Comparison of *Pseudokirchneriella subcapitata, Ceramium tenuicorne* and *Myriophyllum aquaticum* growth inhibition test in the context of sediment and soil quality assessment

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Abstract

Sediments and soils have been shown to be a sink as well as a source for pollutants in the environment. Boats, especially due to their anti fouling paints, are known to release heavy metals and organic compounds like tributyltin (TBT) into the environment. Sediment from a marina in Brunnsviken and soil from its boatyard have been taken and compared to a reference site at *Bergianska botanical garden* approximately 2 km north of the marina. Three different plant bio tests, a microalga test with *Pseudokirchneriella subcapitata*, a macroalga test with *Ceramium tenuicorne* and test with the macrophyte *Myriophyllum aquaticum*, have been used to assess the toxicity of the samples. The two algae tests have been performed with leachates from sediments and soils, the test with *M. aquaticum* was a sediment contact test. Additionally to the bio tests, the samples have been chemically analysed for the concentrations of the metals copper, zinc, lead and tin and for $\sum$16 polycyclic aromatic hydrocarbons and $\sum$10 organo tin compounds.

Both sediment and soil samples caused strong toxic effects especially in the alga tests. Both alga tests showed similar sensitivity to the leachates. Data from chemical analysis reflected these results. Both sediment and soil were highly contaminated with the analysed heavy metals (maxiumum values sediment: Zn 1500 mg/kg (dw); soil: Zn 6000 mg/kg (dw)) and TBT (sediment: TBT 270 µg/kg (dw); soil: TBT 4480 µg/kg (dw)), but concentrations in the soil were even higher than in the sediment. This difference was shown in the test with *M. aquaticum* were the growth in the soil samples was less than in sediment samples. The opposite case was found in the algae tests where the sediment samples caused greater adverse effects. Chemical analysis of the leachates showed higher metal leaching from the sediment than from the soil.

The study showed the advantages of complementing the traditional chemical analyses in sediment and soil quality assessment with biological tests, since only these can take into account the bioavailability of the chemicals.

It is alarming that the activity of pleasure boats pollutes the environment to such a great extent that organisms are severely negatively affected.

1. Introduction

Sediments and soils are huge compartments in the environment which on the one hand store pollutants and serve as a sink, but on the other hand can be a source for toxicants (Hollert et al. 2000, Koethe 2003). For that reason sites with highly contaminated sediments or soils may be needed to remediate.

Especially harbour sediments are known to be highly polluted due to the usage of toxic boat paints and oil/fuel releases of the engines (Eklund et al 2010). Also the soil at boatyards was shown to be highly influenced by this (Eklund and Eklund 2011).

Since there are so many contaminated sites and not all can be remediated there must be a determination of a notably remediation need. So far, this has mainly been done on the basis of chemical analysis but literature suggests more and more a combination of chemical analysis and bio tests (Eisentraeger et al. 2004, Eklund et al. 2010).

All trophic levels should be considered in the testing to get a realistic overview about the hazardous effects (e.g. Directive 98/8/EG). However, primary producers are the basis in the ecosystem and consist of a wide range of species, from microalgae over macroalgae up to higher plants. Hence, it is challenging and important to observe adverse effects at this level properly.

1.1 Boats as source for contamination

Boats are a source of pollution in aquatic systems. Huge ships as well as pleasure boats are painted with paints that leach toxicants in order to prevent fouling. A very commonly used active substance in antifouling paints has been tributyltin (TBT). The use of TBT in antifouling paints started in the mid 60^{th} and reached a worldwide use of 2-3 x 10^3 tons in 1980 (WHO 1990) when first toxic effects on the environment were recognised in France (Alzieu 1986). There the oyster production decreased due to toxic effects on the larvae. Nowadays TBT is known to be among the most hazardous pollutants in aquatic ecosystems, therefore it is on the list of prioritised substances under the water framework directive (2455/2001/EC 2011). Already concentrations of 1 ng/L in the water can cause imposex of the gastropod *Nucella lapillus* (Bryan et al. 1986).

France was the first country to make regulations on the use of TBT in antifouling paints in 1982 (Daehne and Watermann 2009) and more countries followed. In Sweden and the rest

of the EU-countries the use of TBT in paints on pleasure boats has been forbidden since 1989 (Directive 89/677/EEC 1989) and a total worldwide ban of TBT in anti fouling paints was decided by the International Maritime Organisation (IMO) in 2008.

With the regulation of TBT in paints the use of other active substances like copper increased (Dahl and Blanck, 1996). Due to this, copper levels in the environment raised (Claisse &Alzieu 1993). Anti-fouling paints on pleasure boats have been considered by the Swedish authority to be the main anthropogenic source for copper in the environment (KemI, 1993; KemI, 1998a). In 2001 no paints based on leakage of copper were allowed for use on pleasure boats on the east coast of Sweden. Consequently new paints for leisure boats have been launched that were supposed to be biocide free. In a study comparing these paints to copper based paints it has been shown that the biocide free paints release substantially more zinc than copper based paints and showed in some cases even higher toxicity than the copper paints (Ytreberg et al. 2010). Copper and zinc are suggested to be toxic to non target organisms like *Ceramium tenuicorne* in semi-enclosed areas with high boat activity (Ytreberg et al. 2010).

Another heavy metal used in anti fouling paints was lead. It served as biocide, stabilizer and anti-corrosive agent (Martin and Richards 2010).

Besides the boat paints oil and fuel releases can contaminate the environment with polycyclic aromatic hydrocarbons (PAHs) (Eklund et al 2010, Eklund and Eklund 2011). Lead and PAHs are like TBT prioritised substances according to the water framework directive and known to be very hazardous in the aquatic environment (Decision 2455/2001/EC).

1.2 Heavy metals in sediment and soil

In the environment metals can have several different chemical speciations. They can occure as free ions, as solved inorganic/organic complex, as unsolved complex or adsorbed to particles. The bioavailability of metals is dependent on the speciation. The free metal ions are mainly responsible for the toxicity of metals. The chemical speciation of metals is dependent on several parameters like pH, alkalinity, concentration of organic ligands, salinity, concentration of adsorbent surfaces and other environmental chemicals with chelating properties (Fent, 2007). Thus, soil and sediment can be a sink for heavy metals by binding them. On the other hand it can also serve as a source. For example when the

environmental conditions change, like during flood events or heavy rainfall, the pollutants can be resolved (Hollert et al. 2000, Koethe 2003)

Also the organotin compound TBT tends to adsorb to particles because of its lipophilic properties, but this adsorption is reversible as well (Fent 2007).

1.3 Hazard Identification

Due to the potential of polluted soils and sediments to serve as a source for toxicants it is important to make hazard identification, hence, to estimate the potential of these soils and sediments to cause adverse effects. Often only chemical data have been used for classifying sediments or soils as toxic. But chemical analysis have some draw-backs, firstly; does not reflect the amounts of the chemical that are finally bioavailable and secondly; it is never possible to analyse the levels of all existing chemicals. Also the mixture toxicity with synergistic and antagonistic effects cannot be estimated by only looking at chemical data. Literature suggests more and more the use of bio tests to estimate the hazard of polluted sediments (Eisentraeger et al. 2004, Eklund et al. 2010). Several bio tests have been used to assess the toxicity of sediments and soil. Standardised test batteries to investigate the ecotoxicity of sediments use organisms representing three different trophic levels. (HABAB WSV 2000). The classical procedure is a luminous bacteria test, an algae test and a test with daphnia.

It is discussed if microalgae, since they are structurally simple, are sufficient to represent the wide range of species within the primary producers (Wang 1990).

This study will compare three bio tests with primary producers of different development levels in an assessment about the quality of sediment and soil from a pleasure boat harbour. The performed tests were a microalga test with *Pseudokirchneriella subcapitata*, a test with the macroalga *Ceramium tenuicorne* and a test with the with the aquatic plant *Myriophyllum aquaticum*.

Sediment toxicity with microalgae has been tested since the late 80-ies mainly by exposing the algae to leachates (Ross 1988, Ahlf et al. 1989). The macroalga teast with *C. tenuicorne* has been shown by Eklund et al. to be suitable for sediment toxicity assessment (2010). The test with the macorphyte *M. aquaticum* is a relatively new developed sediment contact assay (Feiler et al. 2004).

1.5 Aims

The main aim of this thesis was to compare the three plant growth inhibition tests (*P. subcapitata, C. tenuicorne* and *M. aquaticum)* in testing the toxicity of sediments and soils from a pleasure boat harbour. These sediments and soils were expected to be contaminated with metals and TBT due to boat activities.

The specific objectives were to:

- compare the use of the three plant tests in testing toxicity of sediment and soil.
- compare different ways of exposure in sediment and soil testing.
- underline that chemical analysis alone is not sufficient for sediment and soil quality assessment.
- estimate the influence of boat activities in Brunnsviken.

2. Materials and Methods

2.1 Sampling

The studied area is the lake Brunnsviken in Stockholm. It is an about 3,5 km long and 0,4 - 0,5 km broad lake with a connecting channel to the inner Stockholm archipelago at the eastside of the lake. The lake is mainly surrounded by parks and four boat clubs are located in the lake. Three sediment and three soil samples were taken in a marina in the south of the lake, called *Segelsällskapet Brunnsviken* (SSB), and in the area of *Bergianska botanical garden*. The boat club SSB was founded 1898 and houses currently about 130 motorboats. At the botanical garden only small peers are found where boats can dock temporarily.

In total six sediment samples and six soil samples were taken in the study area Brunnsviken on December 1st 2011. Where the samples were taken is shown in figure 1. The samples from the marina were named with M and the samples from the reference with R. For the soil samples an S was added after the station letter. Additionally two surface water samples were taken in the marina and two in the reference site.

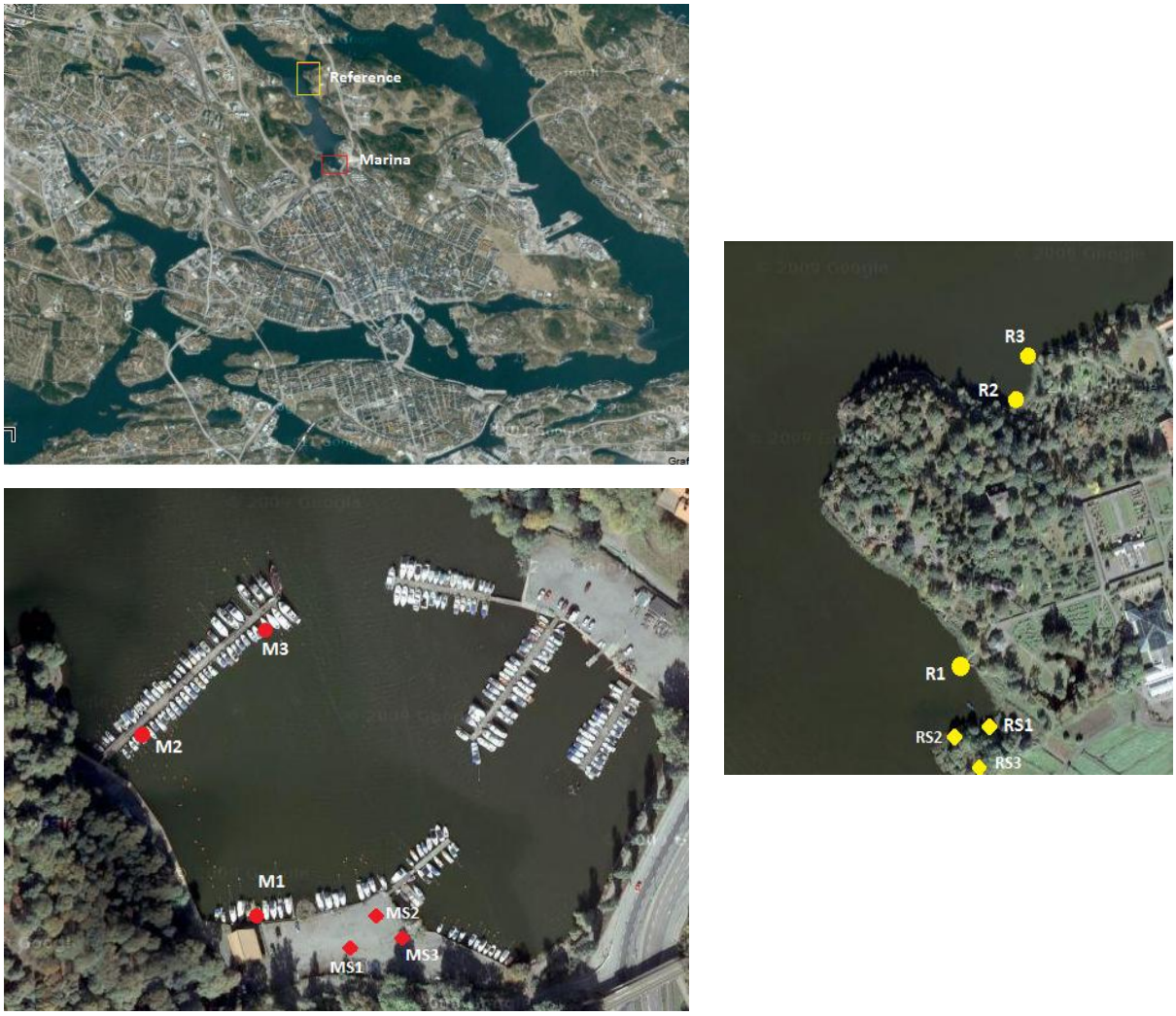

Figure 1: Study area. The figure shows three pictures of the study area Brunnsviken. The picture in the top left shows an overview of the lake Brunnsviken and marks where the marina and the reference area are located. The right picture shows the reference area and the picture bottom left the marina. The dots mark the positions where the sediment samples were taken and the squares the sampling stations of the soil samples. R stands for reference, M for marina and S for soil sample.

The sediment and soil samples were taken to be chemically analysed and to perform biological tests with them. For the biological testing leachates were made from the samples. How sampling and sample use was done is described in the following chapters.

2.1.1 Sediment samples

The sediment samples were taken with a *Villner* core sampler. One to two cores were taken in each sampling point in the marina and in R1. The top 4 cm of the core were sliced off and filled into sealed plastic jars. In M3 the 20-30 cm of the core were taken as well. The samples in R2 and R3 were taken in shallow waters with a shovel because the sediment was very sandy and the use of the core sampler was not possible.

The top 4 cm from the two cores at each sampling site were mixed together and stirred well with a plastic spoon. From this pooled sediment from each sampling station, 7- 18 g (ww) were weighed into small plastic jars and sent to metal analysis. For the analysis of tin organic compounds and PAHs 30 g (ww) from each of the three sampling station in the marina were mixed. The same was done with the samples from the reference site. Finally six samples (three marina and three refrence) were analysed for metal concentrations and two bulk samples (one marina and one reference) for organic compounds. An additional collected sample at M3 from 20-30 cm depth was sent to metal and organic analysis as well. Chemical analysis was carried out by *ALS Scandinavia AB*. The remaining samples were stored in darkness at 4 °C until the leachates and biological testing were done.

2.1.2 Soil samples

The top 1 cm soil was taken with a shovel in the sampling stations marked in figure 1. The samples were filled in sealed plastic jars.

Subsamples of the samples were taken in the lab and sent *ALS Scandinavia AB* for chemical analysis of the metals copper, zinc, lead and tin. For analysis of tin organic compounds and PAHs a pooled sample of equal amounts (ww) from all the three respective stations per site was prepared and sent to *ALS* as well. The remaining samples were stored in darkness at 4 °C until the leachates and biological testing were done.

2.2 Dry matter content and loss on ignition

The water content (W) and the loss on ignition (LOI) of all sieved samples and the bulk sample was determined according to the standard (SS 028113-1). To do this about 2 g wet sample (soil particle size < 3 mm) were transferred to a pre-weighed, burned crucible. Then the crucible and sample were weighed together. For the soil samples three replicates of each sampling station and of the bulk sample (see 2.2.2) were prepared in this way. Concerning the sediment three replicates of the marina samples and two replicates of the reference samples were prepared. The sediment was then dried for 2 ½ days and the soil for 1 ½ days at 105 °C in an oven. After drying the samples were transferred to a desiccator and cooled down to room temperature before weighing again to achieve the dry weight (dw). For the

determination of loss on ignition the dried samples were heated in an oven for 2 hours at 550 °C. The samples were then again cooled down to room temperature and weighed.
The water content, dry matter content (ds in %) and LOI were calculated according to the standard (SS 028113-1).

2.3 Leachates

2.3.1 Sediment

For the preparation of sediment leachates a dilution series was done. 40 g of wet sediment were weighed into an E-beaker and filled up to 250 mL with Brunnsviken surface water (collected from the marina for leachates of marina samples and from the reference area for the reference leachate). The mixture was stirred strongly and then half of it poured into another E-beaker and filled up to 250 mL again. The procedure was repeated to get a dilution series of five concentrations (160, 80, 40, 20 and 10 g/L wet weight). For the reference leachate a pooled sample from three replicate samples was used.
The leachates were shaken on a shaking table for 24 h with 40 rounds per minute. Afterwards the leachates were left unmoved for 3 days in order to let the suspended solids settle down and then filtered through a 0.45 µm pore filter.

2.3.2 Soil

All soil samples were sieved with a 3 mm plastic mesh. Sample MS 2 was spread on a trail to dry for one hour so that it was possible to sieve this sample. A bulk sample of the three sieved reference soil samples was prepared by mixing 180 g of each sample.
Soil portions equal to 45 g dry weight of each sample from the marina and of the pooled reference soil were weighed into 1 L E-beakers and mixed with the leachant (Mili-Q water). In order to get a liquid to solid ratio (L/S) of 10 L/kg dry matter (here 45 g (dw)/ 450 mL) the amount of leachant was calculated according to formula 4 in the standard (ISO/TS 21268-2:2007).
The mixture was shaken for 24 h on a shaking table with 50 rounds per minute and in darkness. Afterwards the leachates were left unmoved for 3 days in order to let the suspended solids settle down and then filtered through a 0.45 µm pore filter.

2.4 Pseudokirchneriella subcapitata test

For the Microalgae growth inhibition test, the freshwater alga *P. subcapitata* was used and the procedure in the ISO-standard (ISO 8692) followed in large. *P. subcapitata* is a planctonic unicellular green alga and one of the most used species in the microalgae test.

Sediment leachates:

The testing of the sediment leachates was done in the course Strategies for environmental Risk and Hazard Identification 2011/2012 (ITM). The materials and methods for this test are described in the report: Sediment and surface water toxicity analysis in Brunnsvikens marina.

Soil leachates:

In total four tests each with 7 leachate concentrations and controls in 20 % Z8 medium were performed. For the preparation of the test solutions the soil leachates of each sample were first diluted five times. This was done by taking 10 ml of the leachate and adding 10 mL 100 % Z8 medium and filling up to 50 mL with Milli-Q water. Then a dilution series with a dilution factor of 2 was prepared by diluting 25 mL of these solutions six times with 20 % Z8 medium. 5 mL of each concentration were taken and filled in a glass tube as blank. To the remaining 20 mL 0.5 mL of algae inoculum was added. For the inoculum an algae pre culture was diluted with Z8 medium so that it had a fluorescence of 13. The final test solution then had a flurorescence of 0.3 which corresponds to a cell density of 1600 cells/mL.

Three replicates of each concentration and control were filled in glass tube. When testing the leachates of MS1 and MS2 6 replicates in the control were prepared. The test volume in each tube was 5 mL. The algae were incubated for 72 hours at 23±1 °C. During the incubation the glass tubes where shaken continuously and exposed to white light (60 – 120 µmol/(m^2 *s)). Fluorescence was measured at test start and after 72 hours. The flourometer was calibrated for each concentration with the corresponding blank. The blanks were kept under the same conditions as the test.

The growth rate and inhibition of growth were calculated according to the standard (ISO 8692 2011). EC_{50} values for each sampling station were determined by using the programme Regtox (Vindimian É).

2.5 Ceramium tenuicorne test

Leachates of the sediment and soil samples from Brunnsviken have been tested for its toxicity in the macro alga test with *Ceramium tenuicorne* according to the standard (ISO 10710, 2010). *Ceramium* is a filamentous red alga that can be found in oceans worldwide and that occurs as a brackish water clone and as a marine water clone along the Swedish coasts. For the tests the brackish water clone of the red alga was used.

2.5.1 Test of the sediment and soil leachates

Two forking tips of the actively growing algae were cut off with a scalpel and collected in a petri dish with growth medium and after finishing cutting the average length from the base forking to the top of 20 tips determined.

Sediment test solutions:

For preparing the test solutions sediment leachates of the samples from M1, M2 and the reference with the concentration 80 g/L (ww) and of M3 with the concentration 20 g/L were taken and NaCl added to adjust the salinity to 7 ‰. Nutrients were added so that it contained 3.46 mg/L nitrogen, 0.76 mg/L phosphorus and 0.1 mg/L iron. Then the leachates were diluted with cultivation medium. Concentration series of 40, 20, 10, 5, 2.5 and 1.25 g/L were prepared. For M3 the concentrations 10 to 1.25 g/L were prepared.

Soil test solutions:

The four soil leachates were first diluted five times. For that 16 mL of leachate were taken, the salinity adjusted to 7 ‰ by adding NaCl and nutrients so that the leachate contained 3.46 mg/L nitrogen, 0.76 mg/L phosphorus and 0.1 mg/L iron. The leachate was then filled up to 80 mL with cultivation medium. 40 mL of this mixture were taken and diluted half with cultivation medium. This was repeated six times, so that in the end a dilution series with the concentrations 0.32, 0.63, 1.25, 2.5, 5, 10 and 20 g/L (dw) was prepared. This was done with all four soil leachates.

In the tests of the sediment and the soil leachates cultivation medium was used as control. In the test of the sediment leachate Water samples from the Brunnsviken marina and the reference station were taken, filtered through 0.45 µm and nutrients added in the same concentrations as to the leachates and tested as well.

The test solutions were filled into petri dishes. Of all concentrations of M1, M2, M3, MS1, MS2, MS3 and soil reference, three replicates with 10 mL each were prepared and of the controls and the sediment reference four replicates. Two tips were transferred into each petri dish. The test was incubated for 7 days at 22±2 °C and 14 hours light (70±10 % µmol $m^{-2}s^{-1}$) and 10 hours darkness per day. At the test end length of every tip from base branch to the top was measured.

The growth rate of the algae in each concentration and the control was calculated after 7 days (ISO 10710 2011). Inhibition of growth was determined as reduction in growth rate relative to the growth medium control. EC values were calculated with the programme Regtox (Vindimian É).

2.6 Myriophyllum aquaticum test

The sediment and soil samples have been tested in a sediment contact test with the higher macrophyte *Myriophyllum aquaticum* according to standard (ISO 16191 2011). *Myriophyllum* is a dicotyledonous freshwater macrophyte which originates from the Amazon River but occurs nowadays worldwide, especially in warm climate regions (ISO 16191 2011).

2.6.1 Pre test

For the control 170 g of soil (50% garden soil and 50% clay) were mixed in a beaker with 90 mL Hoagland nutrient solution (Hoagland 1950). The test sample was soil from the station M2. 250 g of the test soil were mixed with 30 mL nutrient solution. Three times 80 g of the control and the test soil were weighed into three 250 mL beakers. On each beaker three positions were marked.

Whorls with five leaves were cut from an old culture of *M. aquaticum* and collected in a petri dish containing nutrient solution. The whorls were carefully dried, weighed and then planted at marked position. The beaker was covered with a translucent petri dish. The test was incubated under continuous light (60 µmol $m^{-2}s^{-1}$) and 24 ± 1 °C for nine days.

The beakers were irrigated with nutrient solution and water (1:1) and randomised every 48 to 62 h.

At test end the condition of each plant was noted and then carefully taken, cleaned dried and weighed. Growth rate and inhibition of growth rate were calculated according to the standard (ISO DIS 16191).

2.6.2 Test with *M. aquaticum* (ISO/TC 147/SC 5)

Pre culture:

21±3 d before test start a pre culture of Myriophyllum aquaticum was started. For that head whorls of *M. aquaticum* were planted in artificial sediment. Artificial sediment consist of 5% peat powder, 74% Quartz sand, 20% Kaolin clay and 1% $CaCO_3$ powder. 125 g of this mixture were filled into a 1L beaker and mixed with 65 ± 5 mL nutrient solution. It was stirred until it was a homogenous mixture and then condensed by knocking the vessel on the table until all bubbles were removed.

In each beaker seven plants were placed. The pre culture was irrigated every 48 to 62 h with Hoagland nutrient solution and water (1:1) and incubated for 21 days under test conditions. The pre culture for the soil testing was incubated for 29 days.

Preparation of artificial control sediment:

For preparation of the artificial control sediment 12.5 g peat powder were weighed into a beaker and mixed with 100 mL nutrient solution (Hoagland) and 2.5 g $CaCO_3$. The pH was controlled if it was in the range of 5.5 ± 0.5. The mixture was covered with plastic foil and stirred for 3 days. After 3 days the pH was controlled again. It should be 6.0 ± 0.5. Then 185 g of quartz sand and 50 g Kaolin were added to the peat powder mixture and stirred until it was homogenous. The pH was controlled again to be in the range of 7.0 ± 0.5. Finally 400 mL nutrient solution were poured on the sediment, the beaker was covered with a translucent petri dish and kept under test conditions for 7 days.

Test of sediment samples:

Three 250 mL beakers were each filled with 80 g of the artificial control sediment. Three replicates with each 80 g of the R1 sample and two replicates of the marina samples were prepared. Due to insufficient sample material, replicate one of M2 contained only 75 g and replicate two 70 g of sediment sample.

The plants from the pre culture were cut into their whorls and the ones with 5 leaves collected in a petri dish with nutrient solution. The plants were dried with the back side up on tissue paper, weighed and then directly planted at pre marked positions in the prepared beakers. Three whorls were placed in each beaker. Due to insufficient plant material from the pre culture most of the used whorls weighed less than the standard requires. The whorls used in replicate 3 of the reference sample and the control all showed the beginning of a side shoot.

The beakers were covered with petri dishes. The test was incubated for 10 days under continuous light (60 µmol $m^{-2}s^{-1}$) and 24 ± 1 °C. The plants were irrigated with nutrient solution and water (1:1) every 2-3 days. The beakers were randomised when irrigated.

At test end the condition of the plants was noted and then the whole plant removed carefully from the sediment, cleaned in tab water, dried and weighed. The growth rate of *Myriophyllum* and inhibition of growth were calculated as described in the standard (ISO/TC 147/SC 5, 2011).

Test of soil samples:

Seven days before test start 250 g of dry control soil were weighed into a 1 L beaker, mixed with 125 mL Hoagland nutrient solution, covered with a petri dish and kept under test conditions.

At test start three 250 mL beakers were each filled with 80 g of this control soil. Three replicates with each 80 g of the 3 mm sieved soil sample MS1, MS2, MS3 and the sieved and pooled reference sample were prepared. Hoagland nutrient solution was stirred under the 80 g sample until 1-2 mL media supernatant was reached.

The plants were added and the test incubated and finished as described under “test of sediment samples”. Due to insufficient amount of whorls in the required weight range of 19 mg to 31 mg some of the whorls used in the test weighed more than 31 mg, but all less than 40 mg.

3. Results

3.1 Biological test

The results of the tests of the sediment and soil samples with *P. subcapitata, C. tenuicorne* and *M. aquticum* are presented in this chapter.

3.1.1 Pseudokirchneriella subcapitata

The results of the tests with *P. subcapitata* are shown in the figures 2 and 3 and in table 1. The sediment leachates showed with increasing concentration an inhibiting effect on the growth rate of *P. subcapitata*. In the tests of the sediment leachates from the marina samples all tested concentrations inhibited the growth rate more than 50 % compared to the control (figure 2).

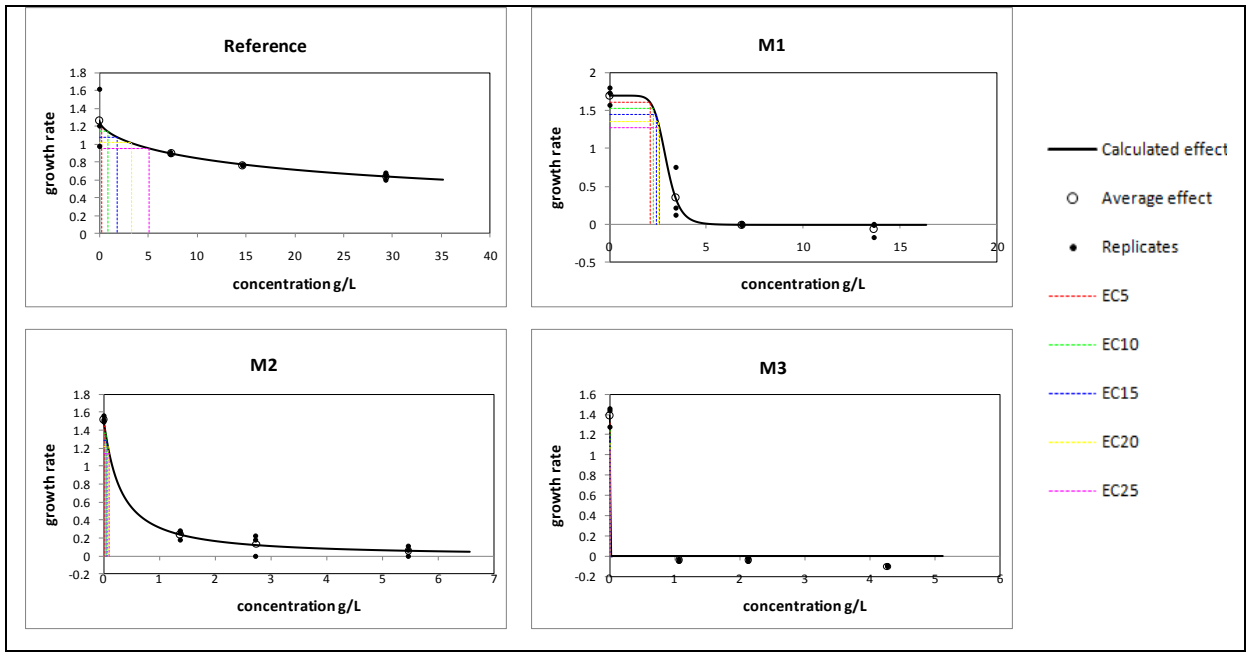

Figure 2: Growth rate of *Pseudokirchneriella subcapitata* in the test of sediment leachates. The figure illustrates four graphs with the relation between leachate concentration [g/L (dw)] and alga growth rate. Each graph shows the results of one sediment sample. Only the growth rate of *P. subcapitata* in the lowest three tested concentrations is presented since hiher tested concentration seemed to be influenced by additional nutrients from the sediement. The graphs show the growth rate in each replicate (filled black dots) and the average growth rate (back circle). Three replicates were used in all tests.

The leachates of the marina soil caused with increasing concentration a decreasing growth rate of *P. subcapitata*. A stimulation was seen in the test of the reference soil, which was observed as a higher growth rate than in the control. The highest tested concentration of the samples MS1 and MS3 and the two highest concentrations of MS2 caused a negative growth rate (figure 3).

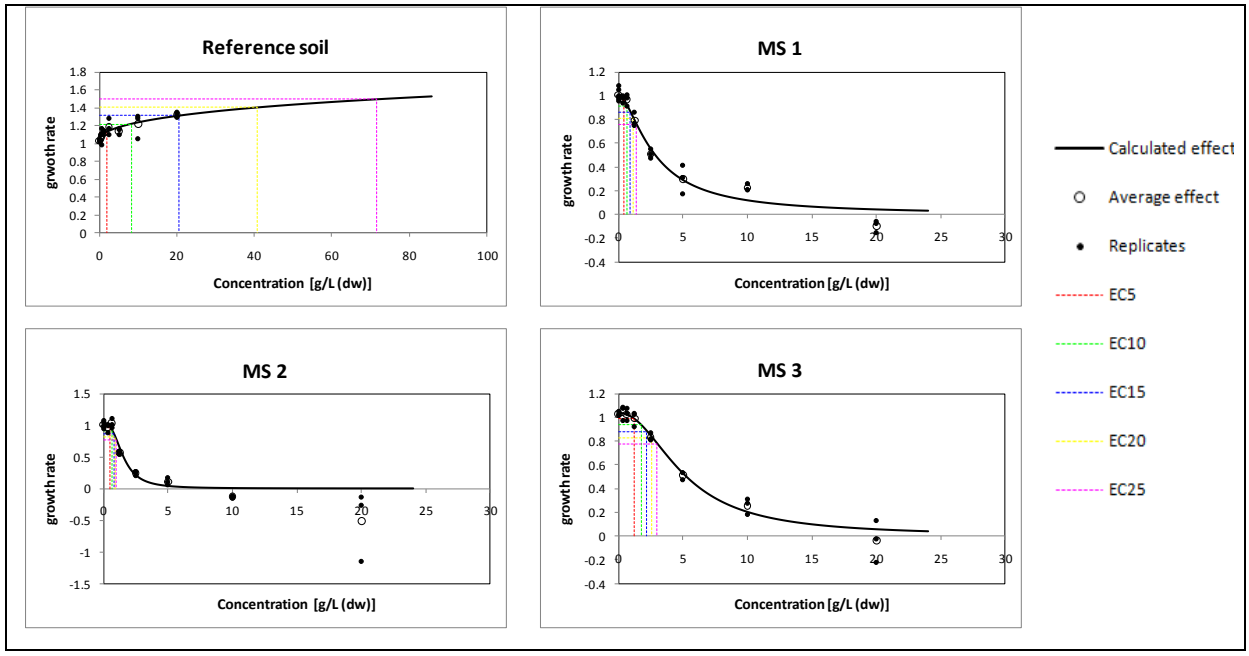

Figure 3 : Growth rate of *Pseudokirchneriella subcapitata* in the test of soil leachates. The figure illustrates four graphs with the relation between leachate concentration and alga growth rate. Each graph shows the results of one sediment sample. The graphs show the growth rate in each replicate (filled black dots) and the average growth rate (back circle). Three replicates were used in all tests except of the control in the test of the samples MS1 and MS2, which was tested in six replicates.

Table 1 shows the EC_{20} and EC_{50} values that were determined in the test of the sediment and soil samples with the *P. subcapitata*. For the sediment reference no effect concentrations could be calculated. The strongest effect was caused by the sediment M3, where the determined EC_{50} was 5.3×10^{-6} µg/L. The effect concentrations in the two other marina sediment samples were more than 50000 times higher.

In the test of the soil leachates the strongest effect was determined in the sample MS2 with an EC_{50} of 1.6 g/L. The effect of the MS1 sample was about three times less.

Table 1: EC_{20} and EC_{50} values in the *Pseudokirchneriella subcapitata* test with Brunnsviken sediment and soil leachates. The table shows the calculated EC_{20} and EC_{50} in the *Pseudokirchneriella* test with leachates of the soil and sediment samples from Brunnsviken marina and the bulk sample from the reference area. The 95 % confidence intervals of the EC_{50} values are shown as well. For the test of the reference sediment no effect concentrations are calculated.

Sampling Station	EC_{20} [g/L (dw)]	95 % confidence intervals [g/L (dw)]	EC_{50} [g/L (dw)]	95 % confidence intervals [g/L (dw)]
Reference sed.	-	-	-	-
M 1	2.7		3.0	0.6 – 3.2
M 2	0.1		0.3	0.03 – 0.64
M 3	4.4×10^{-6}		5.3×10^{-6}	2.7×10^{-6} – 8.4×10^{-6}
Reference soil		stimulation		
MS 1	1.2	0.8 – 1.5	2.8	2.3 – 3.3
MS 2	1.0	0.5 – 1.4	1.6	0.9 – 1.9
MS 3	2.6	2.0 – 3.2	5.0	4.2 – 5.6

3.1.2 Ceramium tenuicorne

The results of the tests with *Ceramium tenuicorne* are shown in the figures 4 and 5 and in table 2.

In the test of the sediment leachates the leachates of the marina samples showed with increasing concentration an increasing effect on the growth of *C. tenuicorne*. In the highest concentrations of the M1 and M2 leachate no growth at all was observed. A growth rate of 0.3 was determined in the highest concentration of M3 leachate. In the reference leachate the growth rate was still inhibited about 50 % in the highest tested concentration (figure 4).

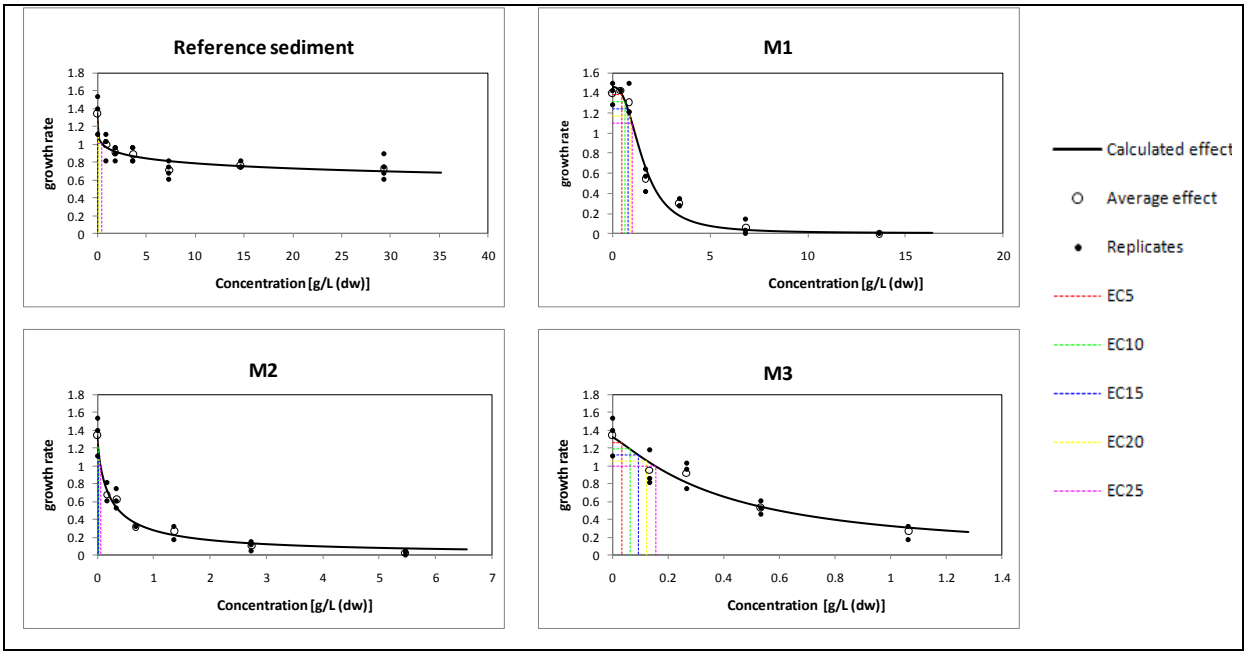

Figure 4: Growth rate of *Ceramium* in the test of sediment leachates. The figure illustrates four graphs with the determined relation between leachate concentration and alga growth rate. Each graph shows the results of one sediment sample. The graphs show the growth rate in each replicate (filled black dots) and the average growth rate (back circle). The leachates of the marina samples and the control were tested in three replicates. The reference leachate was tested in four replicates.

The soil leachates from the marina showed with increasing concentration an increasing inhibition of the growth rate of the macro alga. In the highest tested concentration of 20 g/L (dw) the algae did not grow at all.

In the reference leachate *Ceramium* grew in all concentration. A decreasing growth rate appeared in the highest concentration where the growth rate was inhibited 34 % relative to the lowest tested concentration (figure 5).

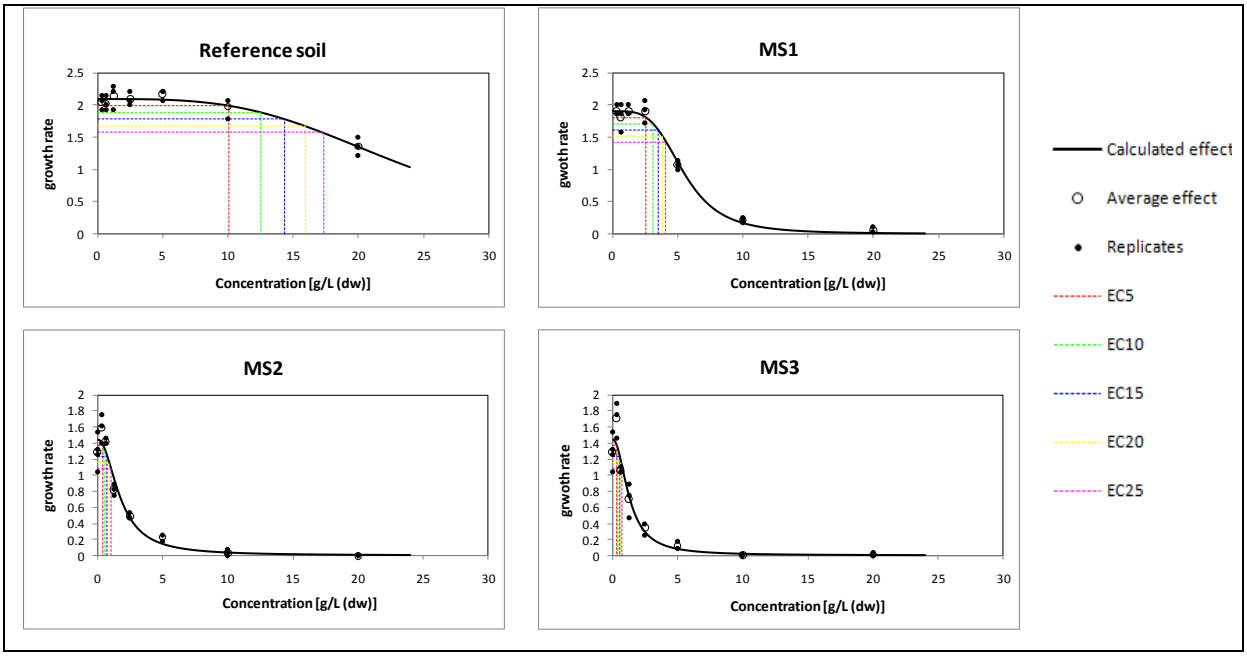

Figure 5: Growth rate of *Ceramium tenuicorne* in the test of soil leachates. The figure illustrates four graphs with the relation between leachate concentration and alga growth rate. Each graph shows the results of one soil sample. The graphs show the growth rate in each replicate (filled black dots) and the average growth rate (back circle). Everything was tested in three replicates, except of the control in the test of the M2 and M3 leachate, which was tested in four replicates.

Generally, the soil samples were about 4 times less toxic than the sediment samples (table 2). Among the sediment samples the M2 showed the strongest effect with an EC_{20} value of 0.04 g/L and EC_{50} of 0.2 g/L. The EC_{50} of M3 was two times higher and of M1 eight times and thus less toxic than M2. The EC_{20} of the reference sediment was 0.1 g/L and the EC_{50} had a value of 37 g/L.

The soil sample MS3 was the most toxic with an EC_{50} of 1.3 g/L and EC_{20} of 0.7 g/L. The sample MS1 had an EC_{50} of 5.5 g/L and in the test of the reference soil an EC_{50} of 24 g/L was determined.

Table 2: EC_{20} and EC_{50} values in the Ceramium test with Brunnsviken sediment and soil leachates. The table shows the calculated EC_{20} and EC_{50} in the *Ceramium* test with leachates of the soil and sediment samples from Brunnsviken marina and the bulk sample from the reference area. The 95 % confidence intervals are shown as well.

Sampling Station	EC_{20} [g/L (dw)]	95 % confidence intervals [g/L (dw)]	EC_{50} [g/L (dw)]	95 % confidence intervals [g/L (dw)]
Reference sed.	0.1	0.006 – 1.3	37	13 – 180
M 1	0.2	0.7 – 1.1	1.6	1.4 – 1.8
M 2	0.04	0.02 – 0.1	0.2	0.2 – 0.3
M 3	0.1	0.1 – 0.2	0.4	0.3 – 0.5
Reference soil	16	13 - 19	24	21 – 30
MS 1	3.8	3.3 – 4.2	5.5	5.1 – 5.8
MS 2	0.9	0.6 – 1.2	1.8	1.4 – 2.1
MS 3	0.7	0.4 – 1.0	1.3	1.0 – 1.6

3.1.3 Myriophyllum aquaticum

The results of the test of the sediment samples with *M. aquaticum* are shown in figure 6 and table 3. The highest growth rate of *M. aquaticum* with a value of 0.103 occurred in the sample M2 and was even higher than in the control where the growth rate was 0.091. The lowest growth rate with 0.052 was in the sample M1.

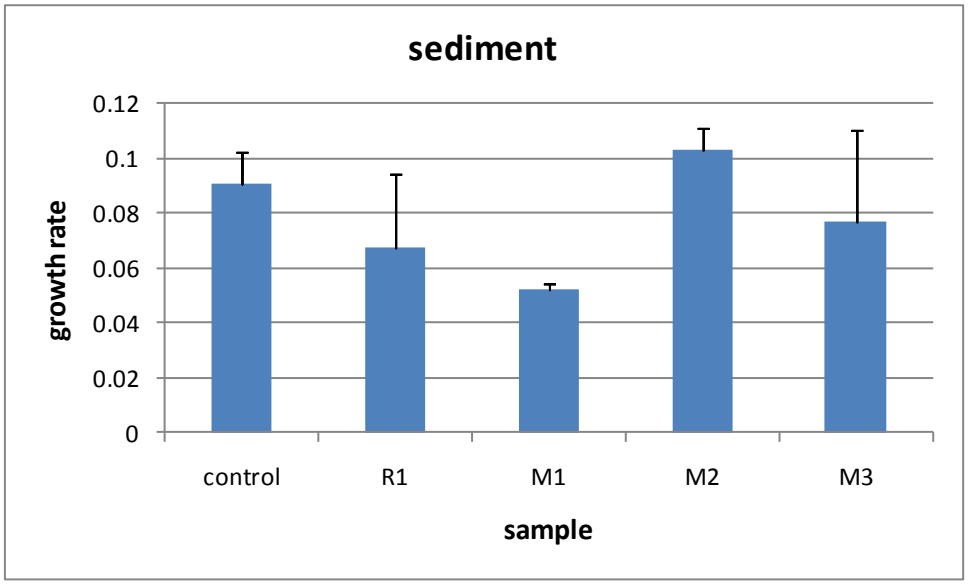

Figure 6: Growth rate of *Myriophyllum aquaticum* in sediment toxicity test. The graph shows the determined average growth rate in the test of sediment samples from Brunnsviken with *Myriophyllum aquaticum*. The mean growth rate of the control and of R1 consists of 3 replicates and of the marina samples of two replicates. The standard deviation is shown as error bars.

In the test of the soil samples the highest growth rate was calculated in the reference soil with 0.141. The growth rate in the control was 0.055. In both samples MS1 and MS2 the growth rate was 0.041. Lowest growth rate of 0.032 occurred in sample MS2.

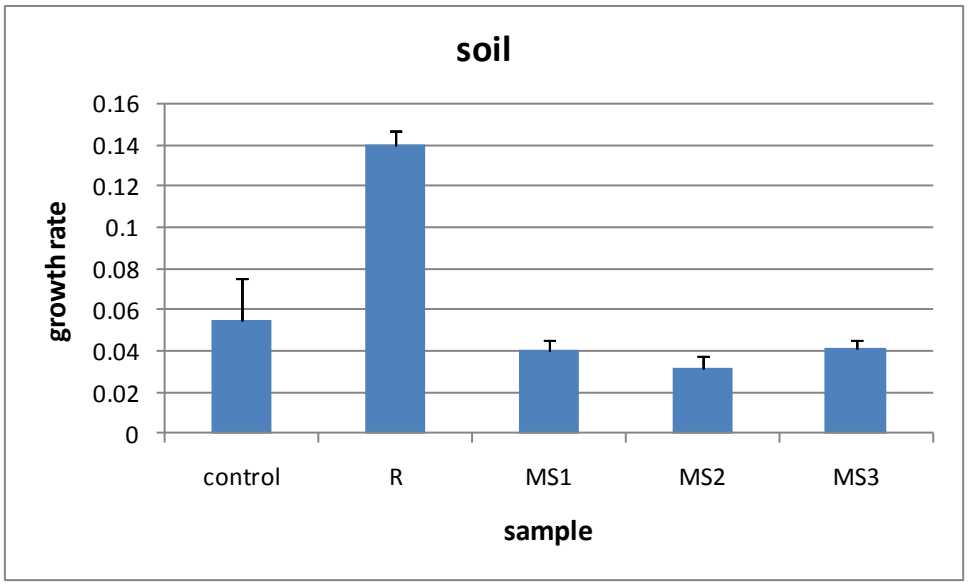

Figure 7: Growth rate of *Myriophyllum aquaticum* in soil toxicity test. The graph shows the determined average growth rate in the test of soil samples from Brunnsviken with *M. aquaticum*. The mean growth rate of the control and of R1 consists of 3 replicates and of the marina samples of two replicates. The standard deviation is shown as error bars.

In the test of the sediments the growth rate of *M. aquaticum* was inhibited in the reference sediment and in the sediment M1 and M2 relative to the control. The strongest inhibition of around 43 % occurred in the sample M1. In the sediment M2 the growth rate was stimulated by about 13 % (table 3).

In the test of the soil the growth of the *M. aquaticum* in the reference soil was stimulated by 157 % compared to the control. In the soil from the boatyard the growth was inhibited in all three samples. The strongest inhibition of growth occurred in sample MS2 with an inhibition of about 42 %.

Table 3: *Myriophyllum aquaticum* sediment toxicity test. The table shows the determined average growth rate and inhibition of growth relative to the control in the tests of sediment and soil samples from Brunnsviken with *M. aquaticum*. In the sediment test the mean growth rate of the control and of R1 consists of 3 replicates and of the marina samples of two replicates. The standard deviation is shown in brackets behind the value. Three replicates were used in all soil tests.

Sample	mean growth rate	Inhibition of growth [%]
Control sed.	0.091 (±0.011)	-
R1	0.068 (±0.026)	25.588
M1	0.052 (±0.003)	42.844
M2	0.103 (±0.008)	-13.089
M3	0.077 (±0.034)	15.251
Control soil	0.055 (±0.021)	-
R soil	0.141 (±0.006)	-157.148
MS1	0.041 (±0.004)	25.151
MS2	0.032 (±0.006)	41.897
MS3	0.041 (±0.004)	24.659
Control pre test	0.042 (±0.014)	-
MS2 pre test	0.024 (±0.006)	43.551

3.2 Dry weight and loss on ignition

Dry weight and loss on ignition was determined for the soil and sediment samples. The calculated dry weight and loss on ignition in the sediment samples are shown in table 4. The dry matter content in the reference samples were clearly higher than in the marina samples. Highest dry matter content of 81.0 % was measured in the sample R2. The dry matter content of the sample M3 was with 10.7 % almost eight times less.

The samples from the marina had a higher content of organic material than the reference samples. The highest organic carbon content was determined in M3 with a loss on ignition of 21.4 %. The loss on ignition in M1 was with 7.8 % almost three times less than in M3. Loss on ignition in R2 and R3 was with 0.7 % and 0.9 % quite similar. The sample R1 deviated from these samples with a loss on ignition more than three times higher. The total organic carbon (TOC) analysed by *ALS Scandinavia AB* is shown as well (table 4). The patterns of TOC follow the once of LOI.

Table 4: Average dry weight (dw), loss on ignition (LOI) and total organic carbon (TOC) of the sediment samples. The average values of the dry weight and the loss on ignition. The average values are based on two replicates. The standard deviation (SD) is shown in brackets behind the values. The table shows also the percentage of total organic carbon relative to the dry matter analysed by *ALS Scandinavia AB.*

Sample	dw [%] (±SD)	LOI [%] (±SD)	TOC [%]
M1	34.1 (±0.5)	7.8 (±0.8)	4.7
M2	13.7 (±0.0)	18.1 (±0.3)	11.3
M3	10.7 (±0.4)	21.4 (±0.6)	12.8
R1	59.3 (±1.1)	3.3 (±0.4)	2.1
R2	81.0 (±1.1)	0.7 (±0.0)	0.4
R3	79.5 (±1.6)	0.9 (±0.0)	1.0

The results for the soil are shown in table 5. The soil from the boatyard had a higher dry matter content then the soil from the reference area. Highest dry matter content with 94.37 % was measured in the sample MS 3. The lowest dry matter content with 70.21 % was determined in sample RS 3.

All samples from the reference had a higher content of organic material than the samples from the marina. Loss on ignition in the samples RS 1 and RS 3 was about 18 % whereas in MS 1 and MS 2 it was less than half of this value and in MS 3 it was 2.2 %. The patterns of TOC follow the one of LOI.

Table 5: Dry weight (dw), loss on ignition (LOI) and total organic carbon of the soil samples. The table shows the average values of dw and LOI. The mean value consists of the data of three replicates. Only the mean of LOI in the pooled R consists of two replicates. The standard deviation (SD) is shown in brackets behind the values. The table shows also the percentage of total organic carbon relative to the dry matter analysed by *ALS Scandinavia AB.*

Sample	mean of dw [%] (±SD)	mean of LOI [%] (±SD)	TOC [%]
RS 1	70.5 (±0.1)	18.0 (±0.2)	10.9
RS 2	74.6 (±0.4)	11.8 (±0.3)	7.1
RS 3	70.2 (±0.3)	18.7 (±0.3)	15.2
pooled RS	71.2 (±0.2)	16.0 (±0.1)	-
MS 1	88.7 (±0.7)	7.7 (±0.4)	4.2
MS 2	93.4 (±0.6)	6.5 (±0.5)	3.3
MS 3	94.4 (±0.1)	2.2 (±0.3)	1.3

3.2 Chemical analysis

The concentrations of the metals copper, lead, zinc and tin as well as 16 PAHs and 10 organotin compounds were analysed by *ALS Scandinavia AB* in the sediment and soil samples. The results of the chemical analysis are shown in the figures 8 and 9 and in the tables 6 and 7. Additionally the concentrations of the metals chromium, copper, zinc, arsenic, cadmium and lead have been analysed in the sediment and soil leachates as well as in the filtered surface water of the reference and the marina by Karin Holm at ITM (table 8 and 9).

3.3.1 Chemical concentrations in sediment and soil

3.3.1.1 Metals

In all sediment samples the concentration of zinc was the highest among the analysed metals. Zinc concentrations up to 1770 mg/kg (dw) was measured in M3. The other two marina surface samples had also high levels of more than 1200 mg/kg. The average concentration in the reference was about 60 mg/kg. Lead had the second highest concentration with a maximum in M1 with 894 mg/kg (dw). The average lead concentration in the reference was about 90 times less than this. Copper was highest with 888 mg/kg in M1 as well, whereas most tin was analysed with 67 mg/kg in sediment M2.

Comparing the surface sediment of M3 and the sample from 20-30 cm, the copper, lead and zinc concentrations in the bottom were about half of the levels in the top. The tin concentrations were about the same (figure 8).

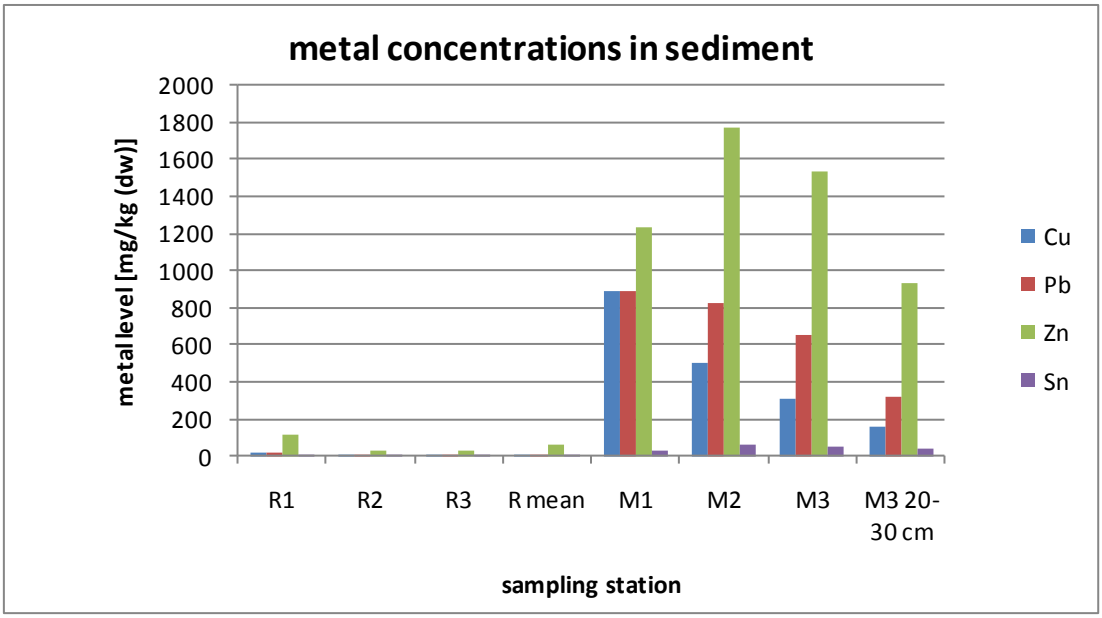

Figure 8: Metal levels in sediment. The graph shows the concentrations of the metal copper, lead, zinc and tin that were analysed in the sediment samples from Brunnsviken marina and reference. For the reference (R) additionally the mean value of the three analysed samples is shown.

Comparing the three soil samples from the marina, all analysed metal concentrations in MS3 were at least four times less than in MS1 and MS2. The sample MS1 had the highest concentrations of copper (6670 mg/kg (dw)) and lead (9520 mg/kg (dw)). This lead content is almost twice of what was measured as second highest concentration in MS2. Sample M2 had the highest concentrations of zinc (5960 mg/kg (dw)) and tin (445 mg/kg (dw)). In the reference zinc had with 58 mg/kg (dw) the highest and tin with 3 mg/kg (dw) the lowest concentration (figure 9).

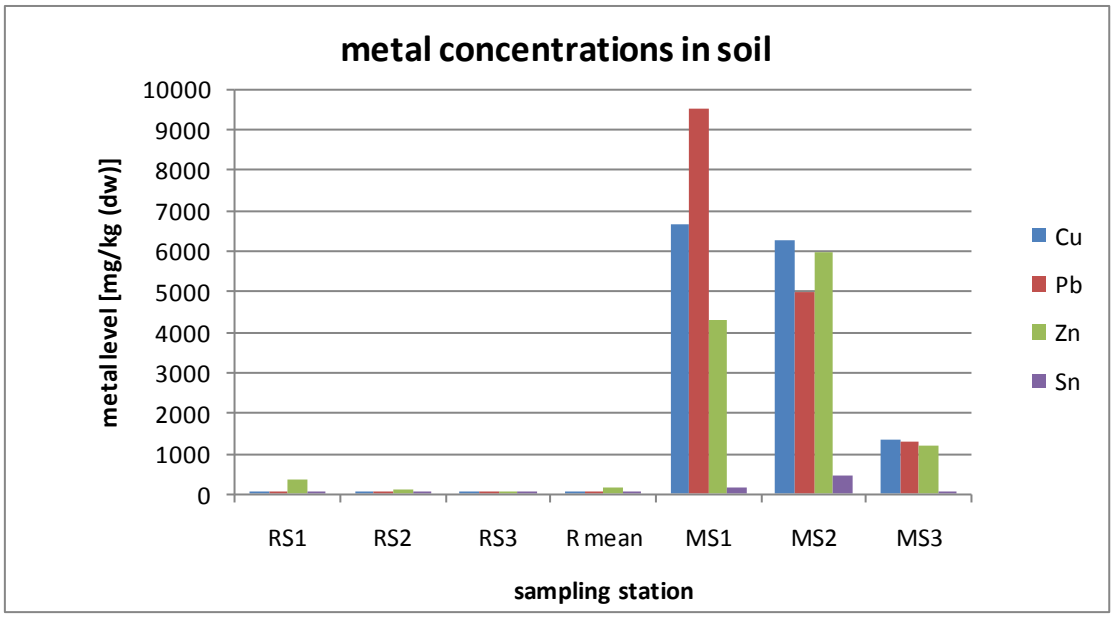

Figure 9: Metal levels in soil. The graph shows the concentrations of the metal copper, lead, zinc and tin that were analysed in the soil samples. For the reference (R) additionally the mean value of the three analysed samples is shown.

Comparing the metal concentrations in sediment and soil, all metals had higher concentrations in the soil samples than in the sediment samples (table 6).

Table 6: Metal concentrations in sediment and soil samples. The table summarises all analysed metal concentrations in the sediment and soil samples from Brunnsviken marina and reference.

Sample	Metal concentration [mg/kg (dw)] (±SD)			
	Cu	Pb	Zn	Sn
R sed mean	9 (± 9)	10 (±8)	58 (±50)	2 (±2)
M1	888	894	1230	29
M2	497	824	1770	63
M3	310	649	1530	47
M3 20-30 cm	162	316	937	40
R soil mean	29 (±13)	51 (±13)	172 (±151)	3 (±2)
MS1	6670	9520	4320	170
MS2	6270	4990	5960	445
MS3	1330	1300	1220	50

3.2.1.2 Organic compounds

The analysed concentrations of PAH (sum 16) and the organotin compounds monobutyltin (MTB) dibutyltin (DBT) and tributyltin (TBT) are shown in table 7. The highest concentrations of PAHs in sediment were measured in the 20-30 cm sediment with 63 mg/kg (dw). Half of this was measured in the marina surface sediment and about 60 times less in the reference. MTB was also highest in the 20-30 cm sediment (1.4 µg/kg (dw)). The levels of DBT and TBT were about 20 times higher in the marina surface sediment than in the marina 20-30 cm. The highest concentration was 358 µg/kg (dw) DBT. Highest organotin level in the reference sediment was 3.02 µg/kg TBT.

The analysed PAH concentration in the marina soil was 5.9 mg/kg (dw) and thus about three times higher than in the reference soil.

The concentrations of MBT and DBT in the marina soil were both about 3000 µg/kg (dw). The concentration of TBT was more than 14 times higher. The highest concentrated organotin compound in the reference soil was 1.31 µg/kg MBT (table 7).

Table 7: PAH and organotin levels in sediment and soil. The table shows the concentration of the sum 16 PAHs and of monobutyltin (MTB), dibutyltin (DBT) and tributyltin (TBT) that were analysed in the pooled sediment and soil samples from the marina and the reference area and in the bottom 20-30 cm sediment in M3.

Sample	PAH (sum 16) [mg/kg (dw)]	MTB [µg/kg (dw)]	DBT [µg/kg (dw)]	TBT [µg/kg (dw)]
R sed. pooled	<1.3	1.26	1.45	3.02
M sed. pooled	31	<1	358	270
M3 sed. 20-30m	63	14.4	33.7	19.9
R soil pooled	1.9	1.31	<1	<1
M soil pooled	5.9	2990	2930	44800

3.2.2 Metal concentrations in leachates

The analysed concentrations of the metals copper, zinc and lead in the leachates and the surface water are shown in table 8. The analysed sediment leachates had a sediment concentration of 10 g/L (ww) which equals dry weight concentrations of 1.07 g/L to 7.33 g/L. The soil leachates all had a concentration of 100 g/L (dw).

The concentrations of all these metals are about the same in filtered surface water from the marina and the reference. Among the sediment leachates M3 had the highest concentrations of copper (6.16 µg/L) and zinc (230 µg/L). In the leachate of the sample M1 the highest concentrations of lead (2.42 µg/L) was measured. The concentrations of all metals were lowest in the reference sediment leachate.

The soil leachate MS1 had the highest concentration of copper (610 µg/L) and lead (301 µg/L). Highest concentration zinc was in leachate MS2 with 2810 µg/L.

Table 8: Metal concentrations in sediment and soil leachates and Bunnsviken surface water. The table shows the analysed concentrations of copper, zinc and lead in the leachates of the leachates of the sediment and soil samples and the filtered surface water from Brunnsviken reference and marina. (Analysed by Karin Holm, ITM Stockholm University)

	Metal [µg/L]		
Leachate	**Cu**	**Zn**	**Pb**
Water reference (filtered)	2.36	8.75	0.14
Water marina (filtered)	2.16	9.68	0.2
R sed. 7.33 g/L (dw)	3.08	10.6	0.21
M1 3.41 g/L (dw)	4.88	68.4	2.42
M2 1.37 g/L (dw)	5.79	58.1	1.24
M3 1.07 g/L (dw)	6.16	230	1.15
R soil 100 g/L (dw)	28.1	75	8.63
MS1 100 g/L (dw)	610	1210	301
MS2 100 g/L (dw)	566	2810	172
MS3 100 g/L (dw)	110	1040	32.9

The percentage of leached metals was approximately twice for the sediment samples than for the soil samples (Table 9). Among the sediment samples the sediment M3 had the highest concentration of leached metal for all three elements. Among the soil sample the

reference soil had the highest percentages of leached metals. Highest calculated percentage of leached metal was for zinc in leachate M3 with 13.5 %. Lowest percentage of leached metal was 0.03 % lead in leachate MS3.

Table 9: Percentage of leached metal. The table shows the percentage of the metals copper, lead and zinc that leached in the water relative to the amount of metals that was contained in the sample used for preparing the leachate.

	Percentage of leached metal [%]		
sample	**Cu**	**Pb**	**Zn**
R sed.	1.08	0.09	0.43
M1	0.09	0.07	1.4
M2	0.53	0.09	2
M3	1.21	0.14	13.5
R soil	0.96	0.17	0.44
MS1	0.09	0.03	0.28
MS2	0.09	0.03	0.47
MS3	0.08	0.03	0.85

Table 10 shows the calculated metal concentrations in the EC_{50} leachates of the two algae test. Highest concentrations had the metal zinc (up to 86 g/L) lowest concentrations were calculated for lead with a highest concentration of 16.6 g/L in the test of MS1 with C. tenuicorne.

Tabelle 10: Metal concentrations in the EC_{50} leachate. The table shows the concentrations of the metals copper, zinc, and lead in the EC_{50} leachate concentration of the sediment and soil leachates from Brunnsviken in the microalga and macroalga test.

	Metal [µg/L]					
Sample	**Cu**		**Zn**		**Pb**	
	P. subcapitata	**C. tenuicorne**	**P. subcapitata**	**C. tenuicorne**	**P. subcapitata**	**C. tenuicorne**
R sed.	-	15.5	-	53.5	-	1.1
M1	4.3	2.3	60.2	32.1	2.1	1.1
M2	1.3	0.8	12.7	8.5	0.3	0.2
M3	0.00003	2.3	0.001	86.0	0.00001	0.4
R soil	-	6.7	-	18.0	-	2.1
MS1	17.1	33.6	33.9	66.6	8.4	16.6
MS2	9.1	10.2	45.0	50.6	2.8	3.1
MS3	5.5	1.4	52.0	13.5	1.6	0.4

4. Discussion

When looking at the results of the biological testing sediment and soil samples from the marina had adverse effects on the three test organisms. However, it was not possible to pinpoint one sediment sample that was the most toxic in all three tests. It has been the same case for the soil samples.

Among sediment samples, sediment M3 had the greatest adverse effects on *P. subcapitata*. The calculated EC_{50} value was 5.3 x 10^{-6} g/L (dw). The test showed that this sample might be the most toxic one, but the calculated EC_{50} value is not that trustworthy since the calculation was based on only three tested concentrations and the effect in all concentrations was higher than 50 %. The calculated EC value seems not to be in a realistic order of magnitude, since it is already below the range of metal ion toxicity (e.g. Cu EC_{50} 130 µg/L, Ni 1070 µg/L (Pereira 2005)).

The growth of *C. tenuicorne* was most affected by the sediment M2 with an EC_{50} of 0.2 g/L (dw), which was quite similar to what was observed with *P. Subcapitata (*0.3 g/L (dw)). Also the EC_{50} values for sediment M1 were in the *C. tenuicorne* test (1.6 g/L (dw)) in the same range as in the microalga test (3.0 g/L (dw)).

In the *M. aquaticum* test the highest growth inhibition of about 43% occurred in sample M1, which had the weakest effects in the algae tests. Sample M2 even slightly stimulated the growth. It has to be mentioned that in this test some average values like the growth rate of the control had a huge standard deviations. And make it difficult to judge about the results.

Of the soil samples MS2 had the strongest effects on *P. subcapitata* with an EC_{50} of 1.6 g/L (dw). The result for this sample in the test with *C. tenuicorne* was about the same (1.8 g/L (dw)). For the sample MS1 the results on the both algae tests were also in about the same range. Only for sample MS3 was the sensitivity in the tests deviating. It was the soil sample that showed strongest effect on *C. tenuicorne*, but in the microalga test the calculated effect concentration was about four times higher and was the least toxic soil from the marina.

The growth of *M. aquaticum* was inhibited as well with about 25% in the samples MS1 and MS3 and 41% in M2. In the reference soil the growth rate was much higher. A stimulation of 150 % relative to the control was calculated. The pre test that was done with the sample M2 gave with an inhibition of 43% the same result as in the main test. This shows the repeatability of the tests and makes it trustable.

All in all the two algae tests had not exactly the same results but both testing of sediment leachates and testing of soil leachates indicated a comparable sensitivity of these bio tests. Whereas the test with *M. aquaticum* was deviating more from the two algae tests in terms of which samples showed the highest toxicity.

The high toxicity of the marina soil and sediment samples and the much lower toxicity in the reference samples were reflected in the data of the chemical analysis.

By looking at the data of the chemical analysis of sediment and soil and comparing the concentrations to threshold values it becomes apparent that both sediment and soil from the marina were highly polluted with the heavy metals copper, zinc and lead and the organic compound TBT.

Sediment with copper concentrations of more than 80 mg/kg (dw) is considered to be highly polluted (SEPA 1999). The measured concentrations in the marina surface sediment exceeded this about four to ten times. The threshold value for lead in sediment of 110 mg/kg (dw) was exceeded six to eight times and the zinc concentrations in the sediment were three to five times higher than the threshold value of 350 mg/kg (SEPA 1999). The reference sediment did not deviate significantly from natural background levels. For TBT in sediment a threshold value of 0.02 µg/kg is recommended (Substance Data Sheet TBT 2005). This was widely exceeded by the marina sediment and even the TBT concentrations in the reference sediment were about 151 times higher than this value.

The metal concentrations found in the Brunnsviken marina sediment were higher than in many other harbours in the Stockholm archipelago (Eklund 2010). Especially the zinc concentrations were at least two times higher than what has been measured as highest concentration in Stockholm city. Also the PAH sum 16 were about two times higher compared to sediments in Stockholm city. However, the TBT concentrations in Brunnsviken were comparable to concentrations found in the boat wash entrance in Stockholm city and about four times lower than the concentrations in another small marina in Stockholm archipelago (Eklund 2010).

The soil samples from the boatyard exceeded widely the threshold value for less sensitive land use (SEPA 2009). For copper a limit of 200 mg/kg (dw) is given and the concentrations in the marina soil were six to thirty-three times higher than this. The lead concentrations were three to twenty-four times higher than the threshold value of 400 mg/kg. For zinc a threshold value of 500 mg/kg (dw) for less sensitive land use is given. The zinc

concentrations in the soil were also two to eight times higher than this. The TBT concentration of 2 mg/L which is suggested as a benchmark value for use on less sensitive land by Eklund and Eklund (2011) is exceeded twenty-two times. The reference soil did not exceed the threshold values for copper, zinc and TBT that are required for sensitive land use (SEPA 2009). The measured lead concentration in the reference soil exceeded slightly the threshold value for sensitive land use (50 mg/kg (dw)).

Both sediment and soil samples were highly polluted, but the measured concentrations of all four metals and TBT were much higher in the soil than in the sediment. This was not reflected in the two algae tests where the sediment leachates showed stronger toxicity than the soil leachates.

The results of these two bio tests are an indication that the metals that were bound to the sediment leached more out than the metals bound to the soil and therefore were higher concentrated in the leachates. The data of the chemical analysis of the leachates support this assumption. When calculating the percentage of metals that leached to the water it becomes apparent that the percentage of leaching metals from the sediment was at least twice as much as from the soil. Also among the sediment samples huge differences in the leaching of the metals were observed. Sample M3 stands out with a higher percentage of leached metals compared to the two other samples. Especially for zinc the percentage is about seven times higher. This higher availability of pollutants in sample M3 could explain why this sample caused, despite lowest metal concentrations among the marina samples, the strongest effect in the microalga test and also strong effects in the macroalga test. Both organisms have been shown to be very sensitive to Zinc. Literature reports for *P. subcapitata* an EC_{50} of 15 µg Zn/L (Heijerick et al. 2005; de Schamphelaere et al. 2004) and for *C. tenuicorne* of 25 µg Zn/L (Ytreberg et al. 2010). The for the EC_{50} M3 leachate concentration in the *C. tenuicorne* test a Zn concentration of 86 g/L was calculated so it is most likely that zinc in the leachate had a strong adverse effect on the macroalga. For the EC_{50} leachate in the *P. subcapitat* test a much lower Zn concentration was calculated, but that this EC value is not realistic was already discussed.

The differences in the leaching could besides different characteristics of the sediment and soil, also be influenced by the leaching procedure. Two different ways of leaching have been

used in this study. Advantages of the way how the soil leachates have been done compared to the sediment leachates are that it is standardised and that it is weighed according to dry weight, which makes it easier to compare the results. All in all there is less variability in the procedure of the soil leachates, which makes it recommendable.

Contrary to the algae tests the growth of the plants of *M. aquaticum* was more affected by the soil than by the sediment. The inhibition of growth relative to the control caused by the sediment and soil samples was in the same range of about 20 to 40 %. However, the growth rates in the marina soil (0.032 – 0.041) were lower than in the marina sediment (0.052 – 0.103).

The control in the soil did not show great growth and some plants showed chlorosis, which indicates that they were not in a good condition. This might be due to a low pH of about 5.3 in the control soil. According to the standard it should be in the range of 7.0 ± 0.5. The plants in the reference soil grew much better. When calculating the inhibition of growth relative to the reference, the growth rate in all marina soils was inhibited by more than 70%.

The *M. quaticum* test differs to the two algae tests, in that the soil had grater inhibiting effects. This difference might be due to the different ways of exposure. Since *M. aquaticum* grows in the sediment it can be affected by both particle bound substances and pore water related contamination. In a study comparing the *Myriophyllum* sediment contact test to *Lemna minor* growth inhibition test on pore water a difference in sediment toxicity due to the two different exposure scenarios was shown as well (Stesevic et al. 2007).

The difference between the algae tests and the *M. aquaticum* test shows that it is important to include different ways of exposure in the hazard identification of polluted sediment/soil in order to get a broad estimation of the possible hazard effects.

Finally the test with *M. aquaticum* seems to be a test that gives another perspective of sediment/soil toxicity and gives quite reproducible results even though it was not possible to perform it exactly like the standard required. There was not sufficient plant material in the required range of weight available. Some plants in the pre culture changed its appearance from green thick leaves to red-brown, thin, feathery form, which reduced the number of plants that looked adequate for the test. The test is also not fully established for testing of soil samples. The control soil used in this study could not offer optimal growth conditions. However, this study showed that in general the *M. aquaticum* test can be used to estimate

the quality of soil. In clean soil like the reference the plants grew well and in polluted soil the growth was affected. Further investigations in testing soil are recommended.

According to the results of this study one of the algae tests and the *M. aquaticum* test should be part in a test battery for sediment hazard assessment. Among the algae tests the algae tests respectively the one with more ecological relevance (fresh water or seawater) can be chosen.

All in all in this study with a combination of bio tests and chemical analysis could show that boat activities in Brunnsviken most likely are the reason to highly polluted sediment and soil in the marina and to such an extent that it is harmful for the environment. The dimension of pollution is quite alarming. If there a several high polluted spots like this marina in a like and the sediment resuspends and spreads in the lake this might harm the ecosystem in the whole lake. The question arises, if fun activities like pleasure boats are worth, to harm the environment in such an extent. Non chemical solutions for anti-fouling that already exist should be promoted more. Also the removal of the old paints should be done in a safe way, so that paint flakes do not just fall to the ground and serve as a long term source in the boatyard soil.

5. Conclusions

- Both sediment and soil in the marina were very polluted by boat activity and exceeded threshold values by many times.

- Sediment leachates showed higher toxicity than soil leachates to both algae tests.

- This study support the necessity of including bio tests in the assessment of sediment and soil quality, since only these can include the bioavailability of chemicals.

- *Ceramium tenuicorne* and *Pseudokirchneriella subcapitata* show comparable sensitivity and usefulness when testing sediment and soil leachates. *M. quaticum* with a different way of exposure gives different results.

- It is important to include bio tests with different ways of exposure in the hazard identification to take into account different factors affecting bioavailability.

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