Experimental zinc stress on benthic diatoms assemblage growth in Fyrisån water

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Zinc stress på bentiska kiselalger från Fyrisån – Laborexperiment

Key Words: Benthic diatoms, heavy metal contamination, valve malformation, heavy metals, DOC, TOC, water assessment.

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Abstract

Benthic diatoms are unicellular, brown algae with siliceous cell walls. They are often used to show water quality in river sand lakes. Benthic diatoms can show elevated nutrient levels, very acid conditions, pollution by organic matter and probably also pollution by heavy metals, which makes them useful biological indicator species. Observed impact of metal pollution on benthic diatoms are reductions in cell number, valve malformation and reduction of some diatom taxa which leads to a change in community structure. The present study is focusing on the impact of the heavy metal zinc.

In aquatic environments, zinc exists in form of divalent cation Zn^{2+}, hydrated zinc (pH=4-7) and moderately weak complexes. At higher pH (7-10) Zn^{2+} is replaced by adsorbed Zinc ($ZnFe(OH)_3$), or aqueous $ZnCO_3$and $Zn(OH)_2$. Zinc release to soil and aquatic environments may be from mining, smelting metals, steel production, waste incineration, burning coal and fossil fuel.

Two hypotheses were tested in laboratory experiments in the present project:

1. The culture of benthic diatoms is possible under the given laboratory (artificial) conditions.

2. High concentrations of zinc (300µg/l zinc) decrease the number of benthic diatoms and increase abnormalities in cell walls.

The first hypothesis "The culture of benthic diatoms is possible under the given laboratory (artificial) conditions." was confirmed. Diatom cell numbers increased during the course of the first experiment.

The second hypothesis that "High concentrations of zinc (300µg/l zinc) decrease the number of benthic diatoms and increase abnormalities in cell walls." was not confirmed by this experiment, possibly due to the loss of the toxic zinc form from solution over time.

Contents List

1. Introduction

Benthic diatoms are unicellular, brown algae with siliceous cell walls. Benthic algae are growing on any surface on the bottom of streams, lakes and also marine habitats. Benthic diatoms are found in shallow water because they need light for photosynthesis and growth [37]. Diatoms are identified by their siliceous cell wall, called frustule [36]. The size of diatoms varies between less than 15 µm to over 1000 µm [37].

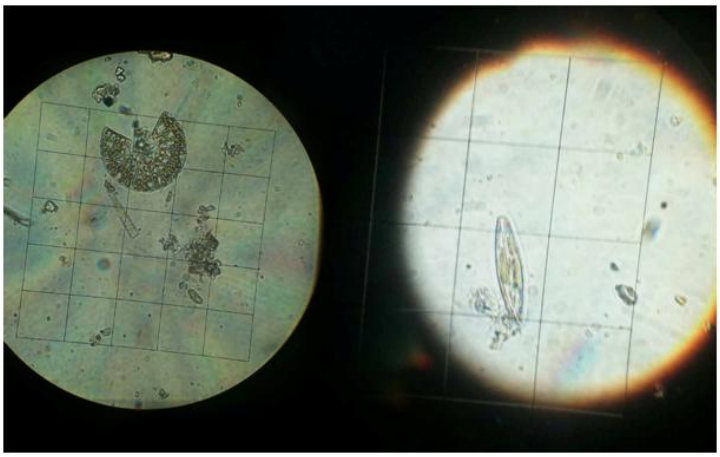

Figure1.Living diatoms of the present project seen in light microscope .Left: Meridion sp., right :Navicula sp. Picture: S. Navid N." Microscope magnification was set on 400 times.

One quarter of photosynthesis in the world is performed by diatoms in general, which includes also the planktonic forms, many of them occurring in the world oceans. [1].Moreover, benthic diatoms are an important link in the food chain in marine and freshwater ecosystems [2]. Chemical, physical and biological factors such as pH, ionic strength, nutrient concentration, light, water velocity and predation levels all have an impact on algal biomass [2]. Climate, geology, topography, and local land-use may all influence these factors [2].

Diatom cell walls comprise two siliceous distinct halves, or valves [14].Contamination has been shown to reduce valve length and increase the number of valve malformations in some studies [15].A malformed valve is also called a teratological valve. Valve malformation can reflect heavy metal pollution in streams and lakes [15].

Natural cycles and anthropogenic disturbances are two main sources of heavy metal pollutions in streams. Anthropogenic disturbances of human beings intensify contamination in stream water equal to or higher than natural cycles [26]. Weathering, soil erosion, waste water, tributary inputs, flood plain, hydrology and water chemistry of the water body may influence the heavy metal concentrations in rivers [26]. All essential metals for organisms have the potential to be toxic in higher concentrations [26].

Benthic diatoms, invertebrates, macrophytes and fish are important indices for water quality assessment. Benthic diatoms are valuable indicators due to their sensitivity and fast reaction to eutrophication and organic pollution and other hazardous environmental conditions [6]. Therefore, there has been some recent research undertaken to demonstrate that benthic diatoms might be useful to indicate the presence of high concentrations of heavy metals [12]. For instance, Duongs work

showed that benthic diatoms are impacted by heavy metals [12]. Among organisms which indicate the influence of metals in water, benthic diatoms might be used to indicate which percentage of different metals can damage and be harmful for biological activity [12]. Species richness and environmental tolerance are valuable characteristics of the organisms in this regard [13].

Some responses may occur after benthic diatoms are exposed to metal pollution; such as reducing number of benthic diatoms, valve malformation and reduction in some species. The relation between metal contaminations and behavior of benthic diatoms against metal pollution is the topic that this study followed. Several studies were carried out regarding influence of heavy metals on benthic diatoms and their responses. Zinc was mostly studied in correlation with cadmium in previous studies. There was a need to study zinc effects on benthic diatoms separately.

In this project 300µg/l zinc concentration was chosen for analysis. This concentration was lower than zinc mining impacted site with zinc concentration (about 800µg/L) [13].It was chosen because of two reasons:

a) 300µg/L was substantially higher about 40 times than zinc concentration in Fyris River and it is higher than190µg/LUSEPA water quality criteria for protection of aquatic life [13].
b) and, because 300 µg/l zinc has been shown to have an effect in a field study in Sweden [10].

Other investigations in Rioumort stream in France indicated that high concentration of zinc (1400 µg/g dry weight) and Cadmium (60 µg/g dry weight) led to reduction of benthic diatoms and increasing number of valve malformations [23]. Reduction of benthic diatoms (cell densities) and deformation of cell walls were observed in Lot River with metal concentration of (Cd15 µg/l and zinc 800µg/l) between 4 and 8 weeks post-contamination [13].

Some environmental stresses such as low current velocity, light intensity, temperature variation, drought and herbicide contamination are known to influence the amount of teratological cells in benthic diatoms while heavy metal contamination and artificial conditions are well-known reasons for teratological forms [4]. Teratological forms in benthic diatoms are phenotypic abnormalities due to non-adaptation of diatoms with environmental stresses [4]. Deformation (teratological forms of diatom valves) of diatoms due to high concentration of metals has been observed in earlier researches [3].

After applying metal or other environmental stresses, benthic diatoms can show different responses. Abnormality of the cell wall of benthic diatoms is an observed reaction of metal influence on them, however in some cases benthic diatoms may adjust to metal contamination. In such adapted diatoms, abnormality of cell walls has not been observed when contaminated by heavy metals [4]. Different species of benthic diatoms can evaluate presence of toxicants or nutrients availability or the interaction between toxicants and nutrients [5]. Increasing some metals such as zinc in rivers and lakes has been shown to be responsible for abnormal valve and teratological forms [5].

This investigation should elucidate whether or not the concentration of zinc has an effect on benthic diatoms and which concentration of heavy metals might have an effect. In this study the response of diatom growth rate and valve malformation to contamination with the metal zinc was tested. Study of

the effects of metals on biological activity in the aquatic environment can potentially be used as a basis to find metal contaminated sites with the help of a diatom indicator.

In detail, this study tried to find the answer on the following two hypothesizes:

1. The culture of benthic diatoms is possible under the given laboratory (artificial) conditions.

2. High concentrations of zinc (300 µg/l zinc) decrease the number of benthic diatoms and increase abnormalities in cell walls.

The overall aims of the thesis were

a) To establish a method to grow naturally occurring diatoms under controlled temperature and light conditions in the laboratory,

b) Study diatom growth and malformation in absence and presence of elevated zinc concentrations,

c) Evaluate the fate of added dissolved zinc throughout time, and test whether diatom growth or malformation effects are due to the occurrence of total, filtered or modeled free zinc in the solution.

1.1. Zinc characterization

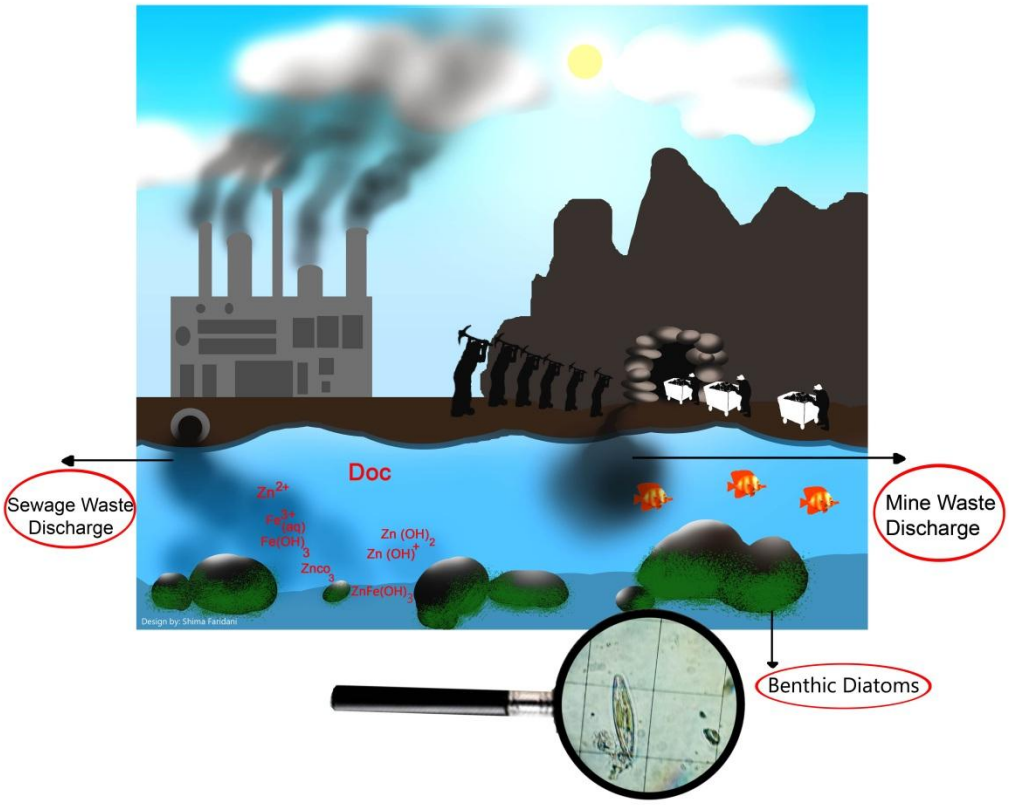

Figure 2 .This figure demonstrate two main sources of zinc. Also, it shows possible species of zinc and some of their complexes in aquatic environment. Picture of benthic diatom was taken by microscope and 400 times magnifying. The figure was designed by Shima Faridani.

Pure zinc is a hard, brittle, bluish–white metal that is part of the transition metals group [19].

Zinc metal is utilized in many industries such as producing batteries, roof covering, coating steels and irons to protect against corrosion (galvanization) [20]. Moreover, zinc oxides are contributed by painting manufactories, rubber products, cosmetics, pharmaceuticals [20]. Zinc is an essential nutrient in low concentration and toxic in high concentration in fresh water ecosystem [32]. In aquatic environments zinc exists in form of divalent cation Zn^{2+}, hydrated zinc (pH=4-7) and moderately weak complexes [20]. At higher pH (7-10) Zn^{2+} can be replaced by sorbed Zn ($ZnFe(OH)^{-}$),or $ZnCO_3$ $Zn(OH)_2$. Zinc release to soil and aquatic environments may be from mining, smelting metals, steel production, waste incineration, burning coal and fossil fuel [20].

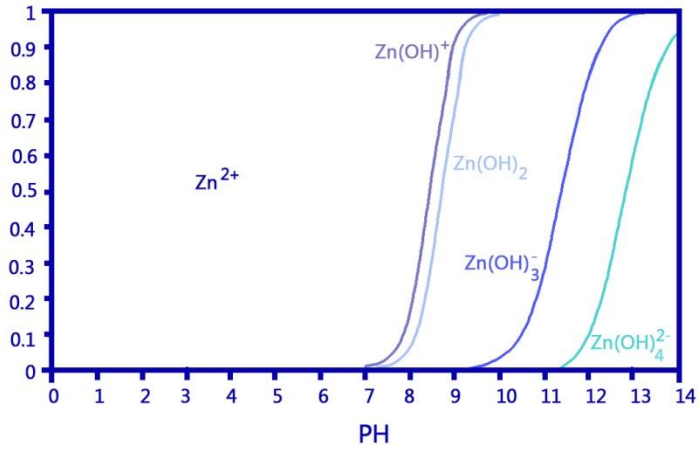

Figure 3.Speciation of zinc as pH function. Metal concentration is 20 ppm at 25 °C [30].This figure was redrawn from another study [30].

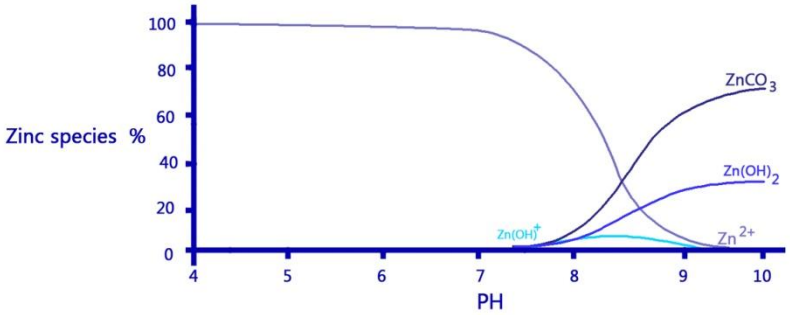

Figure4.Zinc speciation as a function of pH in water runoff by MINTEQA2 and WHAM [31].This figure was redesignedfrom another study[31]. ZN^{2+} is dissolved in water in pH lower than 7, but it is bound to CO_3^{2+} and OH^- when pH increases, and is almost totally solid at pH > 9.Only dissolved Zn^{2+} probably is toxic for the diatoms, as particulate Zn probably cannot been taken up into the cells.].This figure was redrawn from another study [30].

Definitions:

1.2. Total zinc in water

It includes Zinc (metal) existence in water of dissolved metal, colloidal metal, metal desorbed from mineral and organic particles [7]. It is known as non-filtered water sample.

1.3. Filtered zinc in water

It contains part of zinc (metal) existence in the water that can pass 0.45μm filters. Filters should be rinsed and washed by acid before filtering the water [7].

1.4. Dissolved Organic Carbon (DOC)

Dissolved Organic Carbon is a definition of the organic carbon dissolved in water.
Generally, DOC is organic carbon, remaining in a water sample which is soluble or colloidal and it can pass through a 0.45μm filter [16]. Dissolved organic carbon has a function in carbon cycle, energy balance, acid-base chemistry of low-alkalinity streams and light penetration in streams [35] [32].

DOC decreases in lower depth because of chemical adsorption and biological degradation [35]. Furthermore, DOC effects on water color in streams. Dissolved organic carbon increases darker color in water. Vegetation and soil outside of river and biota such as algae and macrophytes are two main sources of DOC in aquatic environments. In different seasons hydrological conditions such as flow rate influence concentration of DOC in aquatic ecosystems [32]. DOC contributes to acidification, light penetration in rivers and lakes, forming complexes with heavy metals and carbon cycle [18][17]. Intense interaction between dissolved heavy metals such as zinc and DOC is important in transport of contaminants [32].

1.5. Total organic carbon (TOC)

Total organic carbon is the gross amount of carbon that can be found in natural water [16]. It contains dissolved organic carbon (DOC) and particulate organic carbon (POC). Particulate organic carbon stands for smaller fraction of TOC(less than 10%)[17]. Particulate organic carbon cannot pass a 0.45-μm filter [17].

1.6. Absorbance

Absorbance (optical density) is a measure of logarithm of ratio light intensity absorbed by solution when light passes through sample [21]. A spectrophotometer is used for measuring absorbance. Water color is an important factor for growing of benthic diatoms due to fact that they need light for photosynthesis, growing and reproduction. Humic substances and soil organic matter lead to various colors in water [34]. Dissolved Organic acids give the streams yellow to black colors [34]. Therefore, measurement of absorbance can help us to observe fate of DOC in this experiment. Dark water due to presence of dissolved organic carbon cause light depletion and may hamper the growth of benthic diatoms.

1.7. Heavy metals

Heavy metals refer to trace elements that have ≥ 3 g/cm^3 densities and may induce toxicity, harmful biological effect or ecological problems [27, 40].From a water quality stand point, metals inducing toxicity in the aquatic environment can be called heavy metals [27]. Heavy metals can appear in aquatic environments in two forms; dissolved and particulate. Dissolved (Filtered) metals more than particulate metal cause damage to organisms due to their free movement and toxicity [27]. Some level of metals is necessary for growing organisms but concentration over essential amount may lead to toxicity in organisms [27]. Sorption-desorption of metals to sediments effect the contamination of an aquatic environment by heavy metals [27].

2. Material and methods

2.1. Fyrisån catchment

Fyrisån is a river located in Uppland province in Sweden. Fyris River (59°51'32.8" N, 17° 38' 4.9") after crossing Uppsala city flows in to in Lake Ekoln (Northern of lake Mälaren) This river has about 80 km length and average mean flow of 13-14 m^3s^{-1} . The Catchment area of Fyrisån River has covered 2000 km² comprises 59% forest, 33% agriculture, 4% wetlands, (~2%) urban areas, and (~2%) lakes [9]. Glacial till in forest land and clay soil in agricultural land are common soil texture in catchment. According to SMHI (Swedish Meteorological and Hydrological Institute) driest month in this catchment is April and wettest and warmest month is July whereas coldest months are December and January [9].

Figure 5.This map shows Fyris River catchment in Uppsala. This map was designed by Shima Faridani. Information was taken from Fyrisån Water Association [38].

2.2. Background pH value and concentrations ofNO_2+NO_3^--N, Fe and Zn in Fyrisån from the Swedish monitoring network

a) pH

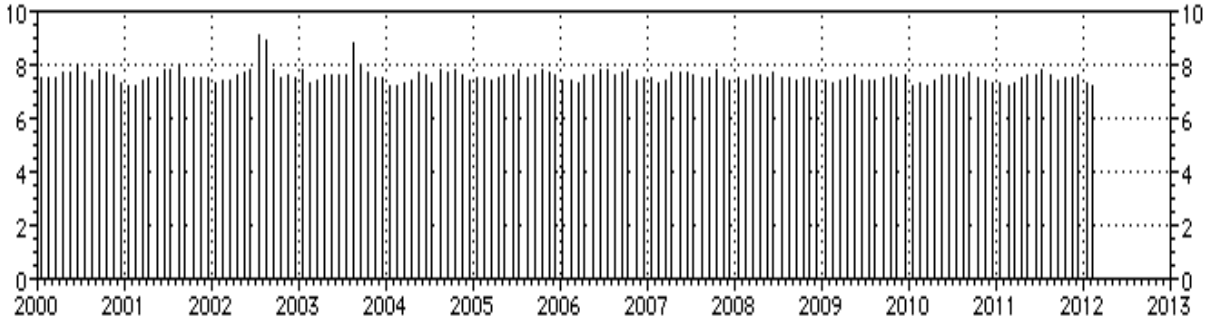

Figure 6.This figure show the fluctuation of pH value between2000-2012 .pH value was measured mostly pH>8. Only in three measurements during 12 years pH value showed pH<8[28].

b) NO_2+NO_3^--N µg/l

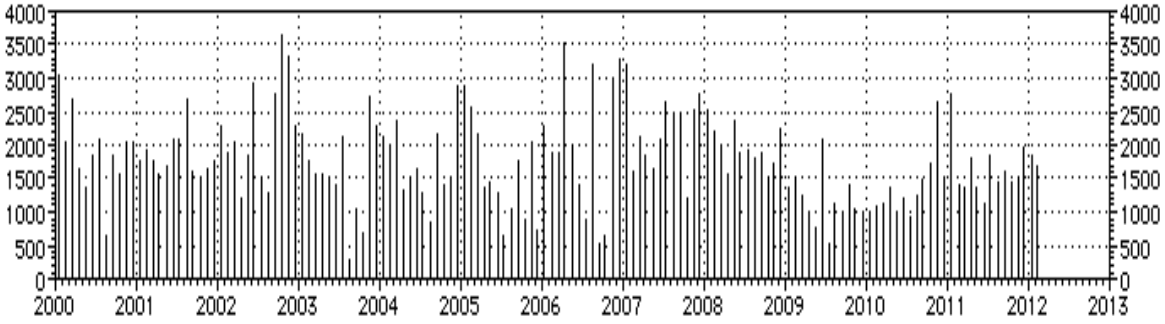

Figure 7.This figure shows the fluctuation of NO_2+NO_3^--N µg/l between2000-2012. The highest concentration was shown NO_2+NO_3^--N >3500 µg/l in 2002 [28].

c)Fe µg/l

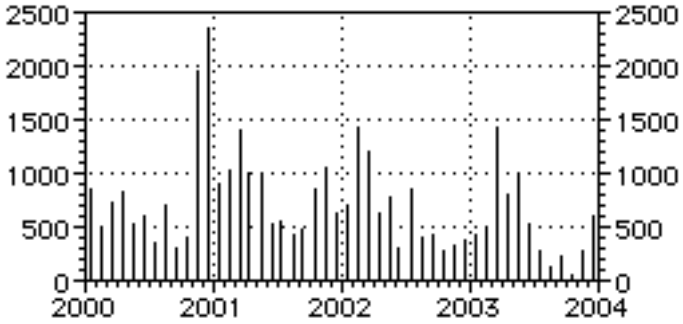

Figure 8.This figure shows the fluctuation of Fe µg/l between2000-2004. The highest concentration was registered Fe = ~2400 µg/l in 2000 [28].

d) Zn µg/l

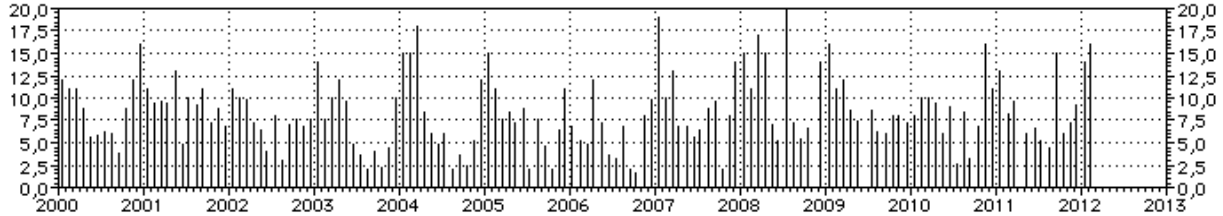

Figure 9.This figure shows the fluctuation of Zn µg/l between2000-2012. The highest concentration was registered Zn = ~20 µg/l in 2008 [28].

2.3. Sampling

Collection of benthic diatoms was carried out twice (2012.03.08 and 2012.04.16) during this project from Fyrisån River at the fish stairs in central Uppsala in Sweden. The stones were chosen from medium water velocity and close to a river bank. Detaching benthic diatoms was undertaken by a toothbrush from the upper part of river's stones as diatoms are photosynthetic and predominantly grow on the upper part due to the presence of higher light levels. Benthic diatoms were stored in river

water and transported at ambient temperature to the laboratory for experiments. Additionally, about 30 liters of Fyrisån water were brought to the laboratory.

2.4. First experiment (05.03 -27.03.2012)

After first sampling on 5th of March four wells of a 24-well tissue culturing plate (1 ml volume) were prepared to observe the growing rate of diatoms and to demonstrate that culturing of diatoms is possible under lab conditions. The temperature fluctuated between 15.1$^{\circ C}$ and 17.8$^{\circ C}$ during the 22 days of first experiment. The diatoms received 12hrs of light followed by a dark period. Light was between 4.6E+15 Q/cm^2.s and 1.2 E+15 Q/cm^2.s during the experiment. A temperature logger registered temperature during this experiment. Due to 2.7$^{\circ C}$ fluctuation, the second experiment was conducted in a constant temperature room with a more consistent temperature.

Figure 10.This figure shows counting of benthic diatoms in Present project. Benthic diatoms were observed in glass chambers. Picture: S. Navid N.

During the first experiment, river water was added to the chambers every second day to ensure sufficient nutrient concentrations for the diatoms. When adding river water to chambers, 1mL surface water was initially removed from chambers by plastic pipette then 1 mL new river water was added.

Counting of diatoms to calculate the growing rate was done every day from the 9th of March till 27th of March except Saturdays and Sundays. Counting was done under a microscope (M 34WILD Heerbrugg) with 200 times magnifications (Figure 14).

2.5. Acid washing

All glassware (Erlenmeyer flasks, beakers) and sample tubes were washed with nitric acid (1%) before usage. The equipment was kept in nitric acid for at least 24 hours and subsequently rinsed with deionized (DI) water. Cleaning glassware by nitric acid is necessary for removing metal contamination. All Erlenmeyer flasks were labeled with numbers from 0 till 57.

2.6. Second experiment

Experimental setup

In total 57 microcosms were set up. 54 Erlenmeyer flasks were filled with a defined volume of diatom suspension and then filled up to 500 mL of total volume with river water filtered through a 65 µm mesh to exclude grazers (predators) from the samples. 500 mL was chosen to have sufficient water sample for biology analyzing such as cell counting, malformed cell counting and chemical analyzing such as nutrients concentration, DOC, TOC, total zinc, filtered zinc and absorbance. Thereafter, three additional Erlenmeyer flasks were filled with 500 mL river water, to study the effects of diatoms on zinc concentrations.

Figure11.Growing of benthic diatoms within 57 Erlenmeyer Flasks in laboratory .Picture: S. Navid N.

All Erlenmeyer flasks were placed under fluorescent lamps light. An automatic light switcher was used to set twelve hours light and twelve hours dark to mimic natural light conditions. Temperature in the constant temperature room varied only slightly, between 9.7$^{\circ C}$ and 10.3$^{\circ C}$. After one week zinc was added to 30of the Erlenmeyer flasks (27 with diatoms and 3 without diatoms). The final zinc concentration was 300µg/L.

2.7. Biological analyses

Sampling was done twice per week, sampled flasks were chosen randomly. Different glass chambers with three different volumes (2, 25and 50 mL volume) were used for counting benthic diatoms in order to get an estimate of sufficient sample volume to count. Sonication is a method used to remove particles from sample by sound wave energy. Erlenmeyer flasks were sonicated to detach the benthic diatoms from Erlenmeyer flasks wall by applying ultra-sonic waves in a water bath.

Figure 12.This picture indicates Sonication of Erlenmeyer flasks in present project. Sound waves were detached benthic diatoms from Erlenmeyer flasks. Sonication was carried out before each sample counting. Picture: S. Navid N.

To find the optimal sonication duration for detaching benthic diatoms from the Erlenmeyer flasks and to check if the number of split diatom cells would increase by ultrasonic vibration, different sonication times were tested. Five minutes sonication was determined to best detach the diatoms from the walls of the Erlenmeyer flasks, without increasing the number of split (damaged) cells.

a) Analyses

One mL Lugol's Iodine solution was applied to all Erlenmeyer flasks in order to kill the benthic diatoms and others organisms. Then, all Erlenmeyer flasks were filled up to 500 mL with deionized water to compensate for the water that has been taken out for chemical analysis. This filling up was done to have fixed volume (500 mL) in case of counting benthic diatoms and malformations. Third Erlenmeyer flasks were put in a sonication bath in order to detach all benthic diatoms from the wall of the Erlenmeyer flasks, to get a precise estimate of the cell numbers in the flasks. Counting of the diatom cell numbers was done in 25 mL and 50 mL chambers, after 24 hours sedimentation time. The rest of the samples were stored in 500 mL plastic bottle for analysis of diatom valve malformations.

These routines were followed twice a week, but cell counts were only made once a week. Counting of benthic diatoms was done with a microscope (M 34WILD Heerbrugg or M75 Carl Zeiss) using 400 times magnification.

b) Counting diatoms (cell density)

In the second experiment, benthic diatoms were grown 672 hours for control and impact samples from16.04.2012 till 14.05.2012. Glass chambers were used for counting of benthic diatoms in entire experiment.

C) **Counting malformation diatoms**

Diatom community composition and diatom valve malformations were analyzed in three samples: one sample from day 0, one control and one treatment sample from the last day. The diatom samples were oxidized with hydrogen peroxide and hydrochloric acid (hot hydrogen peroxide oxidation), and then mounted with Naphrax (refractive index = 1.74, Brunel Microscopes Ltd.) to permanent slides (CEN 2003). About 50 diatom valves were grossly identified and examined for the occurrence of malformations at a 1000 times magnification (Nikon Eclipse 80i microscope, oilimmersion 1,4 Plan Apochromat objective).

Overall experimental design

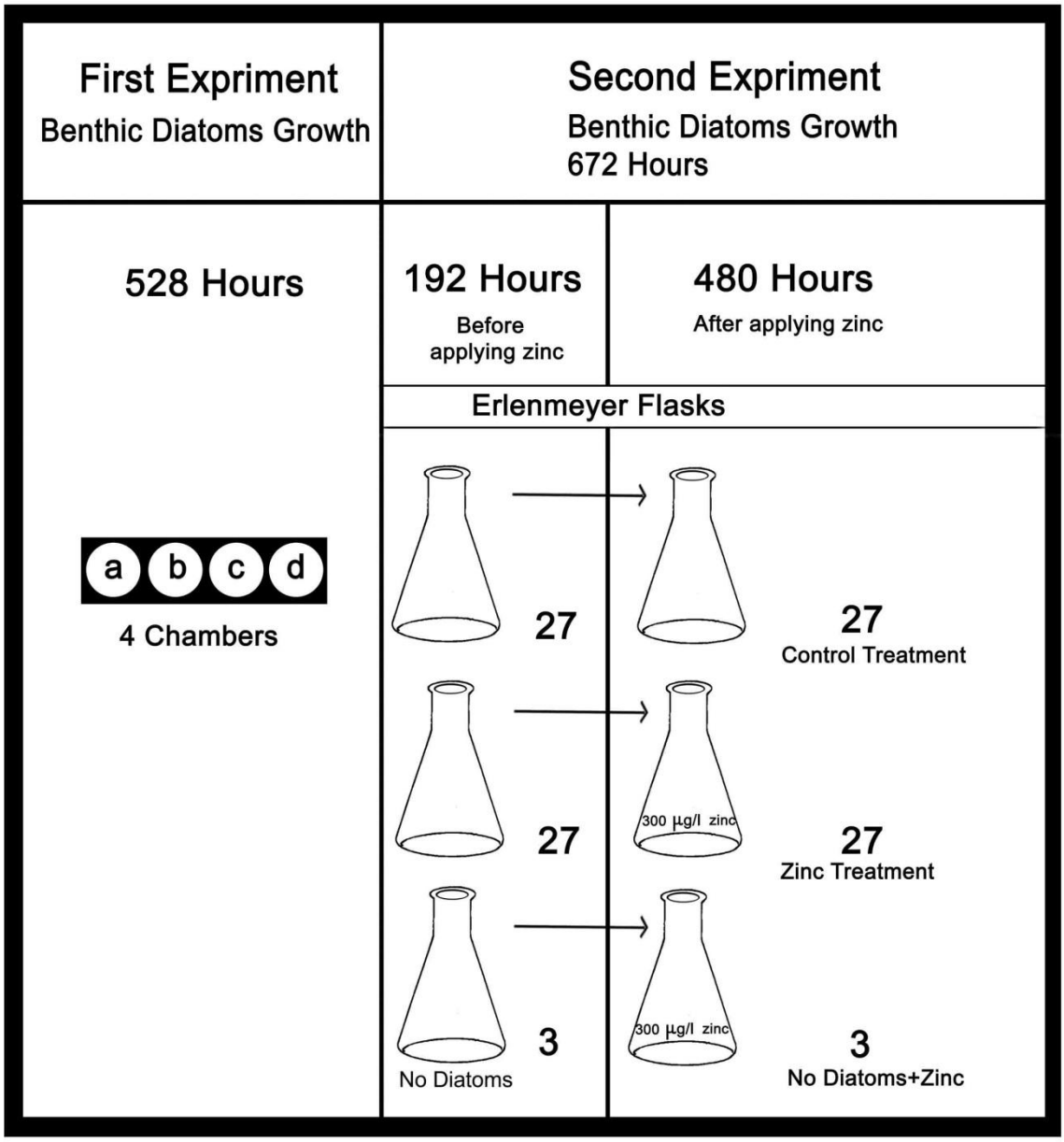

Figure 13.Zinc solution added to 27 impact solutions after192 hours. This figure is designed by Shima Faridani.

In first experiment, 4 chambers were prepared to observe growing of diatoms daily during the 22 days of experiment.

In second experiment 54 Erlenmeyer flasks were prepared to have 6 sample solutions (3control-3 zinc added) for 8 days of sampling and analyzing during 28 days. The remained 6 extra samples were retained as reserved samples. No diatom Erlenmeyer flasks were prepared to observe destiny of zinc in solutions without presenting benthic diatoms. Furthermore, in all sampling days for every treatment 3 replicates was taken to give us additional data. These replicates were prepared to enhance the results of data analyzing.

2.8. Chemical procedure

a) pH measurement

pH and temperature of the solution was measured before harvesting in every beaker during the experiment. pH measurements(WTW pH-Electrode SenTix and pH meter WTW Inolab 720) were carried out under experimental conditions in the constant temperature room. Furthermore, solution temperature was measured by pH meter during measuring pH value at the same time because temperature influences H+ ions activity and as a result of that pH value is changing.

$pH = - \log [H+]$

In process of measuring pH value it was essential to calibrate the pH meter to achieve precise results. Standard buffer solution (pH=4, pH =7) were measured during the calibration procedure. Meanwhile, pH electrodes were rinsed every time of utilizing by Milli-Q water.

b) DOC, TOC, Total zinc, Zinc filtered sampling

During the four weeks of experiment, every Monday and Thursday one sample from control Erlenmeyer flasks and another one from zinc added Erlenmeyer flasks were taken for measuring DOC, TOC, Total Zinc concentration, Zinc filtered concentration. 15mL tubes were used to collect the samples and afterwards samples were stored in a fridge. 10 mL samples were taken by syringes and filters for DOC and filter zinc. For TOC and zinc samples, syringes without filters were used and 15mLwater samples for TOC and 10mL water sample for zinc were taken.

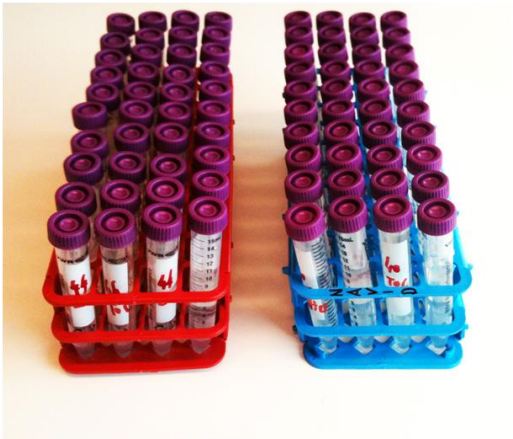

Figure14. This picture shows DOC, TOC, Total zinc, filtered zinc samples .These samples were analyzed in second experiment. Picture: S. Navid N.

1 mL of 0.5 N HNO_3 was added to all of the total zinc and filtered zinc samples in order to preserve them for the duration of the experiment. This 1 mL was not considered in our Total zinc and filtered zinc results.

c) Absorbance

Absorbance of water samples was measured twice a week. In total twenty samples absorbance comprise of control, zinc added solution and river water without diatoms were measured during whole experiment. Spectrophotometer (AvantesAvalight-DH-S-BAL) was used for measuring absorbance.

Three standard steps consisting of dark, reference and river water samples were undertaken to evaluate our samples precisely. Absorbance fluctuations can estimate how much DOC is left in our solutions.

d) Chemistry analyzing for changing nutrients during four weeks

Ten samples from the entire four weeks of experiment were sent to a chemical analysis laboratory for measuring total phosphorus (TP), total nitrogen (TN), phosphate (PO_4^{-3}), ammonium (NH_4^+), silica (Si), nitrate (NO_3^-) and nitrite (NO_2^-).

2.9. Calculating the zinc speciation using the Visual MINTEQ software

Visual MINTEQ is computer program that was designed by Jon Petter Gustafsson for the calculation of speciation, solubility, and equilibrium of solid and dissolved phases of minerals in water solution [39] .This software is usually used for natural waters. Visual MINTEQ was used as chemical equilibrium model for the calculation of metal speciation.

Missing data in our experiment were filled in data set by using simultaneous sample analyzing data in Fyris River for precise establishing our calculations [28].

Four reactions were considered for removal of zinc;

1) $Zn^{2+}(aq) + Fe(OH)_3(s) \rightarrow Zn\text{-}Fe(OH)_3(s) + 2H^+(aq)$

2) $Zn^{2+}(aq) + H_4SiO_4(s) \rightarrow Zn\text{-}H_2SiO_4(s) + 2H^+(aq)$

3) $n\ Zn^{2+}(aq) + (1\text{-}n)\ Ca^{2+}(aq) + CO_3^{2-}(aq) \rightarrow Ca_{(1-n)}\ Zn_{(n)}\ CO_3(s)$

4) $5Zn^{2+} + 2\ CO_3^{-2} + 6\ OH^- \rightarrow Zn_5(CO_3)_2(OH)_6(s)$

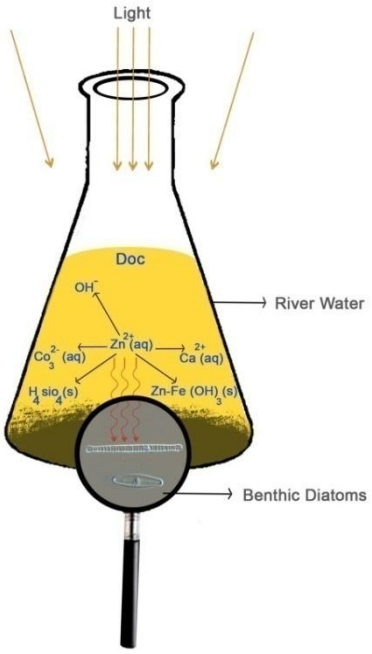

Figure 15.This figure shows possibility of zinc complexes with other chemical substances .These speculations was analyzed by Visual MINTEQ. The figure was designed by Shima Faridani. The picture of benthic diatoms was taken by Steffi Gottschalk.

2.10. Statistics

ANCOVA (analysis of covariance) was used to test the hypothesis if the zinc treatment as categorical factor had an impact on the growth of the diatoms. The method also analyzed if the continuous factor time had an impact on the growth of the diatoms and if there was an interaction between the zinc treatment and time. The method is i.e. testing if the two growth lines have the same or a different inclination. ANCOVA could be used because the single values of the cell numbers were independent, as each value represented a new batch. For analysis, JMP software was used.

A transformation of each observation was needed because the variation increased with increasing cell number. A quadratic function was the best fit for the algal growth curve, so the values were square-root transformed. After transformation, the variation was constant around the now linear regression line, the growth line, which also simplified the analysis.

To test if the two factors had a significant impact on the cell number, the method is assessing the residual variance around regression lines that are adapted using a “dummy variable” which is 0 without zinc and 1 with zinc, and time as factor explaining the growth.

Control, no zinc: y=intercept+b2*time

With zinc: y=intercept+b1+b2*time+b3*time

Then, the regression parameters b1 and b3 and their variation is used to assess if there is a difference between zinc and control, i.e. if the regression lines have the same inclination (b3) or different starting points (b1).

3. Results

3.1. First experiment

Benthic diatoms in four different chambers (replicates) were grown during the entire first experiment. This experiment showed possibility of growing benthic diatoms in all replicates in laboratory and artificial conditions. After 300 hours the growing rate of diatoms increased quickly (Figure 16). Although laboratory conditions were applied similarly the number of cells counted for chamber A was considerably higher than other chambers. These higher cells number in chamber A can be explained by the presence of higher benthic diatoms grazers (predators) in other chambers, because the 65 µm mesh was not used for the first experiment. This experiment was an appropriate step for the second experiment because it was shown that necessary conditions such as sufficient light, nutrients and temperature for growing of benthic diatoms were prepared.

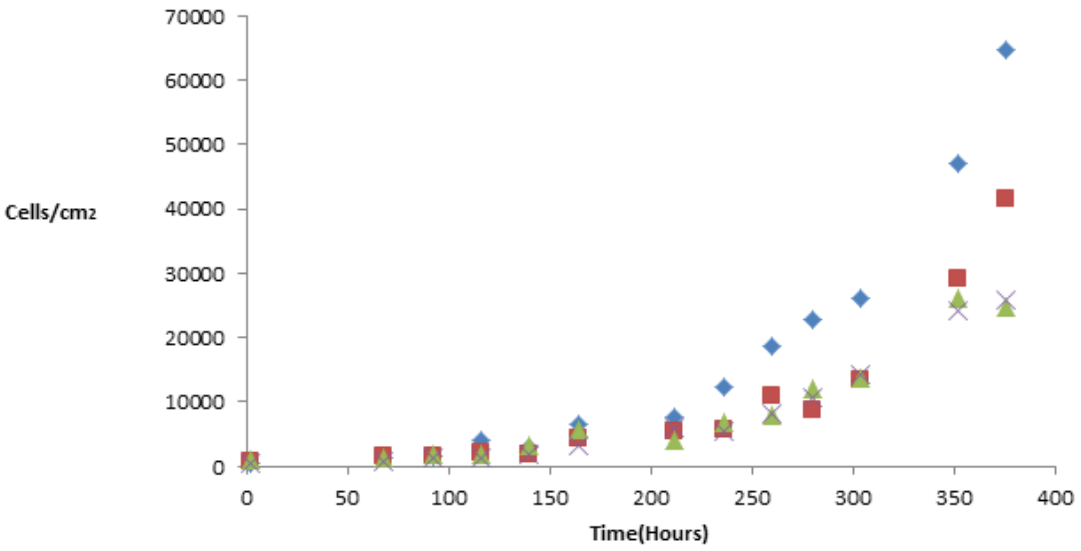

Figure 17.Diatoms growth rate in the first experiment.♦, Chamber A; ■, Chamber B; ▲, Chamber C; ×Chamber D.

3.2. Second experiment

Counting of benthic diatoms in control treatment and zinc added treatment was shown growing of them in both treatments almost identically. Artificial laboratory conditions such as light, temperature, nutrients were retained equally for the whole samples. The maximum cell number was counted in zinc added treatment. This result demonstrated that 300 µg/l of added zinc did not influence the number of benthic diatoms.

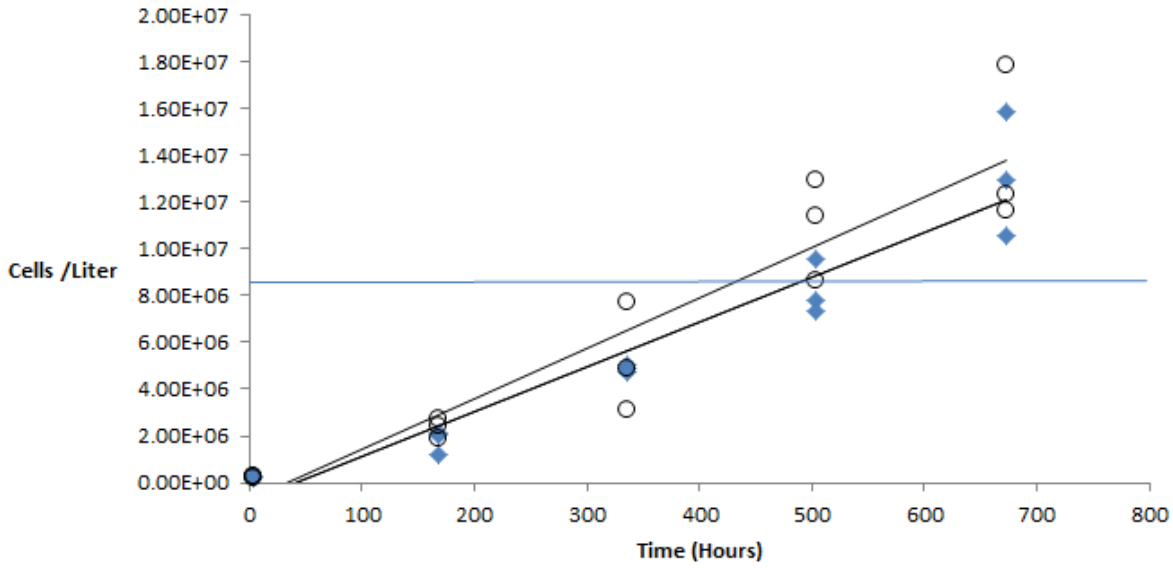

Figure18.Growthrate of cells in second experiment also two regression lines for control and zinc added solution.♦, control treatment; ○ zinc added treatment.

3.3. pH Value

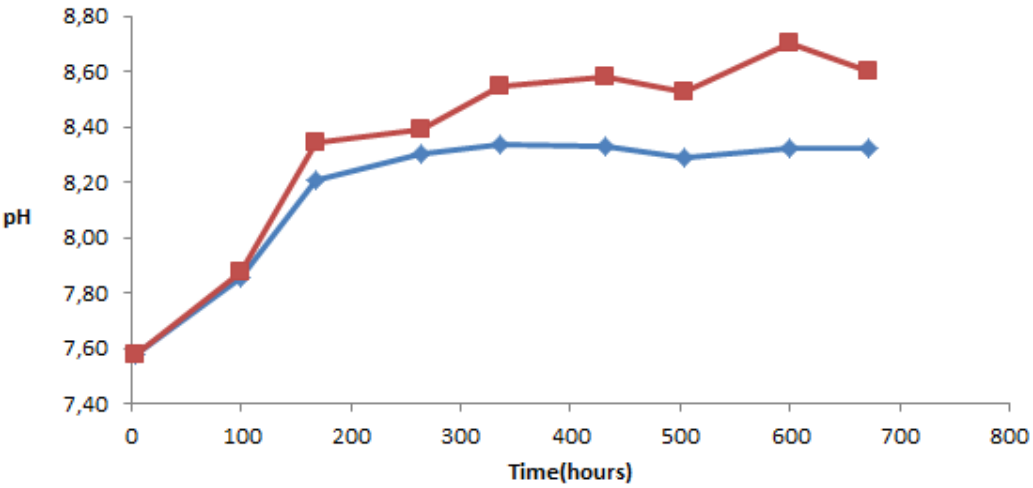

Figure 19.Fluctuations of pH in second experiment. ♦, control treatment; ■zinc added treatment.

Initial pH measurement was shown pH= 7.6 after 3 hours from sampling site.The pH ranged between 7.58 to 8.34 in control treatment. At highest concentration of zinc the pH was shown 8.4 in zinc treatment and the max pH was measured 8.7.

The pH was increased in controls and zinc treatments probably due to releasing CO_2 from river water to air. Fyris River initial data indicated super saturation of CO_2 in water samples. Super saturated CO_2 in water was lost from water to have equilibrium concentration with atmosphere.

Another reason for decreasing CO_2 in water and increasing the pH could be consumption of CO_2, HCO_3^-, CO_3^{2-} by benthic diatoms.

3.4. Dissolved Organic Carbon

The results show DOC in the control treatment decreasing during the experiment as expected, whereas in zinc added solutions and no diatom solutions, DOC did not decrease during the course of the whole experiment.

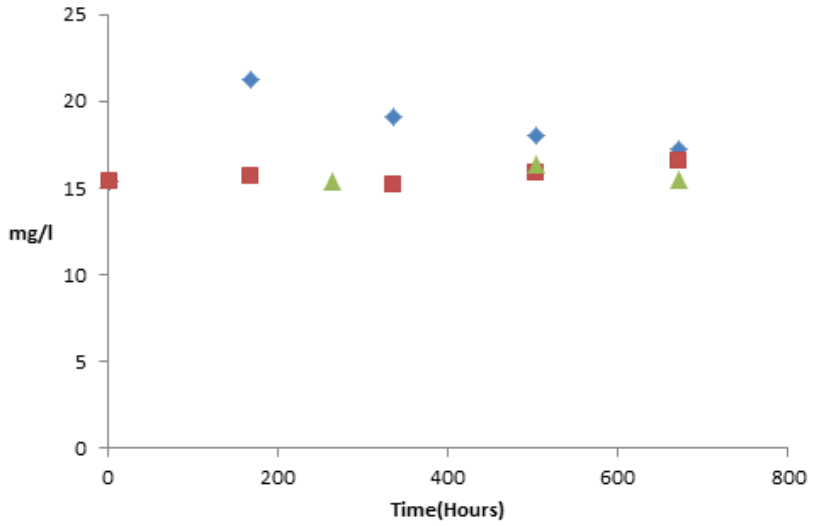

Figure20. Fluctuating of Dissolved Organic Carbon mg/l in second experiment.♦, control treatment; ■ zinc added treatment; ▲, no diatoms + zinc.

3.5 Absorbance

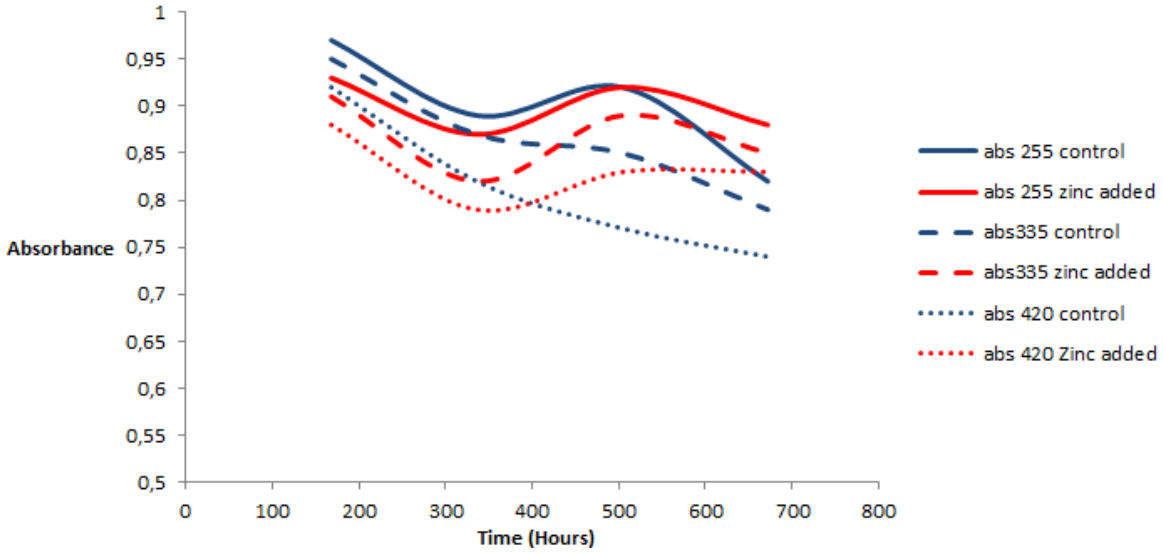

Figure 21.Absorbance was reduced during our experiment. Increasing was observed between 300-500 hours in 255(nm) zinc added and 335(nm) zinc added graphs. Probably, it was occurred due to pollution of photometer or laboratory instruments.

Photometer indicated a 20-25% reduction observed in control treatment solutions. In zinc treatment solutions, less DOC reduction was observed .This decrease may have occurred by photo bleaching in the samples.

3.6 Zinc

Zinc decreased over time in both total zinc and filtered zinc. Total zinc in the control treatment decreased 76.62%, and a 78.62% reduction was observed in the zinc added treatment. Filtered zinc in control treatment was reduced by 46.66%, while an 82.85% decrease was observed in the zinc added treatment.

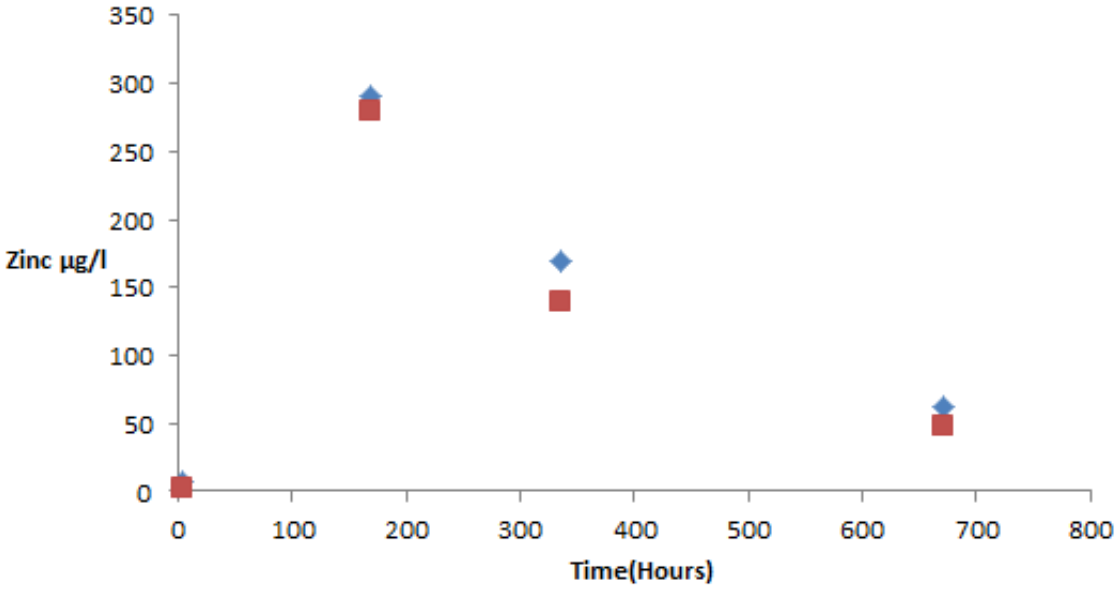

Figure 22.Changing of total and filtered zinc during experiment zinc during experiment. ♦, Total zinc ■, Filtered zinc.

3.7. Silicon

Silicon decreased over time in control and zinc added solution. Silicon concentration decreased intensively in the zinc added treatment. Silicon concentration decreased by 89.3% in the zinc added treatment, whereas in the control treatment a 61.8% reduction was observed.

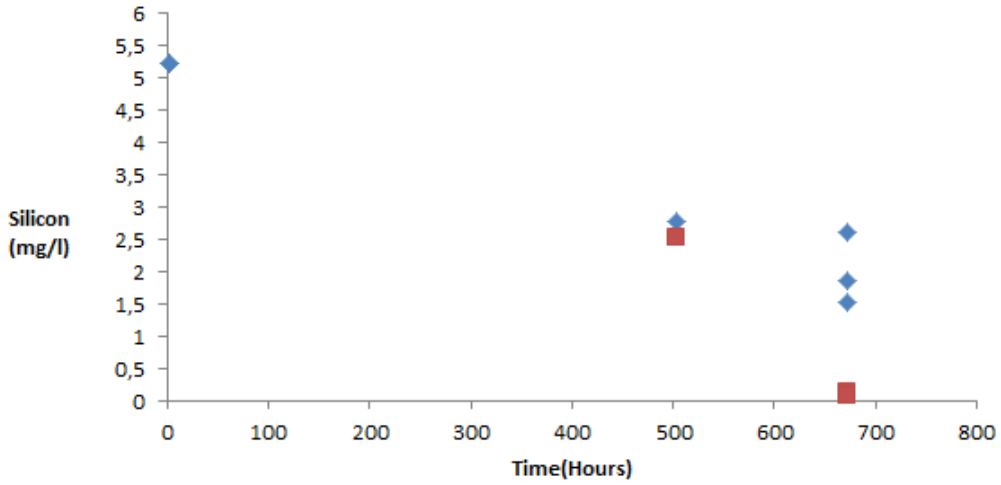

Figure23.Total Silicon changing in second experiment. ♦, control treatment; ■ zinc added treatment.

3.8. Malformations

The dominating diatom found in the zinc sample belonged to the *Achnanthidium minutissimum* (Kützing) Czarnecki complex, additionally valves were found belonging to *Diatoma tenuis* Agardh, *Fragilaria gracilis* Østrup, and the genera *Nitzschia* and *Navicula*s.l. No malformations were observed whatsoever.
The control samples are also dominated by *Achnanthidium minutissimum* (Kützing) Czarnecki complex, additionally valves of *Meridion circulare*, *Fragilaria* spp., *Nitzschia* spp. and *Diatoma tenuis* Agardh were found.

3.9. Statistics

There was no significant effect of the zinc treatment, but there was as expected a significant effect of time: Both control and zinc treated diatoms had the same growth curves (ANCOVA, effect of time $p < 0,0001$, effect of zinc $p = 0,697$). Thus, our working hypothesis was rejected.

4. Discussion

With presented data, the first hypothesis "The culture of benthic diatoms is possible under the given laboratory (artificial) conditions." was confirmed. Diatom cell numbers increased during the course of the first experiment.

The second hypothesis that "high concentrations of zinc (300 µg/l zinc) decrease the number of benthic diatoms and increase abnormalities in cell walls" was not confirmed by this experiment.

4.1. First experiment set up

Growth rate of benthic diatoms increased in all of four glass chambers. First successful result was obtained in the first experiment when benthic diatoms were grown in laboratory with artificial conditions. Establishing a method to grow naturally occurring diatoms under controlled temperature and light conditions in the laboratory was done successfully. Some negative points such as temperature fluctuation and presence of grazers in chambers were shown by first experiment. Counting benthic diatoms and sometimes repetition of counting took 3-4 hours for each chamber particularly during the final days of experiment. Persistency and continuity were required to obtain the precise results. All of our goals were carried out in first experiment successfully and everything was prepared for second experiment. First experiment was formed as base for second (main) experiment. Meanwhile, problems and limitations were known to set up second experiment in optimized conditions.

4.2. Second experiment setup

Benthic diatoms in zinc added solutions were grown in the same way as benthic diatoms in the control solutions. According to growth rate figure no sign of decreasing in number of benthic diatoms in zinc added solutions was observed in whole stages of second experiment. Furthermore, based on our observations, malformation of benthic diatoms did not occur. These two reasons were sufficient to reject second hypothesis of this project.

The second experiment was performed in a constant temperature room giving optimum conditions for growing of benthic diatoms. 28500 mL river water was filtered through a 65 µm mesh to exclude grazers before inserting in Erlenmeyer flasks. Glass chambers were used to observe benthic diatoms by microscope clearly in second experiment. For counting benthic diatoms in second experiment two microscopes were used interchangeably because sometimes glass chambers were replaced by plastic chambers. The reason of replacing chambers was sharing plastic and glass chambers between laboratory colleagues.

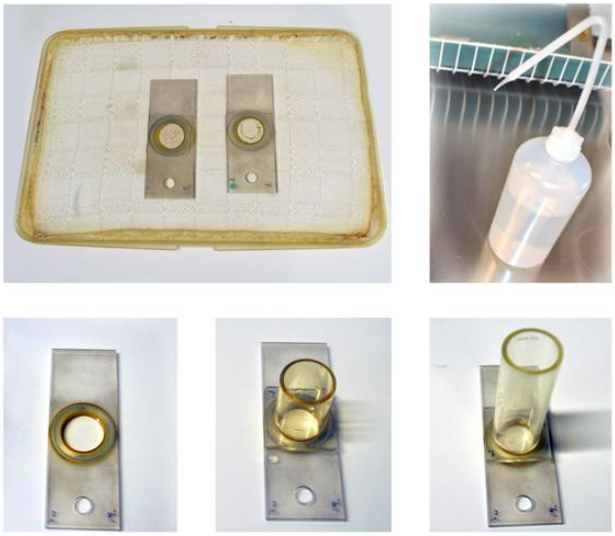

Figure 24 . This picture shows preparation of benthic diatoms slide for counting them by microscope. Picture: S. Navid N.

Preparing benthic diatoms samples due to sedimentation before counting was the sensitive stage in the second experiment due to the fact that benthic diatoms and sample water should not be lost before counting. It was necessary to spend 2 hours for counting samples in the final days of the experiment when the number of benthic diatoms was high.

Meanwhile, 250 mL of the 6 last samples were filtered to separate benthic diatoms from water solution in order to recognize the concentration of zinc in benthic diatoms but time limitation and high expenses for analyzing them were not allowed us to observed new results.

Cell numbers were not significantly lower in zinc treatment compared to the control treatment because total zinc solution which was used for this project did not appear to affect the cell numbers. In addition, deformations of benthic diatoms was not observed in this experiment whereas in previous study in Dalarna region, 4.2% deformation had been observed after applying 300 µg zinc/l[10]. The high value of deformations in the Dalarna study could be explained by the fact that other toxicants, for example other heavy metals than zinc, might have added toxic impact to the zinc and therefore increased the proportion of malformations.

The dominant diatom *Achnanthidium minutissimum* that was presented in our samples is considered as a tolerant species of benthic diatoms and possibly because of this tolerance there was no observed effect of the zinc on either malformation or cell number. Indeed, if domination of *Achnanthidium minutissimum* were replaced by lower tolerant species such as *Nitzschia linearis* or *Ulnaria ulna* the results might have been different due to fact that such sensitive diatoms have been shown not to tolerate artificial growth conditions and zinc stress [4]. However, despite its occurrence in contaminated water, *Achnanthidium minutissimum* has been shown to develop malformations in other studies [10, 4], the present study could not find any because high pH induces the loss of the toxic zinc form from solutions over time.

Malformations and reducing number of benthic diatoms may be observed because of differences between artificial laboratory conditions and natural field conditions [4]. Due to properly controlling light intensity and temperature fluctuations, malformations and reducing the number of benthic diatoms were also not observed in the control bottles of our experiment.

Low pH and high concentration of dissolved metals can reduce number of benthic diatoms [22].Background concentrations of zinc in Fyrisån are 8 µg Zn/ L. Zinc concentration applied in our experiment are about 40 times higher compared to the background values. In fact, it can be expected to observe more reduction of benthic diatoms in case of applying higher zinc concentration (>300 µg zinc/l). Meanwhile, pH was not low in our samples that influence on reduction of benthic diatoms.

4.3. Zinc

The concentration of zinc in control treatment samples did not exceed more than 7.7µg/l during our experiment. It is revealed that our control samples were not influenced by zinc contamination during our experiment.

Zinc toxicity can be changed by presence of water hardness (Ca and Mg) and humic substances [8]. Water hardness and humic substances are known as buffer substances against zinc and heavy metal toxicity [8]. Some metals bound to humic substances strongly and this effects of the fate and effect of metals in the aquatic environment. Interaction between zinc and humic substances can be observed by measuring absorbance [8].

4.4. Calculating the zinc speciation using the Visual MINTEQ software

Four below speculations were analyzed by visual MINTEQ regarding precipitation of zinc in our samples:

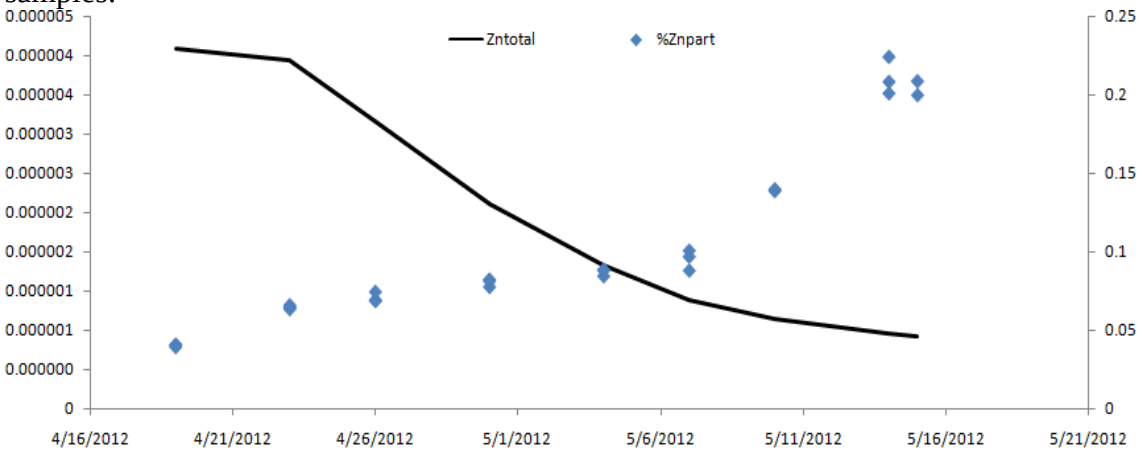

Figure 25.This figure indicates decreasing of total zinc and increasing particles in our experiment.

1) $Zn^{2+}(aq) + Fe(OH)_3\ (s) \rightarrow Zn\text{-}Fe(OH)_3\ (s) + 2H^{+}(aq)$

850 ppb iron was considered for this calculation from simultaneous experiment in FyrisRiver [28]. The model was revealed existence of 25 % of $Zn\text{-}Fe(OH)_3$precipitation in our zinc treatment samples at the end of experiments.

2) $Zn^{2+}(aq) + H_4SiO_4(s) \rightarrow Zn\text{-}H_4SiO_4(s) + 2H+(aq)$
Model was indicated that $Zn\text{-}H_4SiO_4$adsorptionmaybe occurred in our experiment. If $Zn\text{-}H_4SiO_4$ adsorption occurred is appropriate explanation for highly reduction of silicon in our zinc treatment samples.

3) $n\ Zn^{2+}(aq) + (1\text{-}n)\ Ca^{2+}(aq) + CO_3^{2-}(aq) \rightarrow Ca(1\text{-}n)\ Zn(n)\ CO_3(s)$
According to our calculations and model this reaction may were occurred in our sample solutions. Presence of Carbon trioxide and calcium induce zinc precipitation in form of $Ca(1\text{-}n)\ Zn(n)\ CO_3(s)$.

4) $5Zn^{2+} + 2\ CO_3^{-2} + 6\ OH^- \rightarrow Zn_5(CO_3)_2(OH)_6(s)$
The model was revealed this reaction was not occurred in our experiment. $Zn_5(CO_3)_2(OH)_6$ particles were not precipitated in the zinc treatment sample solutions.

4.5. Decreasing organic matter by light

Reducing of TOC and DOC was observed in our experiment. Total organic carbon concentration probably was degraded by light [24].Light exposure induced rapid oxidation of total organic carbon concentration. This oxidation causes transition of organic carbon to inorganic carbon. This conversion led to increasing pH and alkalinity. [24]TOC concentration was decreased 33-50% after 12 days in past studies [24].Moreover, organic carbon may were decreased by microbial activity and consumption.

4.6. Silicon Fate

Although silicon decreased significantly, visually observation of benthic diatoms cell walls by microscope did not show changes in wall thickness during our experiment. Silicon concentration in benthic diatoms was not measured in our study to confirm visual experiment. As zinc is able to form organic and inorganic complexes [25], it is speculated that the extreme reduction of silicon was observed due to bonding with zinc.

Silicon was extremely reduced in the second experiment. Silicon was decreased close to zero concentration in zinc added solution. Although silicon may have been consumed by diatoms for their nutrition’s and reproductions, the reason for disappearance of silicon in zinc treatments remains unclear.

5. Conclusion

300 µg/l zinc concentration did not appear to inhibit benthic diatoms' photosynthesis and hence growth. In addition, malformation of benthic diatom cell walls was not observed. This study assists us in improving our knowledge regarding benthic diatoms as bio-indicators in freshwater streams. For future studies it is necessary to test higher concentrations of zinc in benthic diatom communities, preventing the pH from increasing so that zinc stays dissolved.

Silicon analysis should also be undertaken in order to measure the amount of silicon in diatoms, sediments and Erlenmeyer flasks. Also, it may be possible to observe increasing or decreasing diversity of different species of benthic diatoms. Moreover, benthic diatom samples should have nutrients added during longer experiments in order to prevent nutrient depletion.

Finally, measuring concentration of zinc (heavy metal) in benthic diatom cells will give us more profitable and useful results in future investigations. Following this project it can be ideal if two concentrations of metals and two different metals are tested over longer project durations.

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8. Appendices

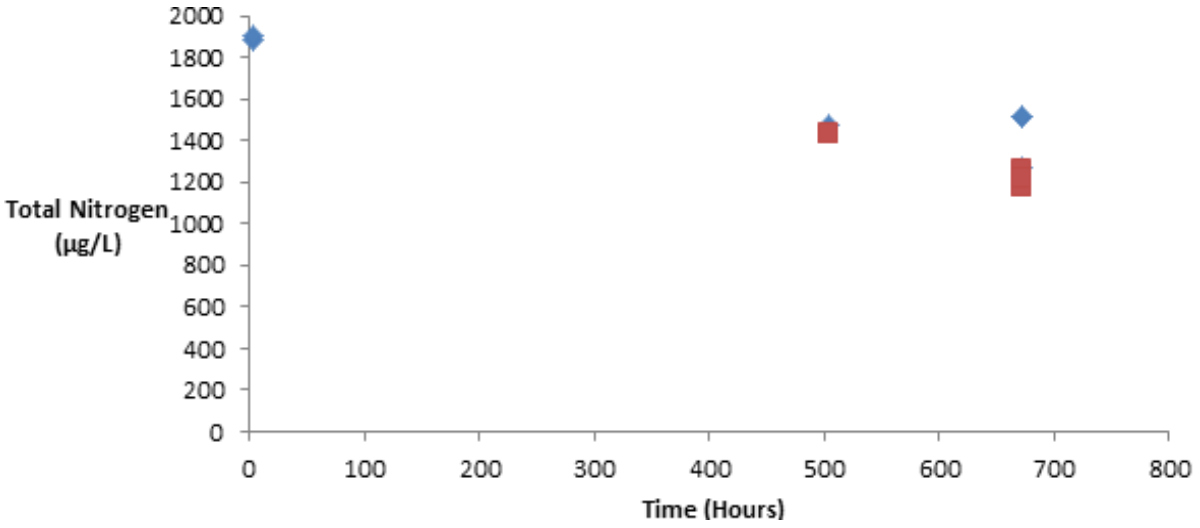

Figure26.Total nitrogen changing in second experiment. ♦, control treatment; ■zinc added treatment. Total nitrogen decreasing slightly in both control and zinc added treatments.

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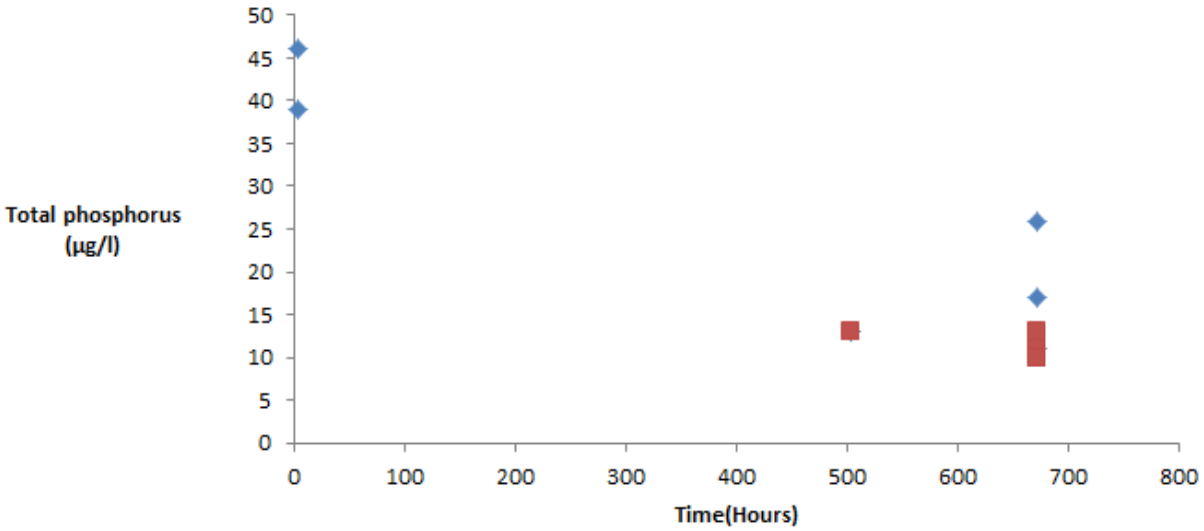

Figure27.TotalPhosphorus was reduced in both control and zinc added solution in second experiment.♦, control treatment; ■ zinc added treatment.

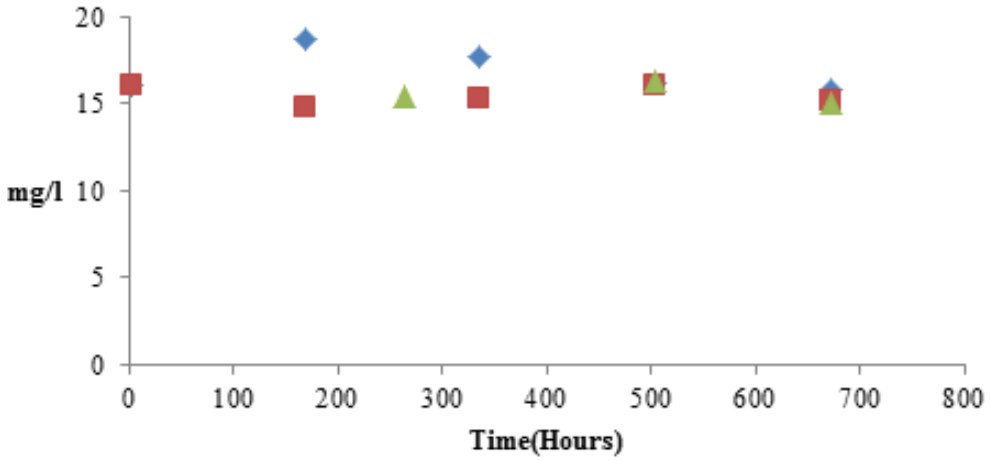

Figure 28.Fluctuation of Total Organic Carbon in second experiment.♦, control treatment; ■, zinc added treatment; ▲, no diatoms + zinc.

Table 1.This table shows growing rate of benthic diatoms in four different chambers.

TIME HOURS	*Chamber A cells in 1,8 cm2*	*Chamber B cells in 1,8 cm2*	*Chamber C cells in 1,8 cm2*	*Chamber D cells in 1,8 cm2*
2	936	1106	1785	963
68	2312	2569	2019	1437
92	2937	2784	3304	2012
116	7373	3885	3242	2233
140	5415	3427	5751	3151
164	11809	7709	10279	5629
212	13828	9515	7342	10524
236	22180	10187	12207	9790
260	33395	19641	14287	14440
280	40720	15480	21476	19243
304	46900	24199	24536	25637
352	84653	52468	46931	43627
376	116502	74802	44636	46411

Table2.Control treatment solutions. This table indicates number of benthic diatoms was grown.

Growing Cells duration(Hours)	*Samples*	*Total cells in 500 ml*	*Date*
3	0a	153,301	16.04.2012
3	0b	145,931	16.04.2012
3	0c	99,056	16.04.2012
168	10	1,015,834	23.04.2012
168	15	606,468	23.04.2012
168	20	1,048,053	23.04.2012
336	12	2,454,337	30.04.2012
336	17	2,507,692	30.04.2012
336	21	2,381,580	30.04.2012
504	2	3,919,178	07.05.2012
504	11	3,676,655	07.05.2012
504	13	4,787,412	07.05.2012
672	14	5,296,711	14.05.2013
672	5	7,922,432	14.05.2015
672	26	6,481,989	14.05.2015

Table3.Zinc added treatment solutions. This table indicates number of benthic diatoms was grown.

This rate of growth was indicated 300 µg/l zinc was not influenced cells growing.

Growing Cells duration(Hours)	*Samples*	*Total cells in 500 ml*	*Date*
3	0a	153,301	16.04.2012
3	0b	145,931	16.04.2012
3	0c	99,056	16.04.2012
168	43	1,374,030	23.04.2012
168	46	1,222,413	23.04.2012
168	50	936,235	23.04.2012
336	35	1,571,551	30.04.2012
336	40	3,860,972	30.04.2012
336	49	2,459,187	30.04.2012
504	32	4,312,066	07.05.2012
504	45	5,713,851	07.05.2012
504	47	6,489,926	07.05.2012
672	53	6,184,347	14.05.2012
672	36	8,946,689	14.05.2014
672	33	5,822,987	14.05.2016

Table 4.Total zinc reduction in control and zinc added solutions.

Treatment	Hours	Zn µg/l
Initial	3	7.7
control	168	3.1
conrol	672	1.8
zinc	168	290
zinc	336	170
zinc	672	62

Table5.Filtered zinc reduction in control and zinc added solutions.

Treatment	Hours	Zn µg/l
Initial	3	3.0
control	168	1.9
conrol	672	1.6
zinc	168	280
zinc	336	140
zinc	672	48

Table 6. Decreasing of silicon concentration in second experiment.

Treatment	Hours	Si mg/l
Control	3	5.24
Control	504	2.78
Control	672	2.61
Control	672	1.86
Control	672	1.54
Zinc Added	504	2.52
Zinc Added	672	0.1
Zinc Added	672	0.15
Zinc Added	672	0.09

Table 7. Fluctuation of light during First experiment.

LIGHT Q/cm2 .s	Light Q/cm2 .s	Light Mmol/m2.s
$0.46*1*10^{16}$	4.6E+15	76.36
$0.34*1*10^{16}$	3.4E+15	56.44
$0.40*1*10^{16}$	4E+15	66.4
$0{,}39*1*10^{16}$	3.90E+15	64.74
$0.39*1*10^{16}$	3.90E+15	64.74
$0.16*1*10^{16}$	1.60E+15	26.56
$0.12*1*10^{16}$	1.20E+15	19.92
	Average	53.59428571
	Max	76.36
	Min	19.92

Table 8.Total zinc measurement in control and zinc added solutions.

Treatment	Date	Hours	Zn µg/l
Initial	4/16/2012	3	7.7
control	4/23/2012	168	3.1
zinc	4/23/2012	168	290
zinc	4/30/2012	336	170
conrol	5/14/2012	672	1.8
zinc	5/14/2012	672	62

Table 9.Filtered zinc measurement in control and zinc added solutions.

Treatment	Date	Hours	Zn µg/l
Initial	4/16/2012	3	3.0
control	4/23/2012	168	1.9
zinc	4/23/2012	168	280
zinc	4/30/2012	336	140
conrol	5/14/2012	672	1.6
zinc	5/14/2012	672	48

Table 10 .Control sample treatments.

Hours	Date	TOC	DOC	abs255	abs335	abs420
3	16.04.2012	16.05	15.36			
168	23.04.2012	18.69	21.26	0.97	0.95	0.92
336	30.04.2012	17.64	19.13	0.89	0.87	0.82
504	07.05.2012	16.12	18.05	0.92	0.85	0.77
672	14.05.2012	15.82	17.19	0.82	0.79	0.74

Table11. Zinc added solutions.

Hours	Date	TOC	DOC	abs255	abs335	abs420
3	16.04.2012	16.05	15.36			
168	23.04.2012	14.84	15.67	0.93	0.91	0.88
336	30.04.2012	15.23	15.22	0.87	0.82	0.79
504	07.05.2012	16.04	15.89	0.92	0.89	0.83
672	14.05.2012	15.12	16.55	0.88	0.85	0.83

Table 12.The table gives us information regarding TOC,DOC, Total zinc, filtered zinc in control treatment samples.

TREATMENT	Date	Sample Numbers	Toc mg/l	Doc mg/l	Total Zinc µg/l	Zinc Filtered µg/l
Initial	16.04.2012	0	16.05	15.36	7.7	3.0
Control	19.04.2012	9				
Control	19.04.2012	25				
Control	19.04.2012	22				
Control	23.04.2012	10				
Control	23.04.2012	15				
Control	23.04.2012	20	18.69	21.26	3.1	1.9
Control	26.04.2012	19				
Control	26.04.2012	23				
Control	26.04.2012	4				
Control	30.04.2012	21				
Control	30.04.2012	17				
Control	30.04.2012	12	17.64	19.13		
Control	04.05.2012	3				
Control	04.05.2012	18				
Control	04.05.2012	16				
Control	07.05.2012	11				
Control	07.05.2012	13				
Control	07.05.2012	2	16.12	18.05		
Control	10.05.2012	6				
Control	10.05.2012	24				
Control	10.05.2012	8				
Control	14.05.2012	5				
Control	14.05.2013	26				
Control	14.05.2013	14	15.82	17.19	1.8	1.6
Control	15.05.2012	1				
Control	15.05.2012	7				
Control	15.05.2012	27				

Table 13.The table gives us information regarding TOC, DOC, total zinc, filtered zinc in zinc treatment and no diatom added zinc samples.

TREATMENT	Date	Sample Numbers	*Toc mg/l*	*Doc mg/l*	*Total Zinc µg/l*	*Zinc Filtered µg/l*
Initial	16.04.2012	0	16.05	15.36	7.7	3.0
Zinc Added	19.04.2012	28				
Zinc Added	19.04.2012	31				
Zinc Added	19.04.2012	52				
Zinc Added	23.04.2012	43				
Zinc Added	23.04.2012	46				
Zinc Added	23.04.2012	50	14.84	15.67	290	280
Zinc Added	26.04.2012	29				
Zinc Added	26.04.2012	30				
Zinc Added	26.04.2012	38				
Zinc Added	30.04.2012	49				
Zinc Added	30.04.2012	35				
Zinc Added	30.04.2012	40	15.23	15.22	170	140
Zinc Added	04.05.2012	39				
Zinc Added	04.05.2012	34				
Zinc Added	04.05.2012	44				
Zinc Added	07.05.2012	47				
Zinc Added	07.05.2012	45				
Zinc Added	07.05.2012	32	16.04	15.89		
Zinc Added	10.05.2012	42				
Zinc Added	10.05.2012	51				
Zinc Added	10.05.2012	48				
Zinc Added	14.05.2012	33				
Zinc Added	14.05.2012	36				
Zinc Added	14.05.2012	53	15.12	16.55	62	48
Zinc Added	15.05.2012	37				
Zinc Added	15.05.2012	41				
Zinc Added	15.05.2012	54				
No Diatom	07.05.2012	56	16.33	16.35		
No Diatom	14.05.2012	55	15.02	15.51		
No Diatom	26.04.2012	57	15.42	15.39		

Table 14.pH values, solution temperatures, room temperatures were measured for all control treatment samples. Meanwhile, pH meter was calibrated before each measurement.

Date	Sample Numbers	Treatment	pH 1 Value	pH 2 Value	Solution Temperature℃	Room Temperature℃	pHmeter Calibration slope%
16.04.2012	0	Initial	7.58	7.58	11,2	10,3	100.2
19.04.2012	9	Control	7.82	7.86	11	9.7	102.8
19.04.2012	25	Control	7.86	7.87	11	9.7	102.8
19.04.2012	22	Control	7.86	7.88	11	9.7	102.8
23.04.2012	10	Control	8.17	8.21	11.1	10.3	103
23.04.2012	15	Control	8.19	8.22	11	10.3	103
23.04.2012	20	Control	8.22	8.25	11.2	10.3	103
26.04.2012	19	Control	8.33	8.35	11.2	10.3	102.7
26.04.2012	23	Control	8.32	8.35	11.2	10.3	102.7
26.04.2012	4	Control	8.19	8.29	11	10.3	102.7
30.04.2012	21	Control	8.33	8.34	11	10.3	102.6
30.04.2012	17	Control	8.38	8.39	11	10.3	102.6
30.04.2012	12	Control	8.27	8.32	10.9	10.3	102.6
04.05.2012	3	Control	8.24	8.31	11.1	10	102.5
04.05.2012	18	Control	8.37	8.38	11.2	10	102.5
04.05.2012	16	Control	8.33	8.36	11.1	10	102.5
07.05.2012	11	Control	8.30	8.31	10.9	10	103.2
07.05.2012	13	Control	8.35	8.35	10.9	10	103.2
07.05.2012	2	Control	8.22	8.23	10.9	10	103.2
10.05.2012	6	Control	8.23	8.26	10.5	10.1	102.6
10.05.2012	24	Control	8.34	8.35	10.5	10.1	102.6
10.05.2012	8	Control	8.36	8.38	10.5	10.1	102.6
14.05.2012	5	Control	8.24	8.30	11.1	10	103
14.05.2013	26	Control	8.28	8.30	11	10	103
14.05.2013	14	Control	8.31	8.33	11	10	103
15.05.2012	1	Control					
15.05.2012	7	Control					
15.05.2012	27	Control					

Table 15.pH values, solution temperatures, room temperatures were measured for all zinc added treatment samples and samples without diatoms (No diatom). Meanwhile, pH meter was calibrated before each measurement.

Date	Sample Numbers	Treatment	pH 1 Value	pH 2 Value	Solution Temperature℃	Room Temperature℃	pHmeter Calibration slope%
16.04.2012	0	Initial	7.58	7.58	11,2	10,3	100.2
19.04.2012	28	Zinc Added	7.87	7.90	11	9.7	102.8
19.04.2012	31	Zinc Added	7.88	7.91	10.9	9.7	102.8
19.04.2012	52	Zinc Added	7.84	7.86	10.8	9.7	102.8
23.04.2012	43	Zinc Added	8.38	8.39	11.2	10.3	103
23.04.2012	46	Zinc Added	8.33	8.35	11	10.3	103
23.04.2012	50	Zinc Added	8.31	8.33	11	10.3	103
26.04.2012	29	Zinc Added	8.30	8.34	11.1	10.3	102.7
26.04.2012	30	Zinc Added	8.29	8.30	11.1	10.3	102.7
26.04.2012	38	Zinc Added	8.56	8.57	11.2	10.3	102.7
30.04.2012	49	Zinc Added	8.65	8.67	11	10.3	102.6
30.04.2012	35	Zinc Added	8.33	8.33	11.1	10.3	102.6
30.04.2012	40	Zinc Added	8.65	8.66	11	10.3	102.6
04.05.2012	39	Zinc Added	8.72	8.73	11.2	10	102.5
04.05.2012	34	Zinc Added	8.26	8.28	11.2	10	102.5
04.05.2012	44	Zinc Added	8.74	8.76	11.2	10	102.5
07.05.2012	47	Zinc Added	8.65	8.65	10.9	10	103.2
07.05.2012	45	Zinc Added	8.71	8.72	11	10	103.2
07.05.2012	32	Zinc Added	8.21	8.22	10.9	10	103.2
10.05.2012	42	Zinc Added	8.63	8.65	10.5	10.1	102.6
10.05.2012	51	Zinc Added	8.64	8.65	10.5	10.1	102.6
10.05.2012	48	Zinc Added	8.82	8.83	10.5	10.1	102.6
14.05.2012	33	Zinc Added	8.40	8.41	11.1	10	103
14.05.2012	36	Zinc Added	8.64	8.67	11	10	103
14.05.2012	53	Zinc Added	8.75	8.77	11.1	10	103
15.05.2012	37	Zinc Added					
15.05.2012	41	Zinc Added					
15.05.2012	54	Zinc Added					
07.05.2012	56	No Diatom	8.48	8.46	11	10	103.2
14.05.2012	55	No Diatom	8.43	8.45	10.9	10	103
26.04.2012	57	No Diatom	8.16	8.25	11	9.8	103.8

Table 16.Nutrient measurements were done during the experiment (initially, middle and end) in control treatment .Also, Alkalinity of some samples were measured. These data were used in visual Minteq calculations.

Date	Sample Numbers	TREATMENT	Alk./Acid mekv/l	NH4_N µg/l	PO4_P µg/l	Tot._P µg/l	Tot-N_TNb µg/l	NO2+NO3_N µg/l	Si mg/l	Övr. P µg/l	Org._N µg/l
16.04.2012	0	Initial	2.65	36	33	46	1881	1389	5.24	13	456
19.04.2012	9	Control									
19.04.2012	25	Control									
19.04.2012	22	Control									
23.04.2012	10	Control									
23.04.2012	15	Control									
23.04.2012	20	Control									
26.04.2012	19	Control									
26.04.2012	23	Control									
26.04.2012	4	Control									
30.04.2012	21	Control									
30.04.2012	17	Control									
30.04.2012	12	Control									
04.05.2012	3	Control									
04.05.2012	18	Control									
04.05.2012	16	Control									
07.05.2012	11	Control									
07.05.2012	13	Control									
07.05.2012	2	Control	2.688	3	4	13	1478	898	2.78	9	577
10.05.2012	6	Control									
10.05.2012	24	Control									
10.05.2012	8	Control									
14.05.2012	5	Control									
14.05.2013	26	Control									
14.05.2013	14	Control									
15.05.2012	1	Control	2.661	23	3	17	1511	806	2.61	14	682
15.05.2012	7	Control	2.67	13	4	26	1516	712	1.86	22	791
15.05.2012	27	Control	2.679	7	3	11	1273	638	1.54	8	628

Table 17.Nutrient measurements were done during the experiment (initially, middle and end) in zinc and no diatoms treatments .Also, Alkalinity of some samples were measured. These data were used in visual Minteq calculations.

Date	Sample Numbers	Treatment	Alk./Acid mekv/l	NH4_N µg/l	PO4_P µg/l	Tot._P µg/l	Tot-N_TNb µg/l	NO2+NO3_N µg/l	Si mg/l	Övr. P µg/l	Org._N µg/l
16.04.2012	0	Initial	2.65	36	33	46	1881	1389	5.24	13	456
19.04.2012	28	Zinc Added									
19.04.2012	31	Zinc Added									
19.04.2012	52	Zinc Added									
23.04.2012	43	Zinc Added									
23.04.2012	46	Zinc Added									
23.04.2012	50	Zinc Added									
26.04.2012	29	Zinc Added									
26.04.2012	30	Zinc Added									
26.04.2012	38	Zinc Added									
30.04.2012	49	Zinc Added									
30.04.2012	35	Zinc Added									
30.04.2012	40	Zinc Added									
04.05.2012	39	Zinc Added									
04.05.2012	34	Zinc Added									
04.05.2012	44	Zinc Added									
07.05.2012	47	Zinc Added									
07.05.2012	45	Zinc Added									
07.05.2012	32	Zinc Added	2.673	2	4	13	1438	813	2.52	9	623
10.05.2012	42	Zinc Added									
10.05.2012	51	Zinc Added									
10.05.2012	48	Zinc Added									
14.05.2012	33	Zinc Added									581
14.05.2012	36	Zinc Added									588
14.05.2012	53	Zinc Added									598
15.05.2012	37	Zinc Added	2.669	8	1	11	1180	591	0.1	10	581
15.05.2012	41	Zinc Added	2.66	7	2	13	1256	661	0.15	11	588
15.05.2012	54	Zinc Added	2.663	5	2	10	1214	611	0.09	8	598
07.05.2012	56	No Diatom	0.333	6	8	50	1936	1225	3.69	42	705
14.05.2012	55	No Diatom									
26.04.2012	57	No Diatom