – Natural Organic Matter
NTNU – Norwegian University of Technology and Science
NUF – Nutrient solution unfiltered
P – Phosphorus
RAS – Recirculating Aquaculture systems
S – Sulphur
SF – Sludge (and waste water) solution filtered
Si – Silicon
SUF – Sludge (and waste water) solution unfiltered
SUVA – Specific Ultraviolet Absorbance
TAN – Total Ammonia Nitrogen (NH_3 + NH_4^+)
TOC – Total Organic Carbon
T_w – water temperature
WF – Waste water solution filtered
WUF – Waste waster solution unfiltered
Zn – Zinc

1 Introduction

The aquaculture industry in Norway is growing and thereby producing more waste, hence it has become more important with water treatment and re-use of water. Advanced technology is already in use and represents a sizeable expense for the industry. It is therefore of interest to adapt easier and less expensive treatments of the waste with technologies such as aquaponics. Aquaponics combines the production of fish with the production of plants. The waste water from aquaculture is treated as a commodity while the need for chemical fertilizers in hydroponics is lowered or eliminated. The goal of this thesis was to investigate the behavior of metals and organic matter in this type of integrated system by using waste water from salmon smolt production and lettuce.

In order to understand the principles behind aquaponics several questions were raised. Can lettuce provide a natural treatment of waste water from aquaculture? If so, will both dissolved organic matter and toxic metals (including waste products) be removed so that the water is suited for re-use or pose a reduced risk of eutrophication upon discharge? Is it possible to enhance waste water treatment by using a rock wool filter? Is the physiochemical composition of the waste water from aquaculture suitable for growing lettuce, and does the waste water contain enough nutrients for the lettuce in bioavailable form? Will the addition of sludge (solid waste) to the waste water result in better growth?

The successful use of aquaponics in other parts of the world, and research done in order to adapt it to a range of fish and plant species is now receiving a great deal of attention from Norwegian researchers. In order to test the possibilities of aquaponics in a Norwegian setting, a small scale system was set up using waste water from salmon smolt production and lettuce from a local producer. The farm that was chosen is a land-based recirculating system with possibilities of expanding the business with integrated production of lettuce. Lettuce was chosen because it is both easy and common to cultivate in Norway and the whole product is marketable. Waste water and waste water with added sludge were tested in order to investigate the treatment effect and lettuce yield. Testing a commercial nutrient solution in parallel made it possible to compare the results to a realistic cultivation. Filters were also of interest because of reports on the beneficial treatment effect. By using one batch of waste water it was possible to generate hypothesis that could be tested with different water qualities at a later stage.

This study was designed to give insight into the treatment effect of lettuce. It was expected that the lettuce would treat the waste water efficiently by taking up the nutrients (elements) and organic matter present in the solution. It was assumed that this would yield a marketable product, but there were some concerns about the low concentrations of nitrogen which is necessary for lettuce growth. It was thought that the waste water solution containing sludge would yield a better product than the solution without sludge. It was also believed that the filtered treatments would have a better treatment effect and a better lettuce yield by enhanced removal of organic matter and metals. The system with the commercial nutrient treatment was assumed to yield lettuce equivalent to lettuce from the producer and result in a marketable product.

2 Theory

2.1 Aquaculture industry

Norway is the world's largest exporter of fish and fish products after China, and the world's largest exporter of farmed salmon ("Farmed Salmon," 2010, 07.05). Atlantic salmon dominates the fish farming industry in Norway, but rainbow trout and some other species are also important. The production sites are located in either net pens along the Norwegian cost and in the fjords or on land close to shore. Norway has been involved in aquaculture since the 1970-s, but the greatest growth has occurred during the last two decades (Seymour & Bergheim, 1991).

Recirculating aquaculture systems (RAS) are used to reduce water consumption and water discharge in land-based aquaculture (Martins et al., 2009). The water quality in RAS is fundamental for optimal fish growth and health. Treatment of recirculating water is therefore one of the limiting factors for production capacity of a fish farm (Bjerknes, 2007). Solid waste management combined with the control of dissolved minerals, organic matter, dissolved gases, pH, temperature and salinity etc. contribute to ensuring good water quality (Cripps & Bergheim, 2000). While use of RAS has reduced the discharge of waste water to the environment, the accumulation of potentially harmful substances within the system is receiving increasing attention. Efficient removal of minerals can prevent their accumulation to toxic concentrations (Bjerknes, 2007; Wood et al., 2012). Aluminum is of special concern because it accumulates on the gill surface at concentrations as low as 10 – 15 µg/L (F. Kroglund et al., 2007). Copper is also toxic to fish in low concentrations, and has been reported to accumulate in RAS (Martins et al., 2009).

Other minerals such as nitrogen and phosphorus are also of concern due to the discharge of aquaculture effluent to natural waterways (Lin et al., 2002). These elements serve as nutrition for organisms such as algae and plants and may cause eutrophication. During recent decades the influence of Silicon in marine eutrophication has also received attention (Anderson et al., 2002; Officer & Ryther, 1980). A variety of models, guidelines, monitoring protocols and environmental quality standards for salmon farming in cold water have been made, and future regulation of aquaculture discharge requires further development of waste water treatment (Maroni, 2000). Such that the increased production of fish will not lead to increasing amounts of waste water discharge.

2.2 Salmon farming

Salmon go through distinct stages of development (Figure 2.1) from roe to an adult salmon (Bjerknes, 2007). Breeding of salmon starts on land in tanks filled with freshwater where the roe is fertilized. After about 60 days the roe is ready to hatch. 4-6 weeks after hatching the fish are moved to larger freshwater tanks where commercial fish feed is used. Around this time the fish undergo a process called smoltification, which allows the fish to adapt to seawater (Folmar & Dickhoff, 1980). After six months the fish are ready to be moved into net pens in either the ocean or the fjords. Here they are kept until they are ready to be harvested or used for breeding.

Figure 2.1 Lifecycle of Atlantic salmon (*Salmo salar*), from broodstock to processing.

In aquaculture, the fish must be fed according to the diet they would encounter in the wild and this diet is called fish feed (Halver & Hardy, 2002). Fish feed is based on proteins, fat, carbohydrates, vitamins, minerals and pigment. These ingredients are either natural products from fishing and farming or made industrially. Salmon is known to be the farm animal that exploits its feed most efficiently, with a feed conversion ratio (FCR) of 1.2 kg feed dry matter per 1 kg of produced fish (Einen & Roem, 1997). Increase in fat and energy content of the diet have been shown to increase growth, feed utilization, nitrogen (protein) retention in Atlantic salmon while reducing nutrient discharges.

2.3 Aquaponics

The word aquaponics is derived from the two words aquaculture and hydroponics. While aquaculture refers to fish farming, hydroponics refers to the cultivation of plants in soilless media. Aquaponics is an integrated system that uses nutrients from the effluent water from fish farming in the production of vegetables, plants and herbs (Homme, 2012). When nutrient rich waste water is channeled into secondary crops it can be of both economic value and provide a cost-effective and environmentally sound alternative treatment of minerals in the water (Rakocy et al., 1997). Aquaponics is a sustainable technology that will become even more valuable as resources become limited.

The integrated use of water in aquaponics can be illustrated by the nitrogen cycle (Figure 2.2). Fish excrete waste nitrogen as ammonia into the water. Nitrifying bacteria then convert the ammonia compound to nitrite and then nitrate. Both ammonia and nitrite are toxic to fish, while nitrate is relatively harmless and is also the preferred compound of nitrogen for plant uptake. Nitrogen is not the only element that can be recycled in this manner, plants are able to recover other nutrients from the waste water as well. This relieves the environmental load and increases the water exchange rate, in turn lowering operational costs.

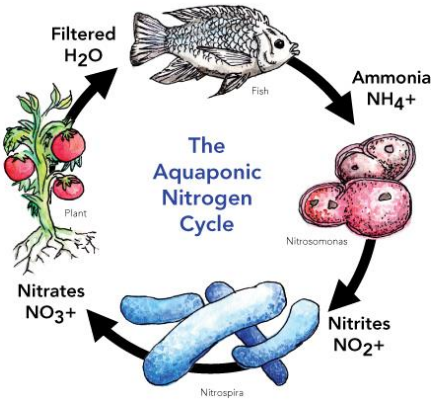


Figure 2.2. The aquaponic nitrogen cycle

2.4 Hydroponics

Hydroponics is a widely and frequently used technique for growing plants without soil (Jones, 1982). The technique makes it possible to maintain complete control over the growing conditions (i.e. light, nutrients, pH, temperature etc.), resulting in larger and more predictable yield of plants for commercial use. The reason why it is possible to grow plants both with and without soil are the mineral elements (Harris, 1992). The mineral elements serve as nutrition for the plant and are absorbed either from soil, sand, gravel or water to provide normal growth. Nutrients are taken up with water by the roots and transported to the leaves where they are needed. Most of the water is lost via transpiration through the leaves, creating a constant water demand in plants requiring them to take up more water and thereby more nutrients. Plants also acquire nutrients by osmosis. Root development and symbiosis with microorganisms are important mechanisms of a plant to improve the nutrient uptake (Alloway, 1995). The physiochemical characteristics of the water and soil may limit the availability of an element. It may also yield favorable conditions for uptake of a non-essential element. In hydroponics nutrients are provided by irrigation with a commercial nutrient solution made by dissolving fertilizer (salts) in water so that the ions dissociate. The nutrient solution may consist of only one or several types of fertilizers.

Elements are usually divided into macro- and micronutrients (Resh, 2012). The macronutrients are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) and are the six elements the plants need the most of. In addition the plants need small amounts of iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B) and molybdenum (Mo), collectively called micronutrients. Carbon, oxygen and hydrogen are also considered as macronutrients, but are taken up through the air. Elements may also be classified as essential or non-essential based on the plants need of the particular element to complete its life cycle (Arnon & Stout, 1939).

Light and temperature affects photosynthesis, translocation and respiration in lettuce which in return affects plant growth (Pramanik et al., 2000). Lettuce also needs a relatively high humidity to grow optimally and humidity is important for nutritional uptake of elements like N, P, K, Ca and Mg (Bævre & Gislerød, 1999).

2.4.1 Nutrient deficiency and toxicity

There are several symptoms of nutrient deficiency associated with more than one element such as tip burn, chlorosis and necrosis (Berry, 2010). Symptoms of stress caused by salinity, pathogens or air pollution are often similar to symptoms of nutrient deficiency. Under condition like these it is common that plants grown in the same environment develop similar symptoms at the same time. Tip burn is indicated by the discoloration of the tip of the leaf, and is a very common symptom. Chlorosis is a general term for the yellowing of leaves through the loss of chlorophyll, while necrosis is a general term for brown, dead tissue (grey/brown areas).

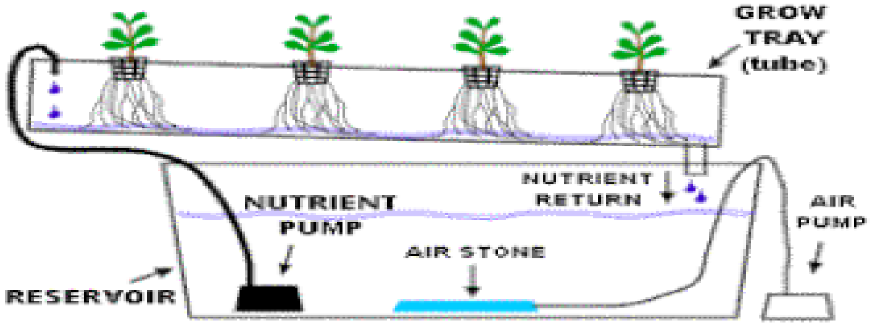
Macronutrients become depleted by rapidly growing plants and must be supplied continuously to prevent acute deficiency (Berry, 2010). For optimal growth the nutrient status of both macro- and micronutrients has to be balanced. However, only one nutrient at a time can limit the overall growth even though the plant may lack several nutrients. If the plant is supplied with the limiting nutrient growth will resume, but another nutrient may become limiting.

During nutrient stress the plant is able to mobilize nutrients from the older leaves to the younger leaves near the growing regions of the plant. This is true for N and K and other very mobile nutrients and results in a depletion of mobile nutrients in old and mature leaves. Uptake and distribution of weakly mobile nutrients such as Ca, B and Mn are dependent on transpiration (H. Marschner et al., 1996). Deficiency will appear in the younger leaves and is usually a result of dehydration. Low concentration of moderately mobile nutrients such as S and Mg will normally cause symptoms over the entire plant (Berry, 2010). Competition among nutrients and from toxic metals is another cause of deficiency. Excessive amounts of Mg will compete with K and Ca uptake. An excess of metals such as Cu, Zn, Cr and Ni competes with Fe. A number of different mechanisms can limit the availability of a nutrient, and the fact that they often work in concert will complicate the work of finding the actual reasons for nutrient deficiencies.

The uptake mechanisms of nutrients in plants are selective, but not all plants are able to regulate their uptake according to metabolic need (Chaney et al., 1994). Plants are able to accumulate toxic concentrations of essential microelements, but they can also absorb high amounts of non-essential elements such as Cd and Pb (Clemens et al., 2002). Non-essential elements are taken up by plants by the same transport mechanisms as essential elements (Alloway, 1995). The use of green plants to remove pollutants from the environment (soil, water or air) or to render them harmless is termed phytoremediation (Garbisu & Alkorta, 2001). The ability to absorb heavy metals is highly dependent on species and cultivars within species. High accumulation of heavy metals or nutrients in edible plants can also pose a threat to human health (Rico-Garcia et al., 2009). It is therefore important to assure that the metal uptake by plants does not exceed the maximum tolerance of humans. This is not a problem when cultivating ornamental plants.

2.4.2 The nutrient film technique

A number of well-established techniques have been used to grow lettuce (Jensen, 2002). The nutrient film technique (NFT) system was introduced as early as the 1960s and is a simple, cheap and easy system to handle. In this system the plants are grown in shallow irrigation troughs (Graves, 1983). A suitable stream of nutrient solution is recirculated over the bare roots of the plant to provide it with adequate water, nutrients, and aeration (Figure 2.3). Usually the plants are grown in a parallel series of sloping troughs. The nutrient solution is pumped up from a reservoir to the upper end of the trough from where it flows past the roots. At the lower end of the trough the solution is simply collected in the reservoir that is placed below. The solution is continuously monitored to make sure that physical/chemical parameters are optimal for plant growth, and is refilled from time to time to provide enough nutrients. One major advantage of NFT is the possibility of an automatic and uniform supply of nutrients that can be made to match the need of a specific plant. The nutrient solution can either be circulated continuously or intermittently. Another advantage of NFT is the efficient use of water and the ability to monitor and control variables such as water uptake, oxygen concentration, temperature etc. Frillice lettuce (*Lactuca sativa* var. *crispa*), a type of iceberg lettuce, has been successfully cultivated by hydroponics in Norway (S. A. Wolff, pers.comm, May 21, 2013). This culture is also suitable for research because it has a short growing period and produces a homogenous biomass that is easy to measure.



Figur 2. Skisse av et NFT system.

Figure 2.3. Sketch of the nutrient film technique (NFT) system.

2.5 Organic matter

Natural organic matter (NOM) is a complex mixture of organic compounds originating from plants and organic waste (Matilainen et al., 2011). In Norway the content of NOM in freshwater is relatively low (Skjelkvale et al., 2007), but an accumulation in RAS has been reported due to accumulation of protein rich wastes (Mook et al., 2012). The content of NOM can be measured analytically as total organic carbon (TOC) and dissolved organic carbon (DOC) (Skoog et al., 1996). Organic particles over 0.45 μm are classified as particulate, and organic particles under 0.45 μm are classified as dissolved (measured as DOC). In surface water the organic matter is mainly dissolved.

Metals and organic molecules can share electron pairs and form stable complexes called chelates (Skoog et al., 1996) and it is widely accepted that DOC present in the water controls the availability of metal ions and will influence the toxicity of the metals (Bjerknes, 2007; John et al., 1987). There is also evidence that organic matter plays an important role in reducing the uptake of metals by plants (Yermiyahu et al., 2002). This is because functional groups in organic matter have a high affinity for metals and thus can change the concentration in the substrate (Baken et al., 2011). Increased amounts of DOC that have a negatively charged surface can reduce the bioavailability of excess nutrients (Fe, Zn, Cu) and positively charged toxic metals (Al, Cd, Pb) by creating organic compounds. This also means that an increased amount of DOC will result in an increased amount of metals. Addition of seawater to the fish tanks containing freshwater is common to raise the pH value (Bjerknes, 2007). Metals bound to organic matter can be mobilized by the rapid change in pH and form metals on labile form, toxic to fish.

The reduced toxicity of metals due to binding to organic ligands can be explained by the free ion model (FIM) (Roy & Campbell, 1997). There are different qualities of NOM and this affects the strength of metal binding. By measuring the specific ultraviolet absorbance, $SUVA_{254}$, it is possible to estimate the dissolved aromatic carbon content in the water, (Weishaar et al., 2003). This will give an indication of how well the organic material binds the present metals over time.

2.6 Physiochemical parameters that influence aquaponics

The physiochemical parameters play an important role in aquaponics. The methods are not emphasized, but the influence the different parameters have on the water chemistry. The parameters control several factors such as solubility, speciation, availability and toxicity of gases, metals and organic matter.

2.6.1 pH

Plants can retrieve nutrients from water, soil or other substrates, and the chemical composition of the medium will therefore influence growth (Berry, 2010). The pH affects the nutrient availability, and both wither too basic and too acidic water pH is undesirable. An analysis of plant material will provide information about what nutrients the plants lack or has in excess. Water and soil analysis provides information about the status of the nutrient supply.

Aquaponics require a balanced pH for plants, fish and nitrifying bacteria (Tyson et al., 2008). A pH value below 6 and above 8 are critical endpoints in aquaculture (Bjerknes, 2007). This is because pH drives chemical speciation, and thus controls toxicity of elements in water. To ensure that the nutrients are available to the hydroponic lettuce the pH range should optimally be within the pH range of 5.0 – 6.0 (Gislerød et al., 2005), but pH levels up to 7 are also adequate for growth (Roosta, 2011). Nitrification is optimal within a pH range of 7.5 – 9.0 (Tyson et al., 2008). An aquaponic system should maintain a pH near 7 because nitrification efficiency decreases at lower pH values while nutrient solubility decreases at higher pH values (Rakocy et al., 1997). The pH is expected to decrease because of the CO_2 produced by nitrification.

2.6.2 Temperature

In RAS the temperature is easy to control. Atlantic salmon have a high temperature tolerance (Elliott & Elliott, 2010). The upper temperature limit for salmon in Norway is 23 – 26 ^{o}C, but optimum temperature for growth is 16 - 20 ^{o}C. To ensure maximum growth and minimize stress, the temperature needs to be maintained in the species optimal range. Optimal growth of lettuce is obtained with day temperatures of 15 – 25 ^{o}C, and night temperatures of 10 – 15 ^{o}C (Grubben, 2004). This means that the optimal temperature for smolt production is within the temperature range required for cultivation of lettuce.

2.6.3 Oxygen

Plants retrieve oxygen as a nutrient from the air. Oxygen in the rooting medium is also required for the metabolic processes involved in root formation and subsequent growth (Soffer & Burger, 1988). Low concentration of dissolved oxygen can decrease water uptake by the roots and thereby decrease leaf growth of lettuce (Yoshida et al., 1997). Irrigation of plants will naturally aerate the water, and in addition aeration devices should be readily available in case of oxygen depletion. Usually conditions are considered hypoxic (low oxygen) when dissolved oxygen is under 65 %. With respect to fish the water should optimally be 100 % saturated with dissolved oxygen (DO) because water is their source of oxygen (Bjerknes, 2007). In addition nitrifying bacteria are aerobic and need oxygen to produce nitrate (NO_3^-) (Henriksen et al., 1981).

2.6.4 Redox potential

Oxidation-reduction reactions (usually termed redox reactions) play an important role in the behavior of various elements in the environment, especially in the transformation of compounds of biological importance (Matia et al., 1991). One of the main parameters which controls these reactions is the redox potential (E_h). In water treatment E_h measurements and redox balances can provide valuable information about elements. However interpretation of E_h measurements must be done with care because of the complexity of the water chemistry.

2.6.5 Conductivity and salt

Seawater is commonly added to freshwater in aquaculture to obtain higher pH values and increase alkalinity. An additional positive effect resulting from high chloride in aquaculture is the competition against nitrite, which is toxic to fish (Atwood et al., 2001). When integrating plants and fish the addition of salt is a challenge. High concentrations of sodium in the presence of chloride are toxic to plants, and high concentrations of sodium are found to compete with the uptake of essential nutrients such as potassium and calcium (Rakocy et al., 1997)). Toxicity, sensitivity and conductivity of sodium and chloride is presented in Table 2.1 (Morris & Devitt, 1991). *Lactuca sativa* var. *crispa* is considered a moderately sensitive plant with respect to salt (Shannon & Grieve, 1998). The general effect of salinity is reduced growth rate resulting in smaller leaves, shorter stature, and sometimes fewer leaves. Uptake of water decreases as salinity increases, and this will dehydrated the lettuce and further inhibit growth (Pessarakli et al., 1989). The degree of reduction in growth is highly dependent on species. Ion toxicities or nutritional deficiencies are common in cases of severe toxicity because of predominance of a specific ion or competition among cations or anions. Electrical conductivity (EC) reflects the amount of dissolved salts the water contains. For lettuce it is recommended that EC should not exceed 2500 µS/cm (Rodriguez-Delfin et al., 2000).

Table 2.1. Toxic values (mg/L) and sensitivity of sodium and chloride to plants. Conductivity range (µS/cm) of toxicity/sensitivity.

Toxicity	Na (mg/L)	Sensitivity	Cl (mg/L)	Conductivity (µS/cm)
None	> 70	Sensitive	< 178	0 – 900
Increasing	> 100	Moderately sensitive	< 178 - 355	900 – 2 700
Significant	> 200	Moderately tolerant	< 355 - 710	2 700 – 6 400
Severe	> 230	Tolerant	< 710	6 400 – 23 700

3 Materials and methods

3.1 Project partners

Waste water (1 000 L) and sludge (10 L) was collected at Hardingsmolt AS located in Tørvikbygd, Kvam municipality in Hardanger. This is a local fish farm that uses an integrated recycling system (RAS) to produce about five million smolts, a young Atlantic salmon, (*Salmo salar*) every year (Tveranger & Johnsen, 2007). The farm uses mechanical, biological and chemical filters to treat the water. $CaCO_3$ (lime) and NaOH (lye) is added to optimize the water quality (pH, salinity etc.). No antibiotics are used. Their water intake is Tørvikvatnet, located near the farm, and the water is discharged to Dragevika, Hardangerfjorden. Water exchange and feeding regime is adjusted after biomass and size of the fish (Appendix 16). The farm has relatively large fluctuations in the water composition of both waste water and sludge during a year (Appendix 17 and Appendix 18). Waste water collected was from a period when the biomass and feeding was relatively low and the water exchange was relatively high (leading to a dilution of nutrients). Hence, the results from this experiment will reflect this particular water quality.

A local producer of lettuce, Kronheim Grønt, located near Bergen airport, Flesland, in Blomsterdalen, provided the experiment with two week old Frillice lettuce (*Lactuca sativa* var. *crispa*). This lettuce type matures early and has rapid leaf growth. Their production is modern with automatic regulation of light, temperature and humidity. Their growth media is soil and the lettuce needs four weeks two grow before it can be harvested.

3.2 System description and treatment determination

A climatic test chamber with possibilities of regulating light, temperature and humidity was utilized for the experiment (Figure 3.1). Fluorescent light was provided to give the lettuce 18 hours of light and 6 hours of dark every day. The temperature was kept constant at about 18 ^{o}C and the humidity between 75 and 85 %. For more information about the climate room contact Ole-Kristian Hess-Erga at NIVA Bergen.

Figure 3.1. Experimental set-up

The experiment started up Friday, September 21st, 2012 and ended Thursday, October 19th, 2012, a period of four weeks. The system consisted of a water reservoir, troughs, plastic tubes to transport the water and an aeration device (Figure 3.2 and Figure 3.3). The solution was pumped up to the troughs to flow through the soil and drain back into the reservoir. Each system had two parallel troughs (10 x 10 x 200 m) with a total of 20 plants. Six different systems were set up like this, three of them connected to a filter between the troughs and the reservoir. Three different solutions of water were used; a waste water solution, a sludge water solution (mix of waste water and sludge), and a nutrient solution. Two reservoirs were used for each solution (1-3). One reservoir was connected to a filter (F) and one reservoir was without a filter (UF). This yielded a total of six different solutions; WUF, WF, SUF, SF, NUF and NF. The nutrient solutions were set-up according to commercially cultivated lettuce as a control. This was used to compare removal effect and lettuce growth with the waste water treatments.

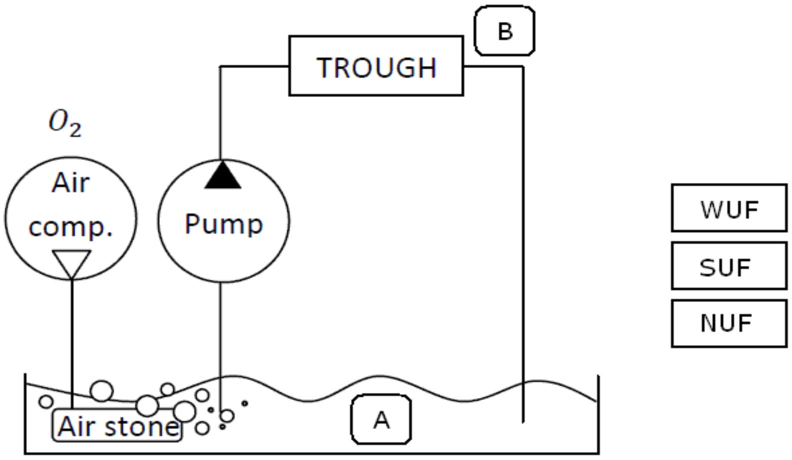


Figure 3.2. Scheme of the recirculating system without filter. Letters A and B indicate where water was sampled (sampling points). The unfiltered waste water solution (WUF), unfiltered sludge water solution (SUF), and unfiltered nutrient solution (NUF) followed this loop.

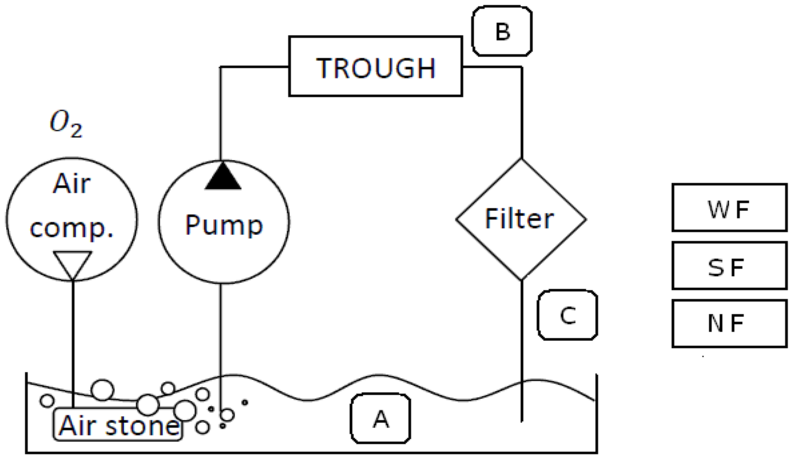


Figure 3.3. Scheme of the recirculating system with filter. Letters A, B and C indicate where water was sampled (sampling points). The filtered waste water solution (WF), filtered sludge water solution (SF), and filtered nutrient solution (NF) followed this loop.

3.3 Operational control and preparation of the system

The filters were made one week in advance in order to acclimate. The waste water was used to prepare two buckets to serve as reservoir solutions for the waste water solutions (WUF and WF). The sludge solutions were made by mixing sludge (50 g, wet weight) into waste water (20 mL). The solutions were used to fill up two buckets to serve as reservoir for the sludge solutions (SUF and SF). Both waste water and sludge used to prepare the reservoirs were kept in a cool, dark place throughout the experiment. *Superex vegetables* and *Calcinit* were used to make the nutrient solution for the lettuce. The nutrient solutions were also used to fill up two buckets to serve as a reservoir for the nutrient solutions (NUF and NF). In a full-scale integrated aquaponic system waste water would supply nutrients continuously. To prevent element depletion and make up for the water removed when sampling and the water utilization (uptake and evapotranspiration) the water reservoirs were completely changed once a week (every Friday). This also allowed for repeated uptake simulation. Similar water utilization was assumed for all six treatments, thus giving the same concentration factor for all the data.

No extra nutrients or buffers were added to any of the treatments. Two week old lettuce (*Lactuca sativa* var. *crispa*) was placed in the troughs and the system was started up the day before the first sampling to establish steady state. The lettuce was grown in pots filled with soil (about 200 g) and irrigated every fourth hour with a constant volume (1000 mL) of water, like it is done commercially at Kronheim Grønt. The soil both provided buffer capacity and contained nutrients. Aeration of the reservoir was done for ten minutes every forth hour (in between irrigation) to keep the oxygen level above 70 %. A camera was set up to take a picture every hour to document the growth of the lettuce throughout the whole period.

A few lettuce plants from NUF and NF was taken out midway during the experiment and supplied with spiked waste water (final concentration 25 mg NO_3-N/L). This was done to see how well the lettuce would do knowing that nitrate was not a limiting factor.

Instrument measurements were done at 9 am every work day (total of 28 days) to record pH, water temperature, oxygen content, salinity, electrical conductivity and redox potential. Redox potential was measured with YSI Ecosense®. The redox instrument was calibrated with a Zobell solution (YSI 3642, 231 mV) from YSI. All other parameters were measured with an Orion 5-Star pH/RDO/Conductivity portable meter (Thermo Fisher Scientific Inc., USA), calibrated by NIVA. The meter was cleaned after every use and stored according to the manufacturer's specifications. Room temperature and humidity were measured continuously by a regular thermometer/hygrometer.

The initial composition of the waste water and the sludge from Hardingsmolt AS, and the initial composition of soil from Kronheim Grønt are shown in Table 3.1. The pH was 7.6, 7.0 and 5.6, and the TOC was 3.3, 420.0 and 94.0 in the waste water, sludge and soil respectively.

Table 3.1 Initial composition of waste water and sludge from Hardingsmolt, and initial composition of soil from Kronheim Grønt

Element		Waste water	Sludge	Soil
		µg/L	mg/kg TS	mg/kg
Macro	N	20 000	23 000	11 000
	Tot-P	2 200	57 000	1 100
	K	16 000	310	930
	SO_4	97 000	380 000	-
	Ca	64 000	240 000	16 000
	Mg	45 000	6 600	1 400
Micro	Fe	<50	2 100	2 000
	Mn	<5	130	50
	Zn	7	580	<72
	Cu	<3	8	22
	B	160	28	<72
	Mo	<1	0	18
Toxic	Al	40	450	870
	Cd	-	1,3	-
	Cr	<1	4	4
	Ni	-	<2.8	<7.2
Other	Na	350 000	2 100	400
	Cl	620 000	1 320	183

3.4 Growth analysis

The last day of the experiment three plants from each treatment were weighed, and measured by leaf length, color and number of leaves. A plant from Kronheim Grønt was also weighed and measured in the same manner as a control. The plants from each treatment were dried at a 100 ^{o}C for about 3-4 hours, weighed and wrapped in paper and then plastic bags. Soil samples were also taken from the pots of the same three plants.

3.5 Sample and chemical analysis

Table 3.2 gives an overview of all the samples collected, including water, lettuce and soil samples. Water samples were collected two times a week from sampling point A (Figure 3.2 and Figure 3.3) throughout the 28-day experimental trial, a total of nine sampling days. Six pre-labeled glass beakers (1 000 mL) were used to collect water from each of the six treatments. A BD Plastipak, sterile syringe (20 mL) was used to collect water samples. Two samples were taken out for analysis of organic material, total content (30 mL) and dissolved content (30 mL, filtered). Two samples were taken out for analysis of elements with ICP-MS, total element concentration (10 mL) and total concentration of dissolved elements (10 mL, filtered). A membrane, polyethersulfone syringe filter (25 mm, w/0.45 µm) from VWR (514-0074) was used for all filtered samples. Water samples for TOC and DOC were collected in sterile, propylene tubes (50 mL) from VWR (89049-176). Water samples for ICP-MS were collected in metal free, sterile, propylene, centrifugal tubes (15 mL) from VWR (89049-172). Samples were stored in a refrigerator until preservation with acid ($HNO_{3,}$ 3 droplets to 10 mL sample) and analysis. The acid (Suprapure) was kept in a metal free Teflon bottle with a dropper tip.

Water samples were also collected every other week from sampling point B and C (Figure 3.2 and Figure 3.3) in the same way as described above. This yielded 3 x 6 samples from point B, and only 3 x 3 samples from point C (treatments without filter did not have a point C). Sampling of point B was done to illustrate how the water was affected by plants, while sampling of point C was done to illustrate how the water was affected by the filter.

Soil samples were accurately weighed (250 – 350 mg) and then dried for two hours. The samples (20 - 30 mg, dry weight) were diluted with HNO_3 (0.6 M) and distilled water (final vol. 108 mL). Lettuce samples were accurately weighed (400 - 500 mg, dry weight), and diluted with HNO_3 (0.6 M) and distilled water (final vol. 60 mL). All samples were decomposed with UltraClave MLS Microwave together with blanks and reference material for both soil (Soil GBW 07408) and lettuce (tea leaves). A total of 12 samples were analyzed for 41 elements (total concentration) with ICP-MS Thermo Element 2. Information about the instrument and procedures can be found at the Faculty of Natural Science and Technology at NTNU. Contact person is Syverin Lierhagen. Nitrogen was not measured. The concentration of elements was divided into macro-, micro-, and toxic elements. Recommended concentration (R) of macro- and microelements (Horst Marschner, 1995) were used to compare with lettuce from the nutrient treatments to determine if the elemental composition was good enough to be used as control. Toxic elements included were Al, Cd, Cr and Ni, and limit values (R) were used to detect toxic concentrations (Alloway, 1995). The Al limit was collected from McLean and Gilbert (1928). Na and Cl were included because of toxic potential to lettuce.

The water samples collected for element analysis were weighed (7.5 mL) and diluted with acid (0.2 M HNO_3, 7.0 mL). A total of 162 water samples were analyzed for 41 elements (total concentration) with ICP-MS Thermo Element 2. The concentration of elements was determined by back calculation for water, soil and lettuce samples. 19 elements were picked out for evaluation in order to limit the discussion. Macro- (N, P, K, S, Ca and Mg) and microelements (Fe, Mn, Zn, Cu, B, Mo) were included because of the importance for plant growth, and Al, Cd, Cr and Ni because of toxic potential to fish. Recommended values (Roberto, 2003), M. Berland, pers. comm., 16.sept, 2012) and limit values were included to compare with the nutrient solutions and determine if the elemental composition was good enough to be used as control. Na and Cl were included because of the high concentration in the waste water received from the fish farm and Si was included because of its role in eutrophication. The same amount of water was sampled from each reservoir, and it was assumed that this had no effect on the data.

Organic material was analyzed with Teledyne Telemar Torch. UV analysis was also carried out for all water samples after analyzing TOC and DOC. Shimadzu UV mini 1240 with a quarts cell (1 cm) was used at a wavelength of 254 nm. Information about the instruments and procedures can be found at the Faculty of Natural Science and Technology at NTNU. Contact person is Øyvind Mikkelsen.

A Spectroquant® Pharo 300 photometer (Merck, Germany) was used to measure NO_3^-. The Spectroquant® photometric Nitrate Cell Test method (14556) was used to analyze the water samples immediately after sampling.

3.6 Data analysis

Microsoft Excel 2007 was used to treat data such as calculation, making tables and charts, and statistical tests. Regression, paired t test of means and ANOVA was performed by using the Data Analysis Add-in in Excel 2007. The confidence interval was set to 5 %, meaning that a p value (the probability) of < 0.05 was considered significant. Regression analyses were performed on all element data to detect significant increasing/decreasing trends. The t-test was used for comparing the means of two groups. This test was used when determining amount of particulate and dissolved metals in the water samples. The t-test was also used for comparing differences between the sample points A and B (lettuce effect) and B and C (filter effect). ANOVA was used to compare differences between the different solutions (waste water, sludge water and nutrient) and the different systems (with/without filter).

Table 3.2. Overview of samples, sampling purpose, sampling days, sampling point, sample treatment, total of samples per day, total of water samples and the total of all samples collected.

Sample	Treatment	**Before start**	**Start**	**Week 1**		**Week 2**		**Week 3**		**Week 4**			
			Fri	**Mon**	**Fri**	**Mon**	**Fri**	**Mon**	**Fri**	**Mon**	**Thu**		
Sludge water solution	SUF		A B	A	A	A	A B	A	A	A	A B		
	SF		A B C	A	A	A	A B C	A	A	A	A B C		
Waste water solution	WUF		A B	A	A	A	A B	A	A	A	A B		
	WF		A B C	A	A	A	A B C	A	A	A	A B C		
Nutrient solution	NUF		A B	A	A	A	A B	A	A	A	A B		
	NF		A B C	A	A	A	A B C	A	A	A	A B C		
Soil and roots (5 grams from each pot)		X 6									X 6		
Lettuce (leaves from each treatment)											X 6		
Analysis	Treatment											water samples (total)	Soil/lettuce included
ICP-MS (10 ml)	no filter		15	6	6	6	15	6	6	6	15	81	101
	syringe filter (0.45µm)		15	6	6	6	15	6	6	6	15	81	101
DOC/UV (30 ml)	no filter		15	6	6	6	15	6	6	6	15	81	81
	syringe filter (0.45µm)		15	6	6	6	15	6	6	6	15	81	81
Back-up (100 ml)	no filter		15	6	6	6	15	6	6	6	15	81	81
Instrument measurements*	Every day											28 days in total	

*Every day at 09 am

4 Results and discussion

4.1 Natural organic material

There was an overall increase in natural organic matter (NOM) in all of the waste water solutions (Table 4.1). Both total (TOC) and dissolved (DOC) organic matter appeared to have increased, but only DOC was significant ($p < 0.03$). Most of the organic matter was dissolved, while a small fraction was particulate. The particulate organic matter appeared to have increased in all of the treatments, but the trend was not significant ($p > 0.4$). No trend in the nutrient solutions was significant ($p > 0.1$).

Table 4.1. Start and end concentration (mg/L) of total, dissolved and particulate matter in the unfiltered (WUF) and filtered (WF) waste water solutions, unfiltered (SUF) and filtered (SF) sludge water solutions, and unfiltered (NUF) and filtered (NF) nutrient soluions.

	Waste water solutions								Nutrient solutions			
	WUF		WF		SUF		SF		NUF		NF	
NOM	start	end	start	end	start	end	start	end	start	end	start	end
total	5.5	12.1	6.3	12.7	6.5	14.3	7.2	12.7	10.4	17.0	10.9	14.0
dissolved	5.0	9.6	6.0	10.0	5.6	11.3	5.9	10.3	10.3	10.4	9.8	9.4
particulate	0.5	2.5	0.3	2.7	0.9	3.0	1.3	2.4	0.1	6.6	1.1	4.6

A wide range of organic compounds is released by the roots of plants (D. Barber & Martin, 1976), and this can explain the increase in particulate matter and DOC (Figure 4.1). The insignificant trend found for the particulate organic matter indicates that it was not removed by the filter. DOC represents the fraction of organic material that is difficult to remove by the filter (< 0.45 µm). Although DOC increases metal solubility, it acts as a ligand to form strong metal complexes that reduce the bioavailability of a metal (Stumm & Morgan, 1995), however this is dependent of the character of the organic matter and the strength of the binding. This metal-ligand formation is beneficial for fish because it lowers toxicity, although the binding does not remove the metals and the water will still have a toxic potential. It was earlier believed that only free metal ions are available for uptake by plant roots. This was questioned by several researchers who found that trace metals such as Fe and Cu are in fact not only taken up as free metal ions, but also in complexed form (Bell et al., 1991; Checkai et al., 1987). Uptake of zinc and cadmium was found to increase in the presence of ligands, though highly dependent on the type of ligand (McLaughlin et al., 1997). This means that some organic ligands can reduce the bioavailability of metals to fish and still be available to the lettuce.

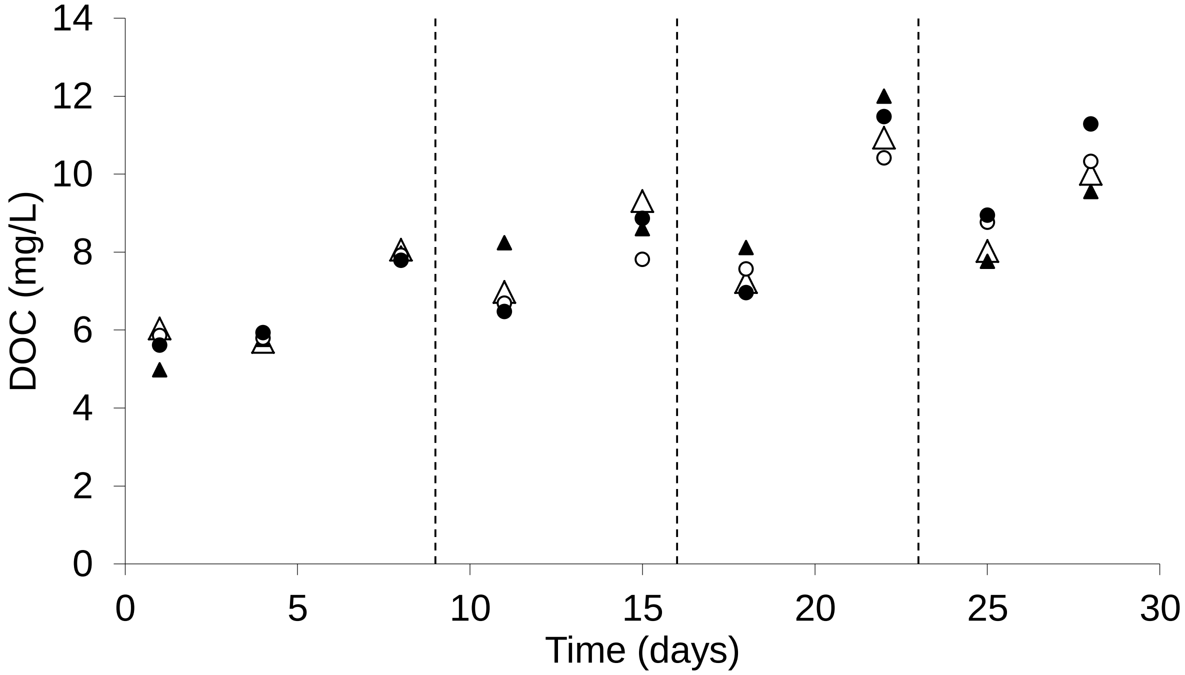


Figure 4.1. Change in DOC (mg/L) with time in the unfiltered (WUF) and filtered (WF) waste water solutions and unfiltered (SUF) and filtered (SF) sludge water solutions over the 28 days experiment. (N=9).

There was a higher concentration of NOM in the nutrient solutions than the waste water. Two types of fertilizer were used, *Calcinit* and *Superex vegetables*. The latter is a chelated fertilizer (LOG, 2013). Chelated fertilizers have been developed to increase micronutrient utilization efficiency (Liu & Hanlon, 2012). This means that the micronutrients (metals) are bound to organic molecules (ligands) to keep them from oxidizing or precipitating in the soil. This would explain the higher concentration of organic matter in the nutrient solutions. Competition from other elements such as calcium for binding to ligands may explain the low uptake of Fe despite the increasing trend seen in the nutrient solutions.

Relatively little information is available about the quality of NOM formed in RAS (Meinelt et al., 2010). SUVA was measured to investigate the qualities of NOM, and the ability to bind metals. However, no evaluation was done because of the low content of NOM and the possibility of interference from nitrate and iron.

Despite the fact that sludge was added to the waste water solution, the low concentration of NOM in SUF and SF may be explained by sedimentation of the sludge on the bottom of the reservoir. Continuous mixing might be a possible way to prevent the sedimentation.

There was no significant difference ($p > 0.05$) between the total element concentration and the concentration of dissolved elements in any of the treatments, except for aluminum in NUF. The average concentration of total aluminum of 19.0 µg/L was significantly different ($p < 0.0007$) from the average concentration of dissolved aluminum of 7.7 µg/L. The low p value indicates that aluminum in NUF was mainly bound to particulate matter and not dissolved. Particle bound aluminum may become toxic to fish if there is a rapid change in pH (Rosseland & Staurnes, 1994). The aluminum can be mobilized into free metal ions that accumulate on the fish gill. The accumulation can lead to reduced marine survival, but only when the concentrations exceed 10 µg AI/L (Frode Kroglund & Finstad, 2003).

No significant difference ($p > 0.05$) was found in NOM in water sampled before (B) and after (C) the filter (Figure 3.3) in any of the treatments. The results show that particles were neither removed from B to C nor throughout the experiment indicating that the filter had no detectable effect.

An increase in the filtered solutions was observed for aluminum (in WF and SF) and for iron (in WF, SF and NF). Although not significant ($p > 0.1$), this trend was not seen in any of the unfiltered treatments (WUF, SUF or NUF). This indicated that the filter was having an effect on the water. Rock wool mainly consists of the minerals SiO_2, CaO, Al_2O_3 and Fe_2O_3 (Nockolds et al., 1978). A two-fold higher concentration of silicon in WF and SF, and six-fold higher concentration of silicon in NF supported the suspicion of filter contamination. Although rock wool is a widely used substrate in the horticultural industry (Edge, 2005; Gibeaut et al., 1997), it is not suited for aquaponics (Bhattarai et al., 2008).

4.2 Element trends

4.2.1 Decreasing trends

The concentration of nitrogen, phosphorus, potassium, manganese, copper and cadmium all decreased in the waste water solutions when comparing start and end concentration (Table 4.2). The highest overall element decrease (in %) for each element was, P:77, K:70, Mn:98, Cu:77 and Cd:51. For nitrate the decrease was 91 %. This gives an indication of how much of each element the lettuce, soil or filter was able to remove from the waste water solutions. Nitrogen was not measured systematically in water samples throughout the experiment and the quality of the data for nitrogen (as nitrate) is therefore not of the same strength as the quality of the other element data.

A significant decrease was found for phosphorus ($p < 0.02$) and potassium ($p < 0.04$) in all waste water solutions. Overall, the concentration decreased by 57 -77 % for phosphorus and 53 - 70 % for potassium. The trends were virtually the same regardless of treatment and therefore the trends are represented by WF only (Figure 4.2) for convenience. The change of reservoir on day 8, 15 and 22 can explain the high concentration of the elements at the start of each week. The first measurement of week 2, 3 and 4 was done three days after the change of reservoir, while the first measurement of week 1 was done one day after reservoir change, thus resulting in a higher concentration compared to the other weeks. If the water reservoir had not been changed the decrease would probably have yielded an almost straight line, assuming sufficient nutrient content. The decrease in both phosphorus and potassium appears to have been nearly the same every week, despite the fact that the lettuce did not appear to grow.

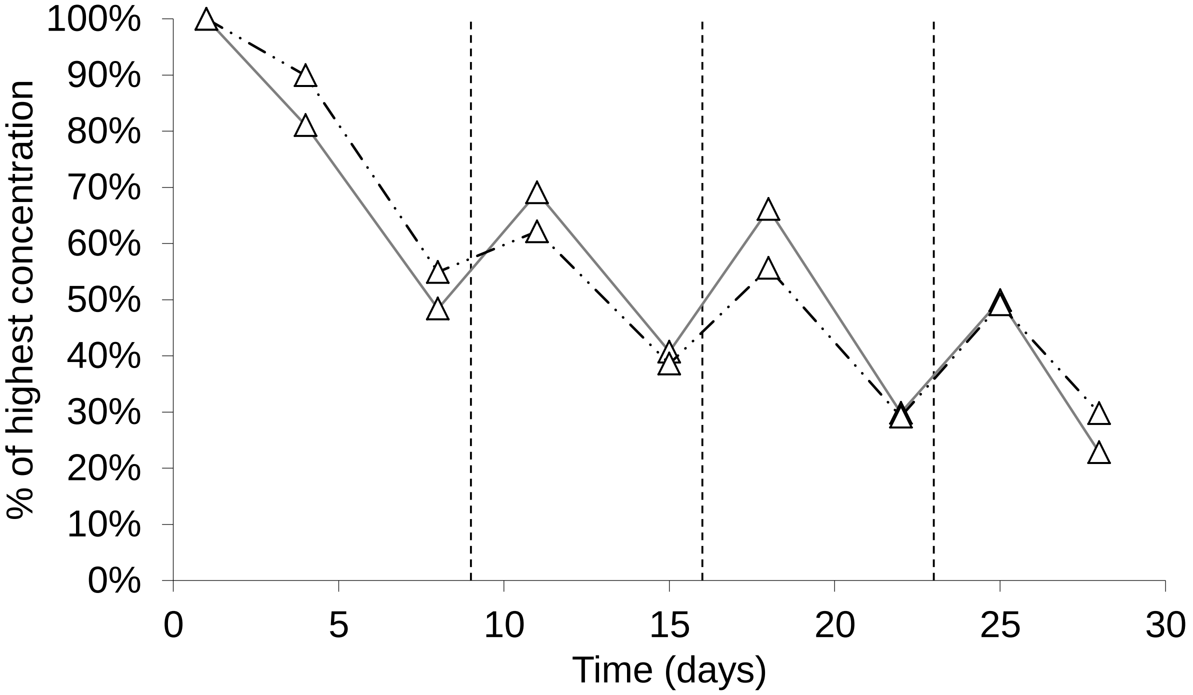


Figure 4.2. Trend for phosphorus (dotted line) and potassium (black line) in the filtered waste water solution (WF) over the 28 day experiment (N=9). Highest concentration set to 100 %. Change of reservoir indicated by vertical, dotted lines.

No significant differences for phosphorus ($p > 0.2$) or potassium ($p > 0.6$) were found between the unfiltered treatments and the filtered treatments. This indicates that the elements were not removed by the filter, but either lettuce or soil. Both elements were below the recommended concentration in the waste water solutions (Table 4.2) and were expected to become depleted in order for the lettuce to get enough nutrition. Sludge and waste water contain high amounts of phosphorus and efficient removal of this element (and also nitrogen) is important to prevent eutrophication caused by aquaculture effluents (Barak et al., 2003). Since phosphorus often is the limiting nutrient for algal growth it is this element that needs to be removed. Plant-based removal of nutrients is not a new method, and the results for phosphorus are supported by earlier findings, where a variety of plants have been used as a natural treatment of waste water (Aoi & Hayashi, 1996; Brown et al., 1999; Stottmeister et al., 2003). Some plants have even been reported to efficiently remove phosphorus from waste water to concentrations less than 100 µg P/L (Adler et al., 2000).

Table 4.2. Elemental composition of the unfiltered (WUF) and filtered (WF) waste water solutions, unfiltered (SUF) and filtered (SF) sludge water solutions, and unfiltered (NUF) and filtered (NF) nutrient soluions (filtered samples, 0.45µm). Start and end concentration (µg/L) of elements, divided into macro-, micro- and toxic elements. Recommended values (R) for optimal growth of lettuce is also included.

			Waste water solutions								Nutrient solutions			
			WUF		WF		SUF		SF		NUF		NF	
Elements		*R*	Start	End	Start	End	Start	End	Start	End	Start	End	Start	End
Macro	NO_3	*200 000*	1 800*	170	5 400*	1 360	2 800*	330	3 600*	720	244 000*	169 000	200 000*	195 000
	P	*40 000*	1 875	457	1 869	426	2 096	901	1 903	743	52 582	56 319	43 211	52 304
	K	*200 000*	15 060	4 607	18 048	5 377	15 549	7 320	17 058	6 329	194 980	154 230	189 059	196 154
	S	*64 000*	34 530	35 592	38 338	39 665	34 604	35 844	38 045	39 636	34 078	46 300	31 776	44 355
	Ca	*150 000*	56 108	65 081	64 977	72 591	58 717	71 768	70 413	83 399	221 720	300 837	210 532	299 629
	Mg	*35 000*	38 932	44 206	44 701	49 655	38 882	45 492	44 312	49 237	21 586	24 352	21 126	25 359
Micro	Fe	*3000*	23,2	22.2	17.0	31.7	19.9	19.6	15.2	27.5	1 656	2 323	1 542	1 957
	Mn	*1000*	13.4	0.4	13.3	0.2	14.7	0.5	6.2	0.6	640	507	512	560
	Zn	*300*	11.5	9.3	11.5	8.7	12.1	8.2	9.6	9.2	244	346	200	117
	Cu	*100*	10.7	2.6	13.3	3.7	3.7	2.1	14.8	3.5	182	195	184	160
	B	*300*	154.8	162.7	169.2	166.0	156.4	157.3	173.6	162.5	387	502	367	500
	Mo	*10*	2.2	3.2	3.6	4.9	2.6	4.2	4.3	4.6	47	60	46	60
Toxic	Al		24.1	6.8	48.2	88.8	7.7	3.0	12.0	40.4	14.5	7.7	10.5	7.1
	Cd		0.03	0.03	0.04	0.03	0.02	0.01	0.03	0.01	0.46	0.23	0.13	0.08
	Cr		0.2	0.1	0.1	0.1	0.2	0.3	0.1	0.1	0.5	0.7	0.4	0.6
	Ni		1.1	1.0	2.4	1.6	0.7	0.9	6.1	1.8	63.5	107.1	6.6	14.8
Other	Na	*230 000***	282 409	292 084	291 892	291 509	287 615	291 509	286 897	291 129	7 072	8 667	7 538	8 507
	Cl	*710 000***	674 314	864 162	806 105	986 592	701 462	883 527	806 845	978 647	9 671	182	9 777	3 848
	Si		601	388	1 147	915	616	445	1 195	1 271	634	1 134	3 519	5 088

* Start concentration of NO_3 is from sampling day 2 of the experiment

** Upper limit before severe signs of toxicity can be observed

Manganese decreased significantly ($p < 0.04$) in all waste water solutions and the concentration was 90 – 98 % lower at the end of the experiment. A significant decrease in zinc ($p < 0.04$) was found in WUF and WF and a significant decrease in copper ($p < 0.008$) was found in WUF, WF and SF. The decrease was 19 - 24 % for zinc and 72 - 77 % for copper. The trends were virtually the same regardless of treatment and therefore they are represented by WF only (Figure 4.3) for convenience.

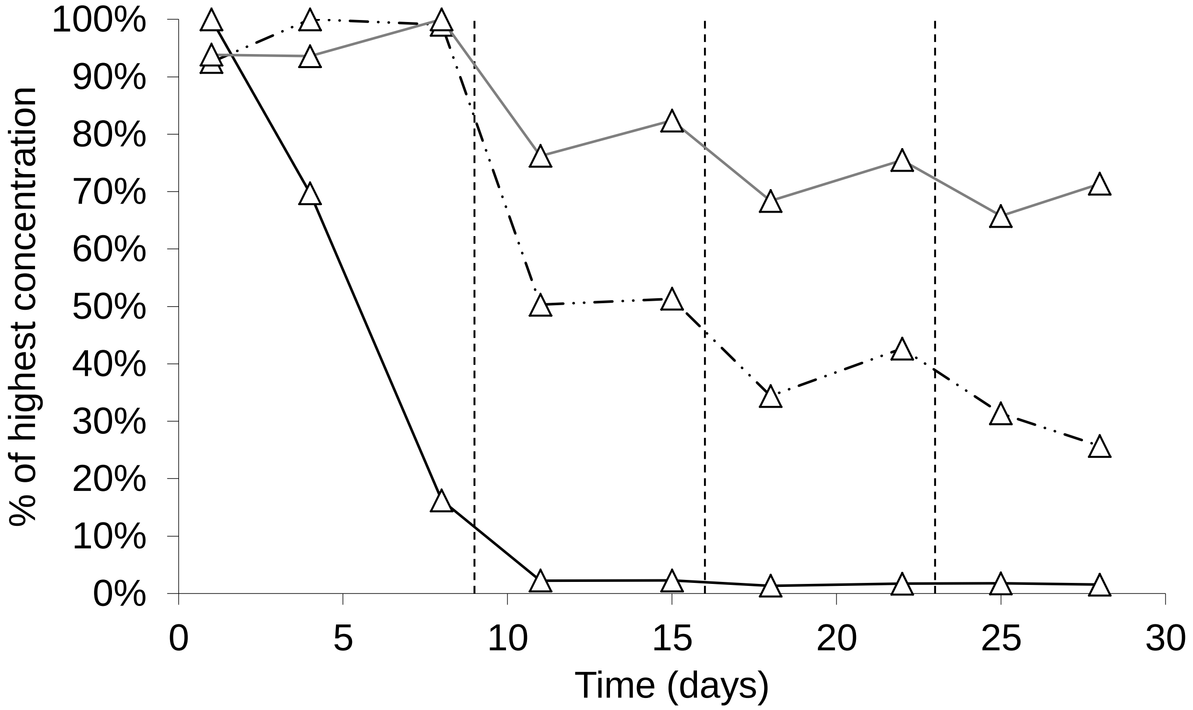


Figure 4.3. Trend for manganese (black line), zinc (dotted line) and copper (grey line) in the filtered waste water solution (WF) over the 28 day experiment (N=9). Highest concentration set to 100 %. Change of reservoir indicated by vertical, dotted lines.

No significant difference for manganese ($p > 0.6$), copper ($p > 0.4$) and zinc ($p > 0.08$) was found between the unfiltered treatments and the filtered treatments. This indicates that the elements were not removed by the filter, but either lettuce or soil. Manganese was found in recommended concentrations within the lettuce (Table 4.4) and may explain the depletion observed in the waste water solutions. Both zinc and copper decreased, but the uptake in plant was not optimal. The presence of organic matter may explain the low uptake of zinc and copper, however this depends on the character of the organic matter (Pinto et al., 2004). Microelements are necessary for lettuce growth, but toxic to fish. Low concentrations of copper have been reported to accumulate in RAS (Martins et al., 2009), hence, it is crucial that waste water treatment removes copper. Zinc is less toxic than copper, and only toxic in high concentrations (Bjerknes, 2007). Manganese does not have any known toxic effect on fish.

For both farmed and wild fish it is important that the concentrations of toxic elements are kept low. There was a decreasing trend of nearly all micro- and toxic elements, but only cadmium was found to be significant ($p < 0.009$), and only in WF. Cadmium is toxic for both lettuce and fish (Ramos et al., 2002), but the concentrations of this element (< 0.04 µg/L) in the waste water solutions was too low to pose any hazard (Haghiri, 1973).

Chloride was found to decrease significantly ($p < 0.03$) in the nutrient solutions, resulting in a concentration 61 – 98 % lower at the end (Figure 4.4). The decrease may be explained by the nutritional status of chloride (Mengel & Kirkby, 2001; White & Broadley, 2001) as long as concentrations are kept below the toxic limit of 178 mg/L (Morris & Devitt, 1991).

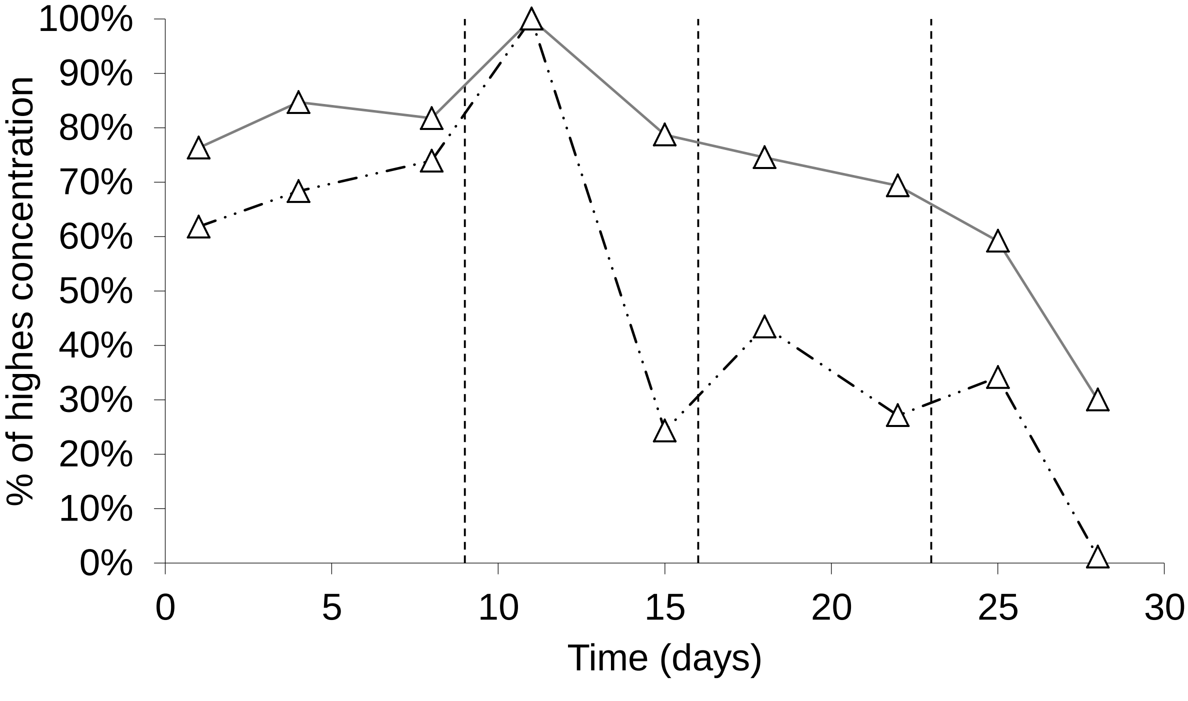


Figure 4.4. Trend for chloride in the unfiltered (dotted line) and filtered (grey line) nutrient solutions (NUF/NF) over the 28 day experiment (N=9). Highest concentration set to 100 %. Change of reservoir indicated by vertical, dotted lines.

4.2.2 Increasing trends

The concentration of calcium, iron, boron and molybdenum was found to increase significantly ($p < 0.05$) in the nutrient solutions. Sulphur and chromium increased significantly ($p < 0.01$), but only in NF. The highest increase (in %) of each element was Ca:42, Fe:40, B:37, Mo:30, S:40 and Cr:53. The trends were virtually the same for all elements regardless of treatment and they are therefore represented by iron only (Figure 4.5) for convenience. No significance ($p > 0.4$) was found between the unfiltered treatments and the filtered treatments indicating that the filter had no effect.

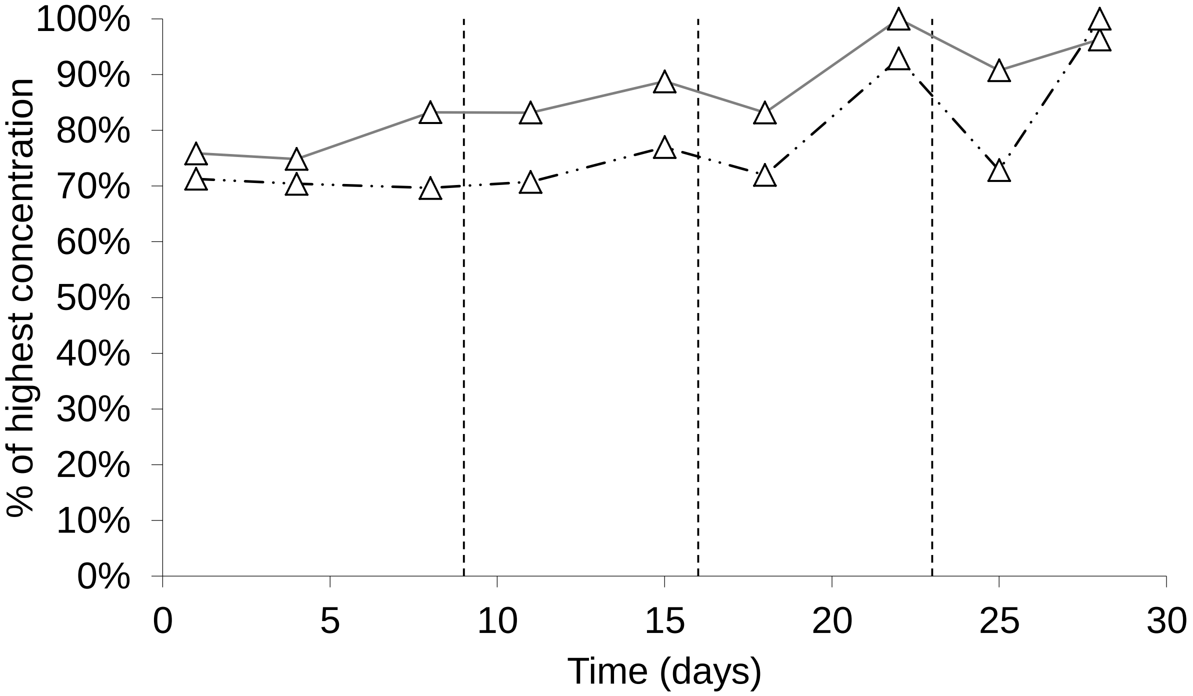


Figure 4.5. Trend for iron in the unfiltered (dotted line) and filtered (grey line) nutrient solutions (NUF/NF) over the 28 day experiment (N=9). Highest concentration set to 100 %. Change of reservoir indicated by vertical, dotted lines.

The concentration of ions depends on the ratio of transpiration to growth (Bugbee, 2003). Transpiration, dependent on humidity, determines the rate of water removal while growth determines the rate of nutrient removal. Bugbee divided the life cycle of a plant into three stages with higher nutrient requirement the first two stages than the last. Refill of the reservoirs were done with the same amounts of nutrients each week, thus imbalance in nutrient refill is cumulative and may explain the increasing trends.

The concentration of iron increased in the nutrient solutions although the concentration of iron in the lettuce was lower than recommended. Iron is readily oxidized into unavailable forms and influenced by the presence of other positively charged elements. This may explain why the uptake of iron was low, but contradicts the increasing trend in the nutrient solutions. However, it could be explained by iron present in the soil, considering that iron is the most abundant nutrient in soil (S. A. Barber, 1995).

Water sampled before and after the lettuce (A and B in Figure 3.2 and Figure 3.3) was used to get an indication of element uptake by the lettuce (and the soil). No significant trend ($p < 0.05$) was observed for any of the elements in any of the six treatments when comparing A and B. This indicates that there was no detectable uptake of elements comprising A and B by lettuce or soil, or direct leaching of elements from the soil.

In contrast to the nutrient solutions the concentration of chloride in the waste water solutions was very high. Chloride increased in all the waste water solutions, but only significantly ($p < 0.02$) in WUF and WF. Magnesium also increased in all the waste water solutions, but only significantly ($p < 0.005$) in WUF and SUF. Chloride decreased by 21 – 28 % and magnesium by 11 – 17 % (Figure 4.6). It seems that both elements were only slightly affected by the change of reservoir. The concentration of sulphur, calcium and molybdenum also appeared to have increased, but not significantly.

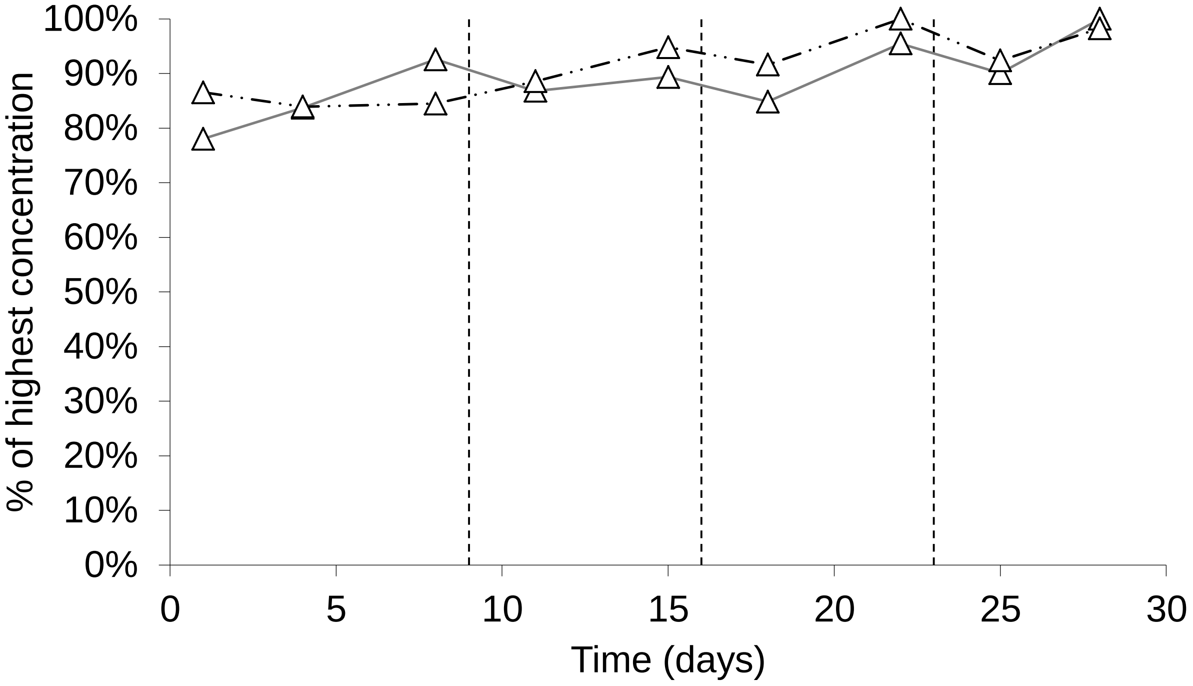


Figure 4.6. Trend for chloride (grey line) and magnesium (dotted line) in the unfiltered nutrient solution (NUF) over the 28 day experiment (N=9). Highest concentration set to 100 %. Change of reservoir indicated by vertical, dotted lines.

There was a significant difference ($p < 0.02$) in the magnesium concentration between the unfiltered (WUF, SUF) and the filtered (WF, SF) treatments. However, this may be explained by a higher start concentration of magnesium in both the filtered treatments (WF and SF), rather than by a filtering effect. The chloride concentration in WUF differed significantly ($p < 0.002$) from WF, but this was also due to a higher start concentration in the filtered treatment (WF). A difference between unfiltered and filtered treatment was not seen in the lettuce (Table 4.4), indicating a similar uptake rate. This can be confirmed by the fact that nutrient uptake is limited by root development (Itoh & Barber, 1983). By assuming similar root development due to similar growth, the uptake rate should be the same.

High levels of potassium can suppress the uptake of magnesium (Mengel & Kirkby, 2001), but this does not seem applicable because there was no relationship between magnesium and potassium in the lettuce (Table 4.4). Uptake of magnesium may be suppressed in acid soils, both because of low availability when pH is low and due to the presence of cationic aluminum species (Grimme, 1983). This did not correspond to the measured aluminum concentrations in the lettuce.

Salinity stress is caused by high sodium and chloride content within the plant (Alam & Pessarakli, 1999). This was found in lettuce from the waste water solutions. Sodium and chloride decrease the solubility and availability of water to the plants by decreasing the free energy of water. The lettuce becomes dehydrated and growth is inhibited (Pessarakli et al., 1989). Uptake of excess sodium and chloride will also contribute to reduced growth because uptake of essential nutrients such as potassium, calcium and magnesium is suppressed (Rakocy et al., 1997). There is a critical concentration of nutrients that can be accumulated by plants (Prasad & Power, 1997). This maximum accumulation cannot be exceeded although there are bioavailable nutrients in the growth media. If the critical limit of chloride accumulation in lettuce was exceeded, this may explain the increase in chloride in the waste water solutions.

4.3 Lettuce

A lettuce plant (Figure 4.7) cultivated under commercial conditions at Kronheim Grønt parallel to lettuce from this experiment was used to compare with lettuce from the nutrient treatments. Although not as compact and tall as the control plant from Kronheim Grønt, lettuce from NUF and NF (Figure 4.8) were similar enough to be used as control. Lettuce from the waste water treatments had mature leaves with a lighter green color and discolored leaf tips (Figure 4.9 and Figure 4.10). Some of the outermost leaves were unusually long and pale, while the young leaves had a fresh, green color and no signs of deficiency. They were smaller than the lettuce from the nutrient treatments and had clear symptoms of nutrient deficiency, stress or both.

Figure 4.7. Harvested Frillice lettuce (*Lactuca sativa* var. *crispa*) from Kronheim grown parallel to the experiment.

Figure 4.8. Harvested Frillice lettuce (*Lactuca sativa* var. *crispa*) from the unfiltered (top) and filtered (bottom) nutrient treatments (NUF/NF) on day 28 of the experiment.

Figure 4.9. Harvested Frillice lettuce (*Lactuca sativa* var. *crispa*) from the unfiltered (top) and filtered (bottom) waste water treatment (WUF/WF) on day 28 of the experiment.

Figure 4.10. Harvested Frillice lettuce (*Lactuca sativa* var. *crispa*) from the unfiltered (top) and filtered (bottom) sludge water treatment (SUF/SF) on day 28 of the experiment.

With respect to measurement (Table 4.3) there was no significant difference ($p > 0.2$) between lettuces from the nutrient treatments, and no significant difference ($p > 0.08$) between lettuces from the waste water treatments. However, the waste water was significantly different ($p < 0.02$) from the nutrient solutions.

Table 4.3. Measurements of different parameters of growth of harvested Frillice lettuce (*Lactuca sativa* var. *crispa*) for the unfiltered (WUF) and filtered (WF) waste water solutions, unfiltered (SUF) and filtered (SF) sludge water solutions, and unfiltered (NUF) and filtered (NF) nutrient soluions at the end of the experiment (N=3). Control plant from Kronheim Grønt is included.

		Waste water solutions				Nutrient solutions		
Parameter		WUF	WF	SUF	SF	NUF	NF	Control
height	cm	7.3	6.3	6.7	6.7	14.0	14.7	19
weight	g	34.0	28.3	25.2	33.3	315.2	264.5	-
sales weight	g	19.8	15.0	12.8	18.3	292.2	243.5	253.0
dry weight*	g	9.5	10.0	8.5	10.0	35.0	43.0	32.5
Color intensity	1 to 5	5	5	5	5	5	4	5
No. of leaves		15	15	15	16	24	24	19

Control = newly harvested plant from Kronheim Grønt, cultivated in a commercial nutrient solution

*Dry weight is based on only one value (N = 1)

The concentration of elements in lettuce from the nutrient treatments (Table 4.4) was close to the recommended values (R) and was used as a control for the lettuce from the waste water. The addition of sludge had a negligible effect on growth, and the results showed that the elemental composition of lettuce from the waste water was relatively similar. Potassium, magnesium and manganese were the only nutrients within the concentration range of NUF and NF. The concentration of toxic elements were generally above the range of NUF and NF, however they were all below the toxic limit. High concentrations of sodium and chloride were found in lettuce cultivated in waste water. Sodium was more than 10 times and chloride more than 5 times higher in the waste water than the upper range of 1 100 µg/L and 1 400 µg/L respectively of NUF and NF. The reasons for reduced growth are most likely a result of several factors, and both nutrient deficiency and nutrient toxicity are possible reasons for reduced growth.

Table 4.4. Concentration of elements in lettuce (dry weight) from the unfiltered (WUF) and filtered (WF) waste water treatments, unfiltered (SUF) and filtered (SF) sludge water treatments, and unfiltered (NUF) and filtered (NF) nutrient treatments at the end of the experiment divided into macro-, micro- and toxic elements (N=3). Recommended values (R) of element concentration in plant dry matter for optimal growth is included.

			Waste water treatments				Nutrient treatments	
Element		R	WUF	WF	SUF	SF	NUF	NF
	N	*1.5*	-	-	-	-	-	-
	P	*0.2*	0.18	0.14	0.15	0.19	0.26	0.32
Macro	K	*1.0*	0.67	0.53	0.56	0.64	0.44	0.83
(%)	S	*0.1*	0.06	0.05	0.06	0.06	0.11	0.14
	Ca	*0.5*	0.35	0.40	0.32	0.39	0.58	0.61
	Mg	*0.2*	0.13	0.12	0.12	0.15	0.15	0.11
	Fe	*100*	30	35	31	39	71	81
	Mn	*50*	57	79	63	75	110	48
Micro	Zn	*20*	9	9	9	12	26	20
(µg/g)	Cu	*6*	1.2	1.2	1.1	1.6	3.8	3.6
	B	*20*	33	43	34	39	39	29
	Mo	*0.1*	0.2	0.3	0.2	0.3	1.0	1.1
	Al	13	3.4	4.5	4.5	8.1	6.6	1.8
Toxic*	Cd	20	0.06	0.06	0.07	0.07	0.04	0.04
(µg/g)	Cr	10	0.41	0.57	0.26	0.14	0.17	0.18
	Ni	30	0.23	0.30	0.14	0.10	0.13	0.44
Other	Na		14 100	11 800	13 800	15 900	1 100	600
(µg/L)	Cl		7 500	7 700	10 200	11 200	1 400	1 300

*The recommended values for the toxic elements are upper critical concentrations that will cause a 10 % depression in yield.

The obvious differences in growth have already been presented above. Figure 4.11 gives an additional image of the difference in macronutrient concentration in the lettuces (recommended values, R, was included for comparison).

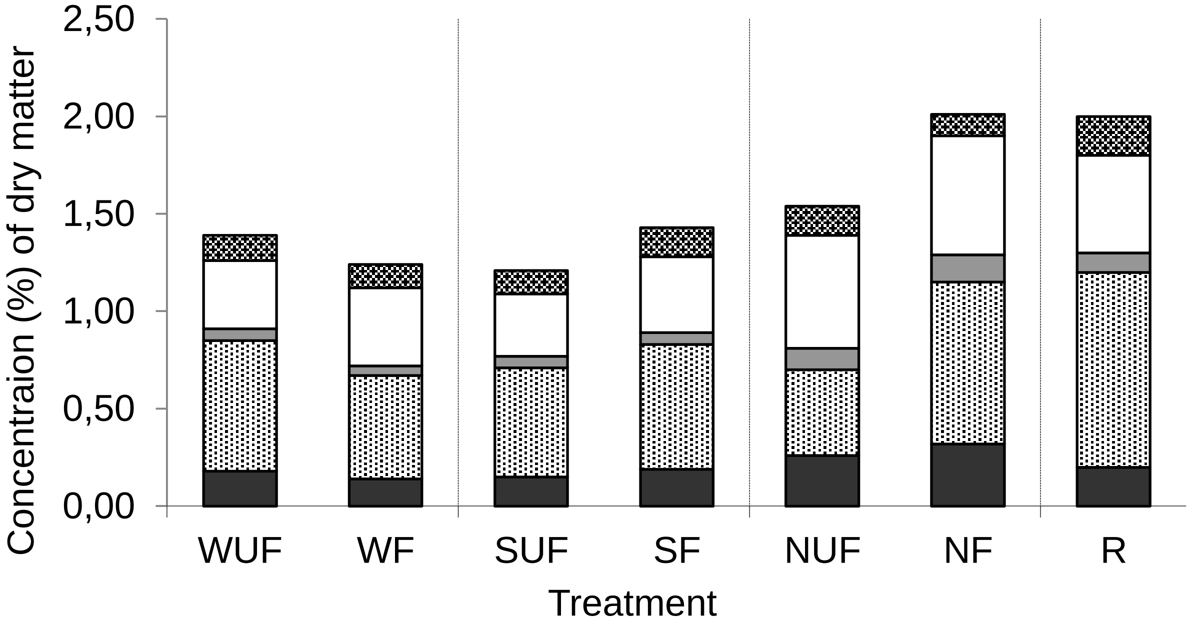


Figure 4.11. Amount (%) of macronutrients in lettuce from the unfiltered (WUF) and filtered (WF) waste water solutions, unfiltered (SUF) and filtered (SF) sludge water solutions, and unfiltered (NUF) and filtered (NF) nutrient soluions. Recommended amount (R) is included. Macronutrients are represented by phosphorus (dark grey), potassium (black dots), sulphur (light grey), calcium (white) and magnesium (grid).

4.3.1 Nutrient deficiency

Low concentrations of many nutrients have most likely contributed to an overall nutrient deficiency. Both macronutrients and micronutrients lead to a number of deficiency symptoms. The most striking symptoms were the pale mature leaves and the severe tip burn that was present on all lettuce cultivated in waste water. The lettuce may also have suffered from necrosis, but no analyses were done to prove this. Mobile (nitrogen, potassium) and moderately mobile (sulphur, magnesium) nutrients found in lower concentration than recommended in the lettuce have probably been moved from mature leaves to the center of the lettuce to yield healthy young leaves, hence resulting in sick mature leaves (Berry, 2010). Less mobile nutrient (calcium, boron) that are dependent on transpiration for uptake were most likely affected by dehydration (H. Marschner et al., 1996). Further evaluation of nutrient deficiency symptoms is beyond this thesis.

Nitrate was not measured in plant dry matter, and the availability or lack of nitrate in lettuce was not possible to discuss from the lettuce data. It is suggested that lettuce is typically not nitrate limited between nitrate concentrations of approximately 80 mg/L to 340 mg/L (Seawright et al., 1998). The concentration of nitrate in the waste water solutions were only 2 – 7 % of the lower limit of 80 mg/L giving reason to believe that nitrate was the limiting nutrient for growth. High FCR ratio of Atlantic salmon gives low concentrations of nitrate compared to other fish used in Aquaponics (Einen & Roem, 1997). Content of nitrate is highly affected by the feed and biomass in the fish tank, and the number of lettuce cultivated with waste water should correspond to the biomass. A full review of the fluctuations in waste water and sludge at Hardingsmolt throughout a year can be found in Appendix 17 and Appendix 18.

An additional experiment was conducted with lettuce from NUF and NF. A few plants from each treatment was taken out of the initial experiment after two weeks and irrigated with waste water with added nitrate (Figure 4.12). After 10 days, the lettuce did not appear as fresh and appealing compared to the control plant or lettuce continuously grown with the nutrient treatment. No analyzes was done, but the high concentration of salt in the waste water may explain the outcome.

Figure 4.12. Frillice lettuce (*Lactuca sativa* var. *crispa*) cultivated with waste water with added nitrate. Day 1 (right), day 5 (middle) and day 10 (left).

4.3.2 Nutrient toxicity:

High concentrations of boron can be toxic to lettuce, but concentrations were lower than the limit of 50 mg/L (McHargue & Calfee, 1933) and it was therefore disregarded as a reason for reduced growth.

The concentration of toxic elements in lettuce was lower than the limit values (R), except for sodium and chloride. The extremely high values of sodium and chloride did without a doubt effected the growth of lettuce cultivated in waste water. Build-up of salt in the root zone may explain why nutrients were not taken up adequately (Brown et al., 1999). It is very well documented that irrigation of vegetables with saline water will decrease both growth and water uptake (Kim et al., 2008; Romero-Aranda et al., 2001; Shannon & Grieve, 1998). It has been suggested that for some species ammonium can compete with sodium for root uptake and thereby minimize the sodium concentration in leaves (Ashraf et al., 2009). The high concentration of sodium and the lack of nitrate in the waste water make this less likely though.

Studies have found that salt tolerance differ between lettuce species, and romaine types have been found to be more salt tolerant than iceberg types (Shannon & Grieve, 1998). Also of interest is that iceberg lettuce irrigated with water having a salinity of 4400 µS/cm was not affected in a field study conducted in Israel. One suggestion is to try asparagus which is considered as the most salt-tolerant vegetable crop commercially available. This vegetable has proved to tolerate EC up to 4100 µS/cm. Green asparagus (*Asparagus officinalis* L.) is well fitted for the Norwegian climate, and the annual consumption is increasing (Vågen, 2005).

4.3.3 Soil

Nutrients present in the soil (Table 3.1) may be able to explain the normal growth of lettuce observed the first week of the experiment. Because soil is the main receiver of salt, due to limited uptake by lettuce, very high concentrations of sodium and chloride in the soil was seen. The use of soil instead of a soilless culture was just a matter of convenience. Soil is used to cultivate lettuce at Kronheim Grønt, thus lettuce in the experiment was cultivated in soil. Soil physiochemical characteristics can impose a limiting factor on the bioavailability of elements (Alloway, 1995). A disadvantage of using soil is that organic substrate will react with the nutrients in the water during irrigation (Olympios, 1999). Also the control of pH and EC is more difficult compared to soilless cultures. Use of a soilless production system like NFT would have provided more precise control of over plant nutrition.

4.4 Physiochemical characteristics of water

The nutrient solutions (NUF and NF) were within recommended values for optimal lettuce growth and were used as a control (Table 4.5). No parameters differed between the waste water solutions (WUF, WF, SUF and SF), but they were different from the nutrient solutions. Small variations in pH were observed due to reservoir change on day 8, 15 and 22 (Figure 4.13). The average oxygen level (day 1 excluded) was above 70 % during the experiment, thus not considered detrimental for lettuce. O_2 measurements from day 1 were excluded because the oxygen levels were too low due to bacterial activity in the system. This was adjusted by aerating the water at set time intervals. The salinity was higher in the waste water solutions than the nutrient solutions, and the electrical conductivity (EC) in the waste water exceeded the recommended value for plant fertilizer of 2500 µS/cm. The redox potential was generally lower in the waste water solutions than the nutrient solutions the whole period, but within the range of +100 to +400 mV regarding oxygenated water.

Table 4.5. Average value of measured physiochemical parameters measured in the unfiltered (WUF) and filtered (WF) waste water solutions, unfiltered (SUF) and filtered (SF) sludge water solutions, and unfiltered (NUF) and filtered (NF) nutrient soluions (N=20).

Parameters		Waste water solutions				Nutrient solutions	
		WUF	WF	SUF	SF	NUF	NF
pH		7.46	7.64	7.56	7.51	6.67	6.73
T_W	^oC	17.9	17.5	17.9	17.5	17.5	17.6
DO	%	71	82	76	71	86	91
EC	µS/cm	2600	2800	2700	2900	2200	2300
Salinity	%	1.3	1.4	1.4	1.5	1.1	1.2
E_h	mV	320	310	310	320	360	360

T_W = Water temperature, DO = Dissolved oxygen, EC = Electrical conductivity, E_h = Redox potential

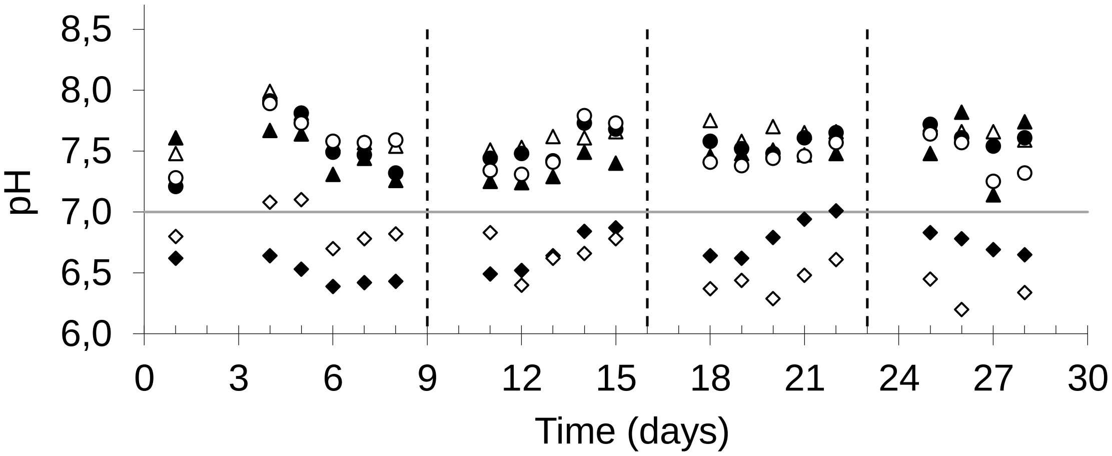


Figure 4.13. pH measurements from all six solutions (N=20). Change of reservoir indicated by vertical, dotted lines. Triangles are unfiltered (closed) and filtered (open) waste water (WUF/WF), circles are unfiltered (closed) and filtered (open) sludge water (SUF/SF), and diamonds are unfiltered (closed) and filtered (open) nutrient solution (NUF/NF). Recommended pH value is indicated by horizontal, grey line.

The pH value of all the solutions were within the recommended value of 6 to 8 for production of salmon (Bjerknes, 2007). However, it is possible that pH affected the bioavailability of nutrients in the waste water because the values were generally above the recommended value of 7 in aquaponics (Rakocy et al., 1997). An increasing trend in EC each week was observed. The change of water reservoir can explain why it appears as EC was lowered at the start of each week (Figure 4.14). By contrast the nutrient solutions EC was relatively stable throughout the experiment, even after the change of reservoir. This indicates similar rates of water uptake (and thereby nutrients) and evaporation from the reservoirs.

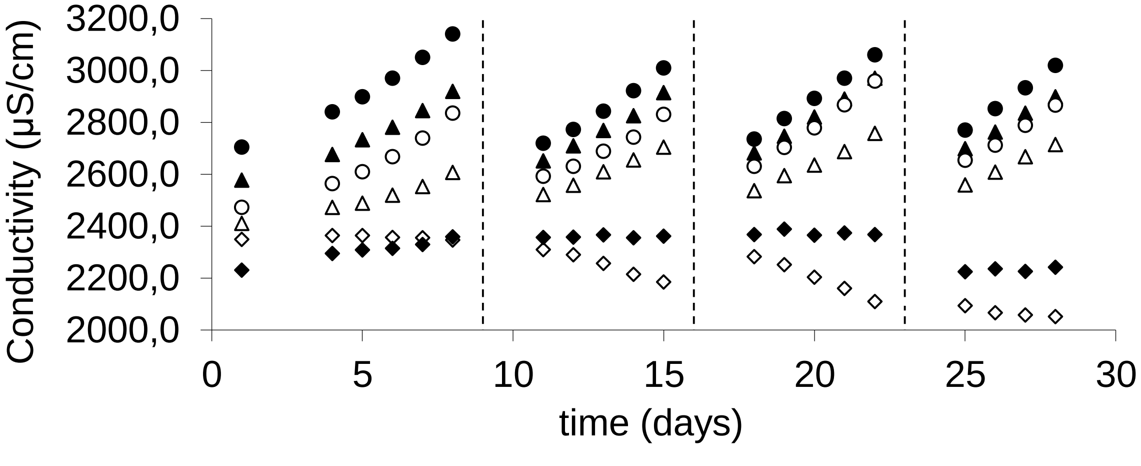


Figure 4.14. Electrical conductivity measurements in all six reservoirs (N=20). Change of reservoir indicated by vertical, dotted lines. Triangles are unfiltered (closed) and filtered (open) waste water (WUF/WF), circles are unfiltered (closed) and filtered (open) sludge water (SUF/SF), and diamonds are unfiltered (closed) and filtered (open) nutrient solution (NUF/NF). Recommended pH value is indicated by horizontal, grey line.

Because of water evaporation from the reservoir there was a concentration of salts (nutrients) in the waste water solutions (Pessarakli et al., 1989). Research have concluded that salinity levels above 2600 µS/cm reduce lettuce growth (Andriolo et al., 2005). This fits well with the average measured EC in all waste water solutions and gives more reason to believe that salt affected the plant growth.

4.5 Further study

Further research should be conducted with either a more salt tolerant plant, or a different batch of waste water. Because of the varying composition of the waste water according to biomass and feeding regime it would have been interesting to use waste water with a more suited nutrient composition.

Further experiments are required to clarify some of the hypotheses presented in this study. In particular evaluation of the character of the organic material could supply information about complexation and availability of nutrients and toxic metals.

A more detailed study on the effect of sludge should be considered, where continuous mixing of sludge is done in order to prevent sedimentation and to keep the nutrients dissolved in the solution. The effect of nitrate is also worth further studies. Nitrate could easily have been added to the waste water solutions and provided more valuable data of nutrient deficiency and suppressed growth.

5 Conclusion

The effect of elevated salinity (sodium, chloride and manganese) in the waste water, and increasing salinity during the experiments due to water evaporation, represents a major challenge. The usage of salt water in RAS-farming of smolt is in most cases necessary. One possible solution to this challenge is to use a more salt tolerant plant species or dilute the waste water with fresh water before irrigation of the plants. However the latter suggestion is not an economically or environmentally good solution because a dilution of salt will also dilute all the nutrients.

There was an efficient uptake of copper and zinc in lettuce which can prevent accumulation of these metals and allow re-use of water. Another treatment effect was the significant decrease in phosphorus. Although the concentration of phosphorus (and nitrate) most likely was insufficient for satisfying lettuce growth, the efficient uptake by lettuce can reduce the risk of eutrophication caused by release of waste water to the environment. Apparently the treatment effect of lettuce was satisfying for phosphorus, copper and zinc (and nitrate). This represents an inexpensive and alternative method for removing these particular elements.

The organic matter in the waste water increased and was found to be on mainly dissolved form, thus indicating removal neither by lettuce nor filter. This increase may reduce the bioavailability of toxic metals in the solution depending on the character of the organic matter, but it does not remove the metals from the water. The metals still represent a toxic potential to the fish if they are not removed by water treatment such as skimming and biological filtration.

The rock wool filter did not appear to have any beneficial effect, and neither metals nor organic matter was removed from the solution. However the filter revealed some unfortunate effects because aluminum, iron and silicon were released to all the filtered solutions. Both aluminum and iron are unwanted in farming of smolt and also unfortunate for the environment because they represent a toxic hazard to fish. It is not known if silicon is a problem in farming of smolt, but high concentrations of silicon may contribute to eutrophication if the waste water is discharged to the natural waterways. It is concluded that rock wool filters should not be used in aquaponics.

Some of the physiochemical parameters were within the recommended range for growing lettuce, but the pH and the electrical conductivity were both too high. The lettuce analysis revealed that nutrients had been taken up, thus indicating that they were on bioavailable form. The addition of sludge appeared to have no effect on growth, but as already discussed this was a result of sedimentation of sludge on the bottom of the reservoirs. Several reasons may be suggested to have affected nutrient uptake and lettuce growth. (1) The uptake of nutrients was affected by the high pH value and should have been adjusted to a pH of 7 or a bit lower. (2) There were inadequate amounts of nutrients present in the waste water solutions from the beginning to yield satisfying growth. (3) The high amounts of salts in the waste water solutions inhibited the uptake of nutrients. It is concluded that the overall depression in growth was a result of all these factors working in concert and that the waste water used is not suited for yielding marketable lettuce. Water samples from two production cycles at Hardingsmolt AS revealed that the waste water composition was highly dependent on stocking density, feeding and season. It is reason to believe that the outcome would have been different if the waste water had been collected at another time of the year. Still, these fluctuations will have to be dealt with if aquaponics are implemented.

6 Bibliography

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7 Appendix

Appendix 1 Total concentration (µg/L) of elements in water samples from the unfiltered waste water solution (WUF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	1 988	15 421	34 122	55 023	39 656	28.8	17.8	12.5	11.3	151	1.99	28.9	0.04	0.21	1.1	283 588	727 007	598
24.9.	1 623	13 013	32 712	57 700	36 899	28.5	18.4	13.7	13.1	152	5.00	21.6	0.04	0.21	1.2	303 285	713 916	583
28.9.	1 101	8 053	38 073	64 091	41 930	27.4	5.5	15.9	12.1	152	10.29	10.0	0.04	0.21	1.5	290 187	764 850	682
1.10.	1 106	11 386	33 415	61 691	39 405	10.2	1.2	12.0	4.0	153	4.21	4.7	0.01	0.12	0.9	294 628	733 670	534
5.10.	612	3 981	38 427	66 339	43 161	27.1	1.0	13.4	5.1	147	7.16	7.3	0.03	0.39	1.2	289 073	851 653	315
8.10.	1 254	8 745	33 969	59 461	42 903	19.3	0.6	9.3	3.8	158	2.39	9.2	0.02	0.16	0.9	291 892	817 236	498
12.10.	494	3 391	38 395	68 329	42 131	32.2	0.8	9.9	5.7	154	4.54	6.8	0.04	0.15	1.3	291 892	836 844	376
15.10.	1 015	8 399	35 195	61 856	43 223	18.7	0.6	9.7	4.6	165	2.05	15.1	0.03	0.21	1.1	293 836	801 545	516
18.10.	497	4 499	36 691	63 405	42 235	23.5	0.6	9.0	2.9	144	3.22	7.9	0.03	0.15	1.1	299 573	789 458	403

Appendix 2. Total concentration (µg/L) of elements in water samples (w/0.45 µm) from the unfiltered waste water solution (WUF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	1 875	15 060	34 530	56 108	38 932	23.2	13.4	11.5	10.7	155	2.17	24.1	0.03	0.20	1.1	282 409	674 314	601
24.9.	1 841	18 163	33 739	56 537	37 753	34.3	16.1	17.1	14.5	182	5.51	13.5	0.04	0.22	1.5	362 121	723 316	702
28.9.	984	7 256	37 095	60 984	38 010	23.4	2.0	16.1	11.3	149	9.93	7.8	0.04	0.20	1.4	310 061	800 017	716
1.10.	1 164	11 652	34 864	59 990	39 838	10.7	1.7	11.3	4.1	150	4.17	6.9	0.01	0.14	0.9	319 903	750 002	515
5.10.	574	4 075	41 753	63 504	42 652	29.0	0.8	12.1	5.3	145	7.10	4.5	0.03	0.18	1.3	304 185	772 037	307
8.10.	1 261	8 408	33 673	59 515	41 208	18.6	0.4	8.9	3.6	156	2.31	9.0	0.03	0.16	1.0	301 079	733 116	521
12.10.	482	3 460	37 190	67 300	44 991	33.5	0.6	11.1	5.5	154	4.21	7.1	0.04	0.21	1.2	300 430	824 990	376
15.10.	1 001	8 123	33 686	64 529	41 554	16.6	0.5	9.6	4.9	153	2.04	9.3	0.03	0.19	1.0	313 807	778 115	477
18.10.	457	4 607	35 592	65 081	44 206	22.2	0.4	9.3	2.6	163	3.23	6.8	0.03	0.14	1.0	292 084	864 162	388

Appendix 3. Total concentration (µg/L) of elements in water samples from the filtered waste water solution (WF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	2 031	18 600	38 754	68 787	44 177	22.4	17.4	12.0	13.5	175	3.42	50.0	0.04	0.17	2.5	287 076	761 857	1 186
24.9.	1 469	15 223	37 582	62 345	39 401	34.5	14.9	11.8	13.7	166	7.55	82.1	0.05	0.16	2.7	283 588	763 230	1 579
28.9.	913	9 342	43 776	75 715	44 052	26.4	2.9	12.4	13.9	166	13.61	82.3	0.06	0.12	3.8	287 795	849 902	2 387
1.10.	1 296	11 472	37 990	63 503	41 285	18.5	0.5	8.6	7.3	154	5.16	58.6	0.03	0.15	1.7	290 751	783 670	1 188
5.10.	972	7 189	43 725	73 214	46 071	39.5	2.3	11.4	7.9	163	9.26	99.8	0.04	0.14	2.8	291 129	873 628	1 539
8.10.	1 250	10 337	37 478	64 711	44 462	23.2	0.3	8.3	4.7	172	3.99	79.0	0.03	0.14	1.4	293 052	874 238	972
12.10.	612	5 197	44 869	82 469	50 700	40.0	0.5	10.2	6.3	187	6.84	91.0	0.03	0.12	2.1	292 663	1 028 337	1 187
15.10.	942	8 962	36 194	69 047	45 912	28.4	0.7	8.3	4.8	171	3.24	77.4	0.02	0.15	1.2	329 487	807 359	832
18.10.	457	5 569	42 227	78 029	49 384	32.9	0.3	7.9	3.9	174	5.37	92.6	0.03	0.15	1.9	289 257	930 386	976

Appendix 4. Total concentration (µg/L) of elements in water samples (w/0.45 µm) from the filtered waste water solution (WF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	1 869	18 048	38 338	64 977	44 701	17.0	13.3	11.5	13.3	169	3.60	48.2	0.04	0.15	2.4	291 892	806 105	1 147
24.9.	1 515	16 230	39 882	70 028	42 906	18.6	9.3	11.5	14.4	180	7.95	63.4	0.05	0.12	2.9	300 000	835 888	1 699
28.9.	905	9 913	43 171	78 609	44 574	26.7	2.2	12.3	14.2	171	13.88	82.2	0.06	0.12	3.6	290 751	855 080	2 434
1.10.	1 291	11 219	37 839	59 834	41 928	17.5	0.3	9.3	7.2	165	5.43	57.8	0.03	0.17	1.8	289 628	821 044	1 257
5.10.	762	6 964	41 945	70 219	45 535	32.2	0.3	10.1	7.4	162	8.59	101.9	0.03	0.15	2.2	289 073	934 307	1 599
8.10.	1 236	10 040	38 374	63 500	43 919	22.4	0.2	8.4	4.9	168	3.69	79.4	0.03	0.14	1.3	290 374	873 997	974
12.10.	558	5 261	41 972	77 317	48 191	38.4	0.2	9.2	6.1	171	6.25	86.3	0.03	0.12	1.9	301 954	912 335	1 186
15.10.	933	8 866	36 783	68 457	44 081	23.2	0.2	8.1	4.5	179	3.10	69.1	0.03	0.15	1.3	295 228	863 324	888
18.10.	426	5 377	39 665	72 591	49 655	31.7	0.2	8.7	3.7	166	4.90	88.8	0.03	0.13	1.6	291 509	986 592	915

Appendix 5. Total concentration (µg/L) of elements in water samples from the unfiltered sludge water solution (SUF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	2 549	15 840	34 929	57 615	39 438	34.7	21.5	16.9	4.9	160	2.7	14.9	0.0	0.2	0.9	293 247	759 966	596
24.9.	2 284	12 762	33 927	61 710	38 469	25.7	15.6	18.4	5.1	157	7.3	8.6	0.0	0.2	0.9	283 758	782 270	654
28.9.	1 329	9 681	36 660	57 399	40 464	17.6	1.9	9.7	5.6	167	3.9	13.3	0.0	0.2	1.0	314 577	785 373	523
1.10.	1 749	8 317	37 207	72 227	41 252	25.0	4.6	21.6	5.5	152	13.6	3.5	0.1	0.2	1.0	377 766	888 186	617
5.10.	1 012	6 717	39 313	69 121	41 762	18.8	1.0	13.8	3.3	141	7.4	4.5	0.0	0.1	1.0	288 522	833 650	440
8.10.	1 818	10 845	33 738	65 549	39 696	17.5	1.7	8.9	3.2	154	2.5	6.8	0.0	0.1	0.8	301 079	786 283	529
12.10.	1 603	6 803	41 465	78 681	47 710	24.5	1.3	12.6	5.4	168	5.5	2.9	0.0	0.1	1.0	290 000	982 148	367
15.10.	2 776	11 050	32 774	69 980	47 705	48.4	5.5	60.6	5.4	167	2.1	27.0	0.2	0.2	0.9	291 129	857 719	422
18.10.	930	7 426	37 222	68 966	48 076	21.7	0.9	8.8	2.3	157	4.1	4.4	0.0	0.1	0.9	310 061	871 104	378

Appendix 6 Total concentration (µg/L) of elements in water samples (w/0.45 µm) from the unfiltered sludge water solution (SUF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	2 096	15 549	34 604	58 717	38 882	19.9	14.7	12.1	3.7	156	2.6	7.7	0.0	0.2	0.7	287 615	701 462	616
24.9.	2 443	14 987	35 936	64 037	39 545	34.2	17.8	18.6	6.4	180	7.7	5.4	0.0	0.2	1.1	292 469	790 899	627
28.9.	1 381	10 688	32 871	57 135	37 968	26.3	4.2	11.2	6.3	150	3.9	9.9	0.0	0.2	1.0	308 371	723 735	576
1.10.	1 861	7 990	39 519	70 199	40 967	25.4	6.2	24.4	5.4	153	14.3	5.2	0.0	0.2	1.1	295 228	868 464	551
5.10.	923	6 922	40 509	71 239	40 873	18.6	0.6	12.6	3.2	139	7.1	3.5	0.0	0.1	1.0	289 257	876 463	491
8.10.	1 664	10 637	32 919	63 168	40 930	15.4	1.3	9.1	3.3	150	2.4	2.6	0.0	0.1	0.8	355 078	798 778	482
12.10.	1 516	6 376	40 084	80 761	46 477	23.5	0.9	11.9	5.8	162	5.7	2.2	0.0	0.1	1.0	302 616	943 041	383
15.10.	1 109	11 100	35 834	63 450	45 080	14.3	0.5	6.0	3.2	164	2.2	4.7	0.0	0.1	0.7	289 814	774 293	609
18.10.	901	7 320	35 844	71 768	45 492	19.6	0.5	8.2	2.1	157	4.2	3.0	0.0	0.3	0.9	291 509	883 527	445

Appendix 7. Total concentration (µg/L) of elements in water samples from the filtered sludge water solution (SF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	1 984	17 960	37 641	69 498	43 446	20.0	8.1	10.8	15.0	173	4.1	18.9	0.0	0.1	6.4	285 135	756 608	1 127
24.9.	1 695	14 423	39 005	74 460	42 571	17.2	3.4	12.0	14.6	171	8.9	28.1	0.0	0.1	6.7	336 501	756 075	1 560
28.9.	1 076	11 435	38 119	68 051	41 077	15.4	2.9	12.3	7.5	150	5.9	16.7	0.0	0.1	3.1	291 509	762 028	1 108
1.10.	1 306	9 076	43 839	80 540	47 471	20.1	2.3	16.1	14.6	180	17.0	44.9	0.1	0.1	7.7	318 539	948 623	2 241
5.10.	934	6 551	43 043	75 395	45 046	26.5	1.0	14.4	6.1	151	9.1	53.7	0.0	0.1	3.5	291 509	902 382	1 288
8.10.	1 587	10 781	37 864	72 146	46 095	24.4	2.6	12.0	5.3	162	3.6	15.3	0.0	0.1	1.9	290 000	875 947	1 022
12.10.	1 342	6 612	44 113	86 078	50 843	34.0	1.8	14.3	5.9	167	6.7	36.6	0.0	0.1	2.3	300 000	1 001 270	1 252
15.10.	1 742	9 599	37 155	76 649	45 984	42.9	4.4	36.2	5.5	168	2.8	32.2	0.1	0.1	1.6	295 833	824 661	1 028
18.10.	836	5 800	43 049	86 356	51 206	30.9	1.0	13.1	4.0	169	4.8	45.1	0.0	0.1	2.0	289 628	956 843	1 251

Appendix 8 Total concentration (µg/L) of elements in water samples (w/0.45 µm) from the filtered sludge water solution (SUF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	1 903	17 058	38 045	70 413	44 312	15.2	6.2	9.6	14.8	174	4.3	12.0	0.0	0.1	6.1	286 897	806 845	1 195
24.9.	1 762	14 707	39 847	68 060	43 538	16.2	2.7	11.5	14.7	171	9.5	24.8	0.0	0.1	6.5	295 833	802 732	1 713
28.9.	1 058	11 439	37 994	71 601	41 779	14.2	2.6	12.9	7.5	158	6.2	11.9	0.0	0.1	3.1	289 628	821 976	1 179
1.10.	1 236	9 231	43 572	80 735	47 635	19.8	1.4	15.6	14.3	170	16.8	36.7	0.1	0.1	7.5	352 312	938 317	2 341
5.10.	934	6 443	42 818	77 586	43 448	25.7	0.7	14.2	6.5	150	9.2	52.8	0.0	0.1	3.5	290 562	927 368	1 257
8.10.	1 463	10 336	36 009	69 783	43 827	22.1	2.0	11.4	5.0	163	3.5	12.5	0.0	0.1	2.0	290 562	824 452	1 038
12.10.	1 247	5 899	42 878	85 783	49 209	32.4	1.5	13.5	5.8	160	6.6	35.9	0.0	0.1	2.5	290 940	1 014 565	1 236
15.10.	879	9 361	37 521	73 586	45 617	22.6	1.8	8.3	4.4	158	3.0	16.2	0.0	0.1	1.5	291 319	862 390	1 076
18.10.	743	6 329	39 636	83 399	49 237	27.5	0.6	9.2	3.5	162	4.6	40.4	0.0	0.1	1.8	291 129	978 647	1 271

Appendix 9. Total concentration (µg/L) of elements in water samples from the unfiltered nutrient solution (NUF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	53 906	189 514	33 904	224 560	21 451	1 682	663	237	177	393	47	39.0	0.5	0.8	61.3	7 367	9 631	620
24.9.	53 398	188 272	34 808	218 981	22 115	1 659	595	501	176	379	38	14.7	0.6	0.7	55.6	8 104	10 710	691
28.9.	52 565	198 039	33 453	206 450	19 598	1 587	599	320	138	386	48	18.6	0.2	0.6	134.7	7 230	11 990	591
1.10.	52 853	193 333	32 973	231 226	22 064	1 699	564	809	169	373	37	8.0	0.7	0.8	56.1	8 766	13 515	780
5.10.	49 962	192 838	36 807	229 477	21 144	1 795	536	403	162	401	55	21.0	0.2	0.6	160.8	8 869	4 627	741
8.10.	50 490	207 692	32 966	232 331	21 312	1 678	560	261	163	410	51	22.4	0.2	0.6	67.5	8 068	8 817	930
12.10.	47 127	194 340	37 291	289 749	22 304	2 088	396	353	212	461	59	12.3	0.2	0.6	102.0	8 629	5 147	904
15.10.	52 269	193 333	33 693	239 566	20 538	1 824	530	255	151	418	55	24.3	0.1	0.6	69.2	7 394	5 772	819
18.10.	52 654	152 408	43 495	283 927	23 164	2 200	464	318	189	490	56	10.9	0.2	0.7	103.2	8 440	1 976	1 088

Appendix 10 Total concentration (µg/L) of elements in water samples (w/0.45 µm) from the unfiltered nutrient solution (NUF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	52 582	194 980	34 078	221 720	21 586	1 656	640	244	182	387	47	14.5	0.5	0.5	63.5	7 072	9 671	634
24.9.	51 889	189 059	34 266	217 259	21 437	1 635	597	504	182	391	40	10.0	0.6	0.6	56.1	7 748	10 694	650
28.9.	52 216	194 213	34 861	215 591	20 160	1 618	594	326	142	388	50	8.2	0.2	0.5	142.2	7 303	11 568	654
1.10.	50 890	241 700	32 418	213 393	20 972	1 643	541	790	161	386	36	4.7	0.7	0.7	54.9	9 071	15 651	749
5.10.	48 749	207 034	34 447	231 747	20 595	1 788	515	393	158	405	51	4.5	0.2	0.6	153.1	9 001	3 802	734
8.10.	49 225	198 870	33 921	240 274	20 407	1 672	531	272	153	413	51	7.7	0.2	0.5	72.2	7 171	6 801	688
12.10.	48 096	195 238	41 033	295 393	21 940	2 158	398	350	208	473	63	4.6	0.3	0.6	100.2	9 349	4 246	865
15.10.	51 811	197 357	33 822	231 098	21 308	1 692	545	247	157	412	52	7.9	0.2	0.5	66.1	7 196	5 345	777
18.10.	56 319	154 230	46 300	300 837	24 352	2 323	507	346	195	502	60	7.7	0.2	0.7	107.1	8 667	182	1 134

Appendix 11. Total concentration (µg/L) of elements in water samples from the filtered nutrient solution (NF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	40 885	189 840	29 989	198 167	19 921	1 494	501	192	176	358	45	17.4	0.1	0.4	6.1	7 170	9 462	3 258
24.9.	37 220	238 614	33 710	209 527	22 924	1 584	472	193	194	389	45	8.0	0.2	0.4	8.3	9 917	13 268	7 088
28.9.	46 967	194 595	35 251	220 170	21 200	1 631	589	230	158	401	54	10.4	0.2	0.4	6.5	7 913	8 707	4 384
1.10.	31 191	207 527	35 927	230 271	25 517	1 714	525	214	207	406	48	8.8	0.2	0.5	12.3	11 360	14 756	12 459
5.10.	42 750	246 444	37 565	247 668	24 348	1 953	592	254	181	425	56	45.6	0.2	0.7	11.4	10 000	11 212	7 838
8.10.	47 528	251 844	31 751	238 216	21 658	1 663	557	223	155	404	52	29.2	0.1	0.5	5.5	7 369	11 034	2 793
12.10.	48 341	197 357	42 097	283 328	24 195	2 067	486	199	183	474	58	9.7	0.1	0.6	12.2	9 050	10 741	5 743
15.10.	51 645	194 723	36 133	244 350	21 336	1 786	585	185	156	415	51	14.0	0.1	0.5	7.6	7 738	7 537	2 726
18.10.	46 861	184 085	42 083	252 149	22 934	1 798	498	104	146	435	53	5.8	0.1	0.5	13.3	7 448	5 754	4 341

Appendix 12. Total concentration (µg/L) of elements in water samples (w/0.45 µm) from the filtered nutrient solution (NF) from the project period 21.09.2012 - 18.10.2012.

	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo	Al	Cd	Cr	Ni	Na	Cl	Si
21.9.	43 211	189 059	31 776	210 532	21 126	1 542	512	200	184	367	46	10.5	0.1	0.4	6.6	7 538	9 777	3 519
24.9.	36 409	212 721	32 396	203 604	22 264	1 521	463	192	189	373	45	5.6	0.2	0.4	8.3	8 582	10 851	6 971
28.9.	48 046	192 470	35 506	221 051	21 674	1 692	617	224	152	390	52	7.4	0.2	0.5	6.5	8 121	10 478	4 358
1.10.	30 277	191 864	36 113	219 369	25 179	1 691	509	207	201	391	48	3.6	0.2	0.4	12.2	10 626	12 817	12 085
5.10.	40 948	219 048	37 239	241 833	23 491	1 805	564	248	174	420	55	5.3	0.2	0.5	10.7	9 442	10 089	7 513
8.10.	48 519	244 330	33 711	235 314	21 602	1 691	594	239	164	427	55	9.5	0.1	0.5	5.9	7 965	9 551	3 020
12.10.	49 932	195 368	41 042	284 337	24 849	2 033	505	198	205	488	61	5.8	0.1	0.6	12.6	9 581	8 890	5 726
15.10.	50 764	193 834	36 315	256 524	22 874	1 845	585	186	155	448	54	9.0	0.1	0.5	7.9	7 447	7 586	2 596
18.10.	52 304	196 154	44 355	299 629	25 359	1 957	560	117	160	500	60	7.1	0.1	0.6	14.8	8 507	3 848	5 088

Appendix 13. Measured UV, calculated SUVA and concentration total and dissolved organic material in the unfiltered (WUF) and the filtered (WF) waste water solution.

	Waste water solutions											
	WUF			WUF (w/0.45 um)			WF			WF (w/0.45 um)		
Date	UV	SUVA	TOC	UV	SUVA	DOC	UV	SUVA	TOC	UV	SUVA	DOC
21.9.	0.12	2.11	5.50	0.09	1.77	4.98	0.12	1.85	6.34	0.10	1.59	6.04
24.9.	0.16	1.98	8.12	0.14	2.41	5.76	0.16	1.95	7.99	0.14	2.53	5.70
28.9.	0.26	3.01	8.51	0.24	2.95	7.98	0.28	2.59	10.96	0.22	2.75	8.06
1.10.	0.16	1.84	8.93	0.14	1.65	8.24	0.19	2.13	9.05	0.14	1.95	6.98
5.10.	0.24	1.88	12.79	0.23	2.65	8.60	0.22	1.92	11.65	0.22	2.34	9.31
8.10.	0.15	1.60	9.17	0.14	1.76	8.12	0.15	1.70	9.04	0.14	1.94	7.23
12.10.	0.22	1.89	11.35	0.22	1.82	12.00	0.25	1.98	12.62	0.21	1.92	10.94
15.10.	0.16	1.91	8.10	0.15	1.88	7.76	0.15	2.01	7.36	0.14	1.72	8.03
18.10.	0.20	1.68	12.09	0.19	1.94	9.55	0.26	2.05	12.66	0.19	1.91	10.01

Appendix 14. Measured UV, calculated SUVA and concentration total and dissolved organic material in the unfiltered (SUF) and the filtered (SF) sludge water solution.

	Sludge water solutions											
	SUF			SUF (w/0.45 um)			SF			SF (w/0.45 um)		
Date	UV	SUVA	TOC	UV	SUVA	DOC	UV	SUVA	TOC	UV	SUVA	DOC
21.9.	0.13	1.92	6.51	0.10	1.78	5.62	0.12	1.68	7.21	0.10	1.71	5.86
24.9.	0.17	1.89	9.06	0.15	2.51	5.94	0.16	1.72	9.32	0.14	2.43	5.80
28.9.	0.25	2.03	12.17	0.21	2.75	7.79	0.23	2.11	11.08	0.20	2.50	7.92
1.10.	0.15	1.99	7.45	0.13	2.01	6.48	0.17	1.71	9.66	0.13	1.88	6.69
5.10.	0.19	1.96	9.55	0.20	2.22	8.87	0.18	1.59	11.45	0.18	2.24	7.82
8.10.	0.21	0.00	9.70	0.14	1.98	6.96	0.15	1.44	10.09	0.13	1.74	7.57
12.10.	0.22	1.62	13.66	0.19	1.65	11.48	0.25	1.93	12.82	0.18	1.73	10.42
15.10.	0.15	1.82	8.00	0.17	1.89	8.95	0.20	1.93	10.25	0.14	1.64	8.76
18.10.	0.23	1.60	14.27	0.20	1.80	11.29	0.22	1.74	12.68	0.19	1.83	10.32

Appendix 15. Measured UV, calculated SUVA and concentration total and dissolved organic material in the unfiltered (NUF) and the filtered (NF) nutrient solution.

	Nutrient solutions											
	NUF			NUF (w/0.45 um)			NF			NF (w/0.45 um)		
Date	UV	SUVA	TOC	UV	SUVA	DOC	UV	SUVA	TOC	UV	SUVA	DOC
21.9.	0.37	3.55	10.37	0.34	3.30	10.28	0.35	3.16	10.93	0.30	3.02	9.85
24.9.	0.31	2.55	12.19	0.40	3.94	10.24	0.37	3.07	12.10	0.36	3.35	10.73
28.9.	0.48	3.16	15.17	0.46	3.70	12.31	0.43	3.16	13.73	0.42	3.45	12.20
1.10.	0.38	3.30	11.36	0.37	3.05	12.10	0.38	3.20	11.99	0.38	3.28	11.53
5.10.	0.42	2.82	14.98	0.18	2.72	6.55	0.44	2.79	15.90	0.20	2.70	7.30
8.10.	0.38	2.93	13.04	0.41	3.71	11.11	0.39	2.81	13.85	0.39	3.48	11.16
12.10.	0.53	2.74	19.35	0.16	2.12	7.44	0.25	3.10	8.13	0.50	3.14	15.79
15.10.	0.40	3.20	12.40	0.37	3.08	12.14	0.40	3.74	10.70	0.38	3.73	10.19
18.10.	0.53	3.09	17.04	0.32	3.08	10.42	0.46	3.26	13.96	0.30	3.21	9.36

Appendix 16. Results from the monitoring program from Hardingsmolt AS. Values concerning water input and output, biomass and fish size, feed and amount of sludge produced. Water collected for this experiment is colored in grey.

		Flow through	Total biomass	Fish size	No. of fish	Feed/day	New water			Rinse water	Sludge production/day (theoretical)	
Date	Sample	L/min	kg	g (mean)		kg	L/min	L/kg fôr	%	L/min	kg sludge w/10% TS (1.75 kg sludge/kg fôr)	kg TS (15% av feed)
15.11.11	1	max 500	59 390	47.0	1 259 068	822	540	946	39	150	1 439	123
29.02.12	2	max 500	97 426	97.0	1 049 667	761	575	1 088	41	150	1 332	114
14.03.12	3	max 500	105 361	101.0	1 038 359	427	720	2 428	52	150	747	64
28.03.12	4	max 500	97 298	103.0	945 533	142	840	8 518	60	150	249	21
20.06.12	5	max 500	16 397	12.0	1 355 843	703	336	688	24	150	1 230	105
09.07.12	6	max 500	30 807	22.0	1 391 710	951	312	472	22	150	1 664	143
25.07.12	7	max 500	51 069	37.0	1 380 045	861	300	502	22	150	1 507	129
15.08.12	8	max 500	63 328	47.0	1 343 314	870	320	530	23	150	1 523	131
04.09.12	9	max 500	88 418	68.0	1 283 098	720	660	1 320	48	150	1 260	108
19.09.12	10	max 500	34 866	45.0	775 249	574	510	1 279	37	150	1 005	86

Appendix 17. Values of different parameters, compounds and metals in the waste water from Hardingsmolt AS. Waste water collected for this experiment is colored in grey.

	pH	Kond	TURB	ALK	TOC	CO2	NH4-N	Cl	SO4	Tot-P	Tot-N	K	Ca	Mg	Na
Date		*mS/m*	*FTU*	*mmol/L*	*mg C/L*	*mg/L*	*µg N/L*	*mg/L*							
15.11.11	6.8	244	0.9	0.6	6.0		210	840	116	3.2	24	15	58	44	360
29.02.12	6.3	2 450	3.7	0.6	8.8	25.7	650	9 400	1 300	4.0	26	140	220	550	4 700
14.03.12	7.2	2 360	1.7	2.1	4.5	7.3	450	2 700	450	2.2	18	96	210	510	4 500
28.03.12	*7.2*	*1 980*	*3.4*	*2.6*	*4.3*	*8.4*	*510*	*6 900*	*920*	*0.7*	*14*	*140*	*200*	*470*	*3 900*
20.06.12	7.4	233	0.3	1.2	3.6	11.7	120	660	98	1.7	30	17	79	42	350
09.07.12	7.7	263	2.1	2.3	7.0	13.6	310	650	100	3.8	39	17	130	46	380
25.07.12	7.4	175	1.0	3.1	6.4	19.5	1 000	340	64	3.9	54	14	140	26	220
15.08.12	7.3	297	0.8	2.3	4.6	12.8	1 900	970	130	2.6	22	22	100	63	520
04.09.12	7.3	326	1.7	2.6	11.0		620	920	130	4.8	71	22	130	60	480
19.09.12	7.6	207	0.4	1.6	3.3		11	620	97	2.2	20	16	64	45	350
06.11.12	7.7	290	0.8	1.9	4.4		9	810	120	2.8	20	20	82	57	460
04.12.12	7.6	462	0.6	1.9	3.5		1 200	1 400	200	2.8	19	32	92	99	800

	Cu	Cr	Zn	Al	B	Fe	Mn	Mo	Si	PO4-P
Date	*µg/L*	*µg/L*	*µg/L*	*µg/L*	*µg/L*	*µg/L*	*µg/L*	*µg/L*	*µg/L*	*µg/L*
15.11.11	2.80		12	17	180	25	1.8	0.9	550	2600
29.02.12	<1	12.0	37	20	2100	510	13.0	<5	370	3500
14.03.12	1.8	<1	16	15	1600	170	6.0	<5	330	490
28.03.12	*<3*	*<1*	*16*	*16*	*1600*	*<50*	*<5*	*5.1*	*390*	*710*
20.06.12	<3	<1	11	110	150	<50	<5	<1	340	1600
09.07.12	<3	<1	15	26	160	<50	<5	<1	180	3700
25.07.12	<3	<1	16	10	91	<50	<5	<1	340	3700
15.08.12	<3	<1	12	11	200	<50	<5	<1	<2	2500
04.09.12	<3	<1	21	28	230	<50	<5	1	420	3800
19.09.12	<3	<1	7	40	160	<50	<5	<1	620	1700
06.11.12	<3	<1	11	8	180	<50	<5	<1	620	890
04.12.12	<3	<1	10	15	340	<50	<5	1	600	2600

Appendix 18. Values of different parameters, compounds and metals in the sludge from Hardingsmolt AS. Sludge collected for this experiment is colored in grey.

	DM	Al	B	Ca	Cl	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Si	Zn	Pb	Cd	Ni
Date	%	mg/kg DM																
29.02.12	40	120	46.0	85000	14800	3.3	<2	740	1800	2500	80	<2.5	62000	250	220	1.9	0.7	<2.5
14.03.12	31	140	31.0	44000	16000	2.3	2.9	370	2600	1700	49	<3.3	70000	340	180	<1.7	0.4	<3.3
28.03.12	22	190	31.0	92000	14900	1.6	8.2	800	1100	5000	51	<4.7	13000	380	320	<2.4	0.7	<4.7
20.06.12	30	240	5.4	130000	1360	1.8	7.3	1800	470	2400	96	<3.4	2100	330	440	<1.7	1.4	<3.4
09.07.12	35	180	7.2	110000	1100	1.7	5.9	830	250	3300	85	<2.9	2500	200	380	1.7	1.0	<2.9
25.07.12	37	340	8.8	240000	496	1.1	5.9	990	300	9400	190	<2.7	2800	270	800	2.0	2.0	<2.7
15.08.12	40	280	9.5	210000	1400	3.2	3.4	1200	330	13000	130	<2.6	2700	210	560	<1.3	1.6	<2.6
04.09.12	48	240	7.5	240	1030	2.4	4.5	2400	230	5500	120	<2.1	2000	230	420	<1.1	1.3	<2.1
19.09.12	36	450	<28	240000	1320	3.8	8.3	2100	310	6600	130	0.4	2100	260	580	2.1	1.3	<2.8
06.11.12	19	390	<52	150000	5510	2.1	11.0	1200	640	4800	180	0.6	4900	320	770	<3.6	2.2	<5.2
04.12.12	22	260	<46	120000	5840	47.0	6.8	1100	550	3200	130	<0.46	4500	250	450	<2.3	1.4	17.0

	DM	pH	SO4	NH4-N	PO4-P	NO3-N	P	Tot-N	TOC	ALK
Date	%		mg/kg DM					*g/100 g DM*	*g/kg DM*	*mmol/L*
29.02.12	40	7.2	3 500	8 900	250	<0.25	92 000	4.0	190	0.0
14.03.12	31	6.7	3 900	3 100	220	1	66 000	5.8	240	11.4
28.03.12	22	6.7	2 300	16 000	790	<0.47	33 000	7.3	-	16.1
20.06.12	30	6.0	2 700	5 700	590	370	43 000	5.0	-	13.0
09.07.12	35	7.1	460	3 800	110	34	51 000	3.1	-	11.2
25.07.12	37	7.2	800	4 400	75	42	82 000	3.0	-	12.0
15.08.12	40	7.0	550	3 200	76	72	73 000	2.1	120	14.5
04.09.12	48	7.1	310	1 900	33	48	55 000	2.2	62	11.6
19.09.12	36	7.0	380	3 300	540	65	57 000	2.3	420	11.2
06.11.12	19	6.1	2 500	6 500	3 600	900	68 000	7.4	270	6.2
04.12.12	22	6.2	1 400	4 100	3 700	760	50 000	3.7	400	7.7