



Metal Removal in Wastewater

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ABSTRACT

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Bachelor's thesis 24pages, appendices 13pages

The aim of this work was to study Copper removal capacity of different algae species and their mixtures from the municipal wastewater. This project was implemented in the greenhouse in the laboratories of Tampere University of Applied Sciences and the wastewater used was the one from the Tampere municipal wastewater treatment plant. Five algae species and three mixtures of them were tested for their Copper removal potential in wastewater in one batch test run. The most efficient algae mixture was tested in semi continuous ATS run, the mixture of *Selenastrum Capricornutum*, *Pediastrum Simplex*, *Scenedesmus Spiriluna*, *Haematococcus* respectively. The ATS system reduced Copper concentration with a 50-55% in this study. Copper concentration decreased in the first week of the ATS experiment, but increased after that. Wastewater addition caused algae to grow again and Copper concentration decreased as a consequence of algal growth. Algal growth and the most efficient Cu removal took place within two days from the addition of wastewater. These results suggest that in the ATS it is necessary to add new wastewater in spans of 2-3 days to provide algae the necessary nutrients and to assure efficient Cu removal.

Key words: Algae Turf Scrubbers, Wastewater, metal removal, algae, copper

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ABBREVIATIONS AND TERMS

| | |
|---------------------------------|--|
| AAS | Atomic Absorption Spectrophotometer |
| BOD | Biochemical oxygen demand |
| N | Nitrogen |
| NO ₃ ⁻ -N | Nitrate Nitrogen |
| P | Phosphorus |
| pH | Logarithmic H ⁺ concentration |
| PO ₄ ³⁻ | Phosphate |
| TN | Total nitrogen |
| TP | Total Phosphorus |
| Cu | Copper. |

1 INTRODUCTION

Wastewater production is inevitable because of human activity, all the industrial factories produce it. Since wastewater is released back into environment, it causes pollution of natural water bodies if not treated properly. Wastewater without treatment is very harmful for the environment and for the public health, because of the germs and bacteria that the wastewater contains.

The wastewater treatment is a set of physical biological and chemical operations. These seek to eliminate as many contaminants as possible before the effluent being discharged, so that the levels of remaining contaminants in treated effluents meet the existing legal limits.

In this project we studied the use of algae for wastewater treatment. Wastewater contains Nitrogen, Nitrates, Phosphorous, Copper, Ammonia, and Ammonium, and with algae these elements could be removed.

The term microalgae refers to the aquatic microscopic plants (organisms with chlorophyll a and a thallus not differentiated into root, stem and leaf), and the oxygenic photosynthetic bacteria, that is, the cyanobacteria, formerly known as Cyanophyceae (Ander- sen, 2005). Micro-algae are microscopic photosynthetic organisms that are found in both marine and freshwater environments.

The microalgae which exist in the freshwater environment and the oceans are important in global ecology, extremely efficient, and taxonomically diverse (Brown and Zeiler, 1993). These microalgae (phytoplankton) in the oceans live in an environment which comprises more than 70% of the earth's surface and is responsible for at least 32% of global photosynthesis (Whittaker, 1975).

Algae are very important bioremediation agents, and are already being used by many wastewater treatment facilities in United States and other parts of the world. The potential for algae in wastewater treatment is however much wider in scope than its current role since there is much more room for further research and development. (Oilgae, 2009; Grobler, 2013)

Industrial wastes, geo-chemical structure and mining of metals create a potential source of heavy metal pollution in the aquatic environment (Gumgumet *al.*, 1994).

Some algae species are used for metal removal in wastewater, are shown in thenTable 1:

| Metal | Organism |
|---------|--|
| Cd(II) | <i>Chlorella vulgaris</i> , <i>Chlorella salina</i> , <i>Scenedesmus obliquus</i> , <i>Chlamydomonas reinhardtii</i> , <i>Asterionella Formosa</i> , <i>Fragilaria crotonensis</i> , <i>Thalassphaere elongate</i> |
| Pb(II) | <i>Chlorella vulgaris</i> , <i>Euglena sp.</i> |
| Zn(II) | <i>Chlorella vulgaris</i> , <i>Chlorella regularis</i> , <i>Chlorella salina</i> , <i>Chlorella homosphaera</i> , <i>Euglena sp.</i> |
| Au(I) | <i>Chlorella vulgaris</i> |
| U(II) | <i>Chlorella vulgaris</i> , <i>Chlorella sp.</i> , <i>Scenedesmus obliquus</i> , <i>Scenedesmus sp.</i> , <i>Chlamydomonas sp.</i> , <i>Dunaliella</i> <i>tertiolecta</i> , <i>Ankiistroesmus Sp.</i> , <i>Selenastrum sp.</i> |
| Cu(I) | <i>Chlorella regularis</i> , <i>Euglena sp.</i> , <i>Cricosphaere elongate</i> |
| Ni(I) | <i>Chlorella regularis</i> , <i>Thalassiosira rotula</i> |
| Co(II) | <i>Chlorella regularis</i> , <i>Chlorella salina</i> |
| Mn(II) | <i>Chlorella regularis</i> , <i>Chlorella salina</i> , <i>Euglena sp.</i> |
| Mo(I) | <i>Chlorella regularis</i> , <i>Scenedesmus sp.</i> , <i>Chlamydomonas reinhardtii</i> |
| Tc(II) | <i>Chlorella emersonii</i> , <i>Scenedesmus obliquus</i> , <i>Chlamydomonas reinhardtii</i> |
| Zn(II) | <i>Chlorella emersonii</i> , <i>Scenedesmus obliquus</i> , <i>Chlamydomonas reinhardtii</i> |
| Hg(II) | <i>Chlorella sp.</i> |
| Al(III) | <i>Euglena sp.</i> |

Table 1: Microalgae involve in metal removal from wastewater. (Priyadarshani et al, 2011).

The presence of heavy metals in the environment is a major concern because of their toxicity to flora and fauna. Moreover, recovery of heavy metals from industrial waste streams is becoming increasingly important as society realizes the necessity for recycling and conservation of essential metals. Heavy metals are major pollutants in marine, ground, industrial and even treated wastewaters.

There are some problems caused by heavy metals, in larger amounts cause acute and chronic toxicity, heavy metals have inhibitory effects on the biological treatment process at the wastewater treatment plants (Ranboll, 2013).

Metals are taken up by algae through adsorption. At first, the metal ions are adsorbed over the cell surface very quickly just in a few seconds or minutes; this process is called physical adsorption. Then, these ions are transported slowly into the cytoplasm in a process called chemisorption. (Dwivedi, 2012).

The ability of algae to absorb metals has been recognized for many. In natural environments, algae play a major role in controlling metal concentration in lakes and oceans, (Dwivedi, 2012).

2 PRINCIPAL AIM

The aim of this project was to use previously selected algae species and their mixtures in municipal wastewater treatment and to study their Copper reduction potential in the wastewater environment. This project was done in the greenhouse in the laboratories of Tampere University of Applied Sciences, TAMK, Tampere, Finland.

3 MATERIALS AND METHODS

3.1 Materials.

Seven algae species were cultivated in 2 L bottles. Table 2 gives information on the specific species present in each bottle, and table 3 shows the algae species used in the study.

| | |
|--------------|---------------------------|
| Sp1: | Selenastrum Capricornutum |
| Sp2: | Pediastrum Simplex |
| Sp3: | Synechococcus Spiriluna |
| Sp4: | Anabaena cylindrical |
| Sp6: | Scenedesmus Spiriluna |
| Sp7 | Chlorophyta Spiriluna |
| Sp8: | Purpuraemus Spiriluna |
| Sp10: | Haematococcus |

Table2: Species present in each bottle.

| | |
|----------------|------------------|
| Bottle1 | Sp1-Sp10 |
| Bottle2 | Sp1+Sp2+Sp6+Sp10 |
| Bottle3 | Sp4+Sp6 |
| Bottle4 | Sp1 |
| Bottle5 | Sp2 |
| Bottle6 | Sp6 |
| Bottle7 | Sp4 |

Table3: Algae species

The wastewater used in this study was taken from the municipal wastewater treatment plant in Tampere. Four places were selected, (Appendix 1), The P, N and Cu concentrations of the wastewater samples from the four different sampling points are presented in Table 4. Sampling place number two was selected as wastewater source for the project, because place number two was the most contaminated, we can see it in Table4.

| | Cu | Total Ph. | Total N |
|----------------------|--------------|------------------|----------------|
| Sample1(mg/l) | 0,198 | 2,090 | 6,807 |
| Sample2(mg/l) | 0,253 | 2,710 | 5,880 |
| Sample3(mg/l) | 0,096 | 0,804 | 11,570 |
| Sample4(mg/l) | 0,103 | 0,073 | 0,672 |

Table 4: Wastewater samples.

3.2 Methods

Two test runs were performed in this study one batch run and one semi-continuous in which algae turf scrubber (ATS) were used.

In the first batch test run the single algae species and their mixtures were tested for their Cu removal potential from the municipal wastewater. For each algae specie and algae mixture one blank and one test bottle was used. The blank bottles contained only wastewater and nutrients (Table 5). To each test bottle 10 mL of Copper solution was added to assure 33,5 mg/l concentration in the test bottles (Table 6). This concentration was found as “optimum” in a previous workdone on the same algae species but no wastewater environment (Benchraka, 2014). This test run lasted for three weeks.

| Bottle | Waste water added (ml) | Algae added(ml) |
|---------------|-------------------------------|------------------------|
| B1 | 400 | 10 ml from bottle 1 |
| B2 | 400 | 10 ml from bottle2 |
| B3 | 400 | 10 ml from bottle3 |
| B4 | 400 | 10 ml from bottle4 |
| B5 | 400 | 10 ml from bottle5 |
| B6 | 400 | 10 ml from bottle6 |
| B7 | 400 | 10 ml from bottle7 |

Table5: Blanks Bottles

| Bottle | Waste water added (ml) | Cu (33,5 mg/l) added | Algae added(ml) |
|---------------|-------------------------------|-----------------------------|------------------------|
| T1 | 400 | 10ml/bottle | 10 ml from bottle1 |
| T2 | 400 | 10ml/bottle | 10 ml from bottle2 |
| T3 | 400 | 10ml/bottle | 10 ml from bottle3 |
| T4 | 400 | 10ml/bottle | 10 ml from bottle4 |
| T5 | 400 | 10ml/bottle | 10 ml from bottle5 |
| T6 | 400 | 10ml/bottle | 10 ml from bottle6 |
| T7 | 400 | 10ml/bottle | 11 ml from bottle7 |

Table6: Test bottles



Picture 1: Algae Project.



Picture 2: Different Algae.

Additionally liquid commercial plant fertilizer (Substral) was transferred into each bottle. This step was done to promote the algae growth. The addition of nutrient and the date of addition are shown below (Table 7).

| Date Addition | Amount of nutrients(Subtral)(ml) |
|---------------|----------------------------------|
| 17/02/2014 | 2 |
| 24/02/2014 | 2 |
| 28/02/2014 | 1 |
| 26/05/2014 | 2 |

Table7: Amount of nutrient added.

In the second test run three Algae Turf Scrubber (ATS) reactors were used to test the Copper removal capacity of the selected algae mixture. Selection of the algae mixture

was based on the batch experiment. The selected mixture was the one in the bottle 2, because the efficiency it showed in consuming Copper during the first test run.

Two of the ATS was run with Copper, wastewater and algae, and the last one only with wastewater and algae and was used as “blank” in this experiment. ATS test was realized like a semi-continuous process.

In this test wastewater was added in different days, these amounts are shown in Table 8.

| Date Addition | Amount of Ws Added to ATS(l) |
|---------------|------------------------------|
| 13/05/2014 | 4 |
| 14/05/2014 | 4,5 |
| 16/05/2014 | 3 |
| 20/05/2014 | 15 |
| 23/05/2014 | 5 |

Table8: Wastewater added

3.2.1 Copper measurements

Copper concentrations in the batch reactors and ATS effluents were measured by Atomic absorption spectrometry (AAS)

AAS is an analytical technique that measures the concentrations of elements, the technique makes use of the wavelengths of light specifically absorbed by an element (Makinen, 2012).

The main operating idea of the atomic absorption spectroscopy (AAS) is to decompose the sample into atoms by vaporising it in high temperature, and then to measure the characteristic wavelength of radiation to obtain the correct atom concentrations. The atomic absorption spectroscopy is a very sensitive method and it can be used to analyse a specific analyte in a complex sample. The analyte is measured at parts per million to parts per trillion levels, and samples with high concentrations must be diluted to reach these levels. (Harris, 2007)

The basic requirements for flame atomic absorption spectroscopy analysis are the fuel-oxidant flow, the flame, the lamp which is the hollow cathode lamp or electrodeless discharge lamp (EDL), the sample in a liquid form, the monochromator, the amplifier, and the

detector, as well as the readout device, which is usually a computer with software. (Harris 2007.)

The lamp works as a radiation source by emitting a sharp line spectrum characteristic of the analyte element. Then the emission beam coming from the radiation source is modulated, and this modulated signal passes through the atomic vapour where the atoms of the analyte absorb radiation. The monochromator selects the desired spectral line, after which the isolated analyte lines fall onto the detector, which gives the electric signal converted from the light signal. The signal is amplified by a selective amplifier and the signal is recorded with the computer or other readout device. (Lajunen&Perämäki 2004.)



Picture 3: Atomic Absorption Spectrophotometer

During the three week batch test runs, AAS were done each week. 14 samples were analyzed, 7 for blanks, without Copper, and 7 with Copper. The calibrations of the Standards are in Appendix 5. The wavelengths used in the first run in AAS, was 216, 15 nm and in ATS experiment 324 nm. For each sample triplicate measurements were done.

4 RESULTS AND DISCUSSION

4.1 First Test Run

The first test week started on the 7th of April, and was finished the 25th of April. The measured Copper concentrations in the blank samples are presented in Table 9 and in the test samples in Table 10.

| | B1(mg/l) | B2(mg/l) | B3(mg/l) | B4(mg/l) | B5(mg/l) | B6(mg/l) | B7(mg/l) |
|--------------|----------|----------|----------|----------|----------|----------|----------|
| Week1 | 0,378 | 0,417 | 0,488 | 0,359 | 0,319 | 0,383 | 0,249 |
| Week2 | 0,378 | 0,209 | 0,273 | 0,381 | 0,306 | 0,245 | 0,318 |
| | 0,23 | 0,134 | 0,029 | 0,224 | 0,238 | 0,065 | 0,000 |
| Week3 | 0,345 | 0,255 | 0,222 | 0,365 | 0,330 | 0,320 | 0,464 |

Table9: Copper concentration in blanks samples of batch experiment

As from the Figure 1 can be seen, in the bottles without Copper (blanks) two species proved efficient Cu removal. These are Specie 3 representing the mixture algae *Synechococcus Spiriluna*, and Specie 6 representing single algae *Scenedesmus Spiriluna*.

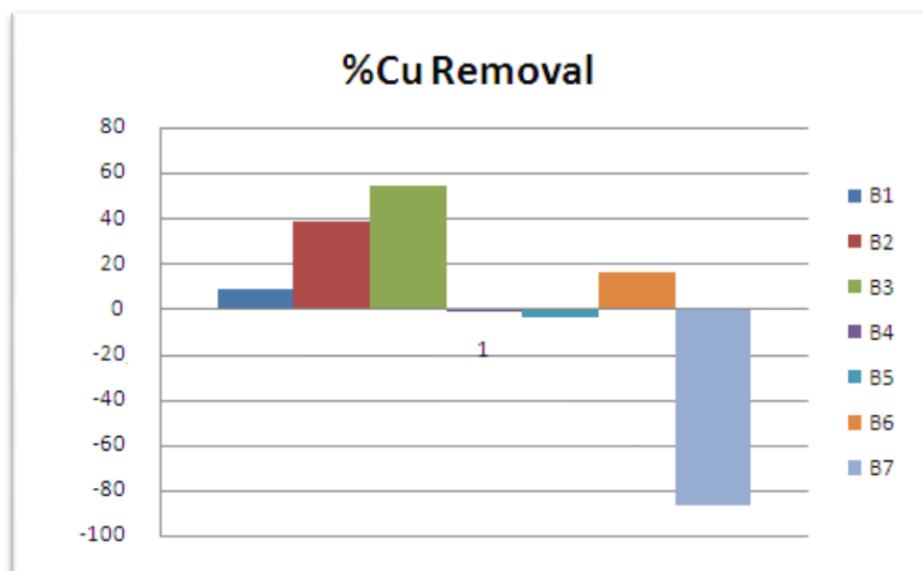


Figure1: Percentage of copper removal in blank samples in batch experiment

Dwivedi (2012) found previously that *Scenedesmus* reduces with 0,5 mg/L the Cu concentration in farm runoff wastewater. We used this algae specie in two bottles; bottle1 and bottle3. *Scenedesmus* is a fresh water green alga, which can produce spinycellex-

tensions and long bristle like structures that make the organism appear large to herbivores and help reduce the sedimentation (Graham and Wilcox, 2000 p48)

Figure 1 shows that specie 7 did not work well, so in the second test run was eliminated, because the removal percentage was negative.

The average Copper concentrations found in test bottles are presented in Table 10. The triplicate Cu concentration measurements are presented in Appendix 3, Copper concentration in test bottles as compared to blanks are illustrated in Figures 2-8.

| | T1(mg/l) | T2(mg/l) | T3(mg/l) | T4(mg/l) | T5(mg/l) | T6(mg/l) | T7(mg/l) |
|--------------|----------|----------|----------|----------|----------|----------|----------|
| Week1 | 0,837 | 0,761 | 0,805 | 0,636 | 0,862 | 0,764 | 0,377 |
| Week2 | 0,371 | 0,230 | 0,474 | 0,382 | 0,389 | 0,322 | 0,515 |
| | 0,578 | 0,332 | 0,403 | 0,055 | 0,339 | 0,305 | 0,589 |
| Week3 | 0,524 | 0,374 | 0,401 | 0,173 | 0,270 | 0,356 | 0,671 |

Table10: Copper concentration in test samples of batch experiment

As Table 10 and Figures 2-8 show, during the first test run weeks, Copper removal was observed only during the first two weeks of the experiment. The reason could be because algae died after more or less ten days.

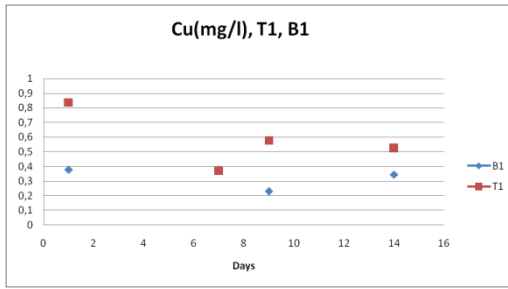


Figure 2: Cu concentration in T1, B1

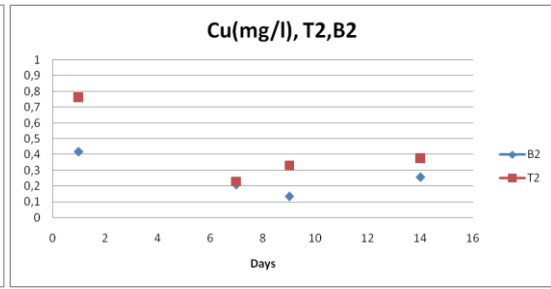


Figure 3: Cu concentration in T2, B2

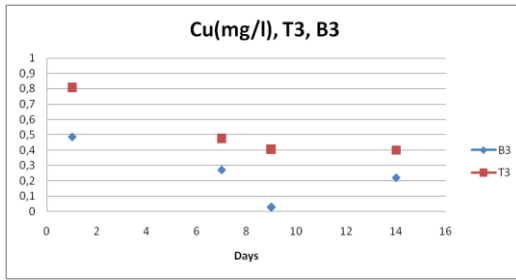


Figure 4: Cu concentration in T3, B3

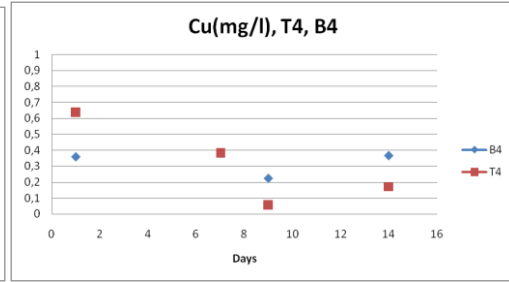


Figure 5: Cu concentration in T4, B4

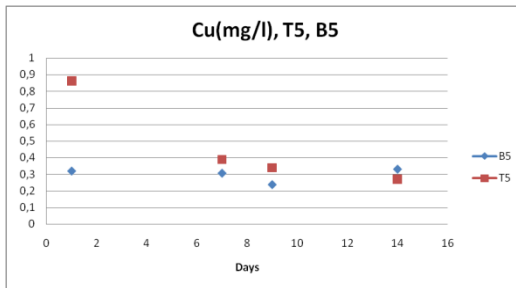


Figure 6: Cu concentration in T5, B5

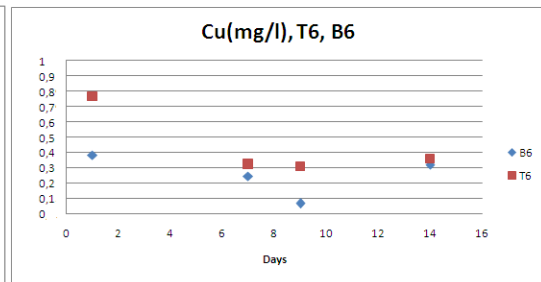


Figure 7: Cu concentration in T6, B6

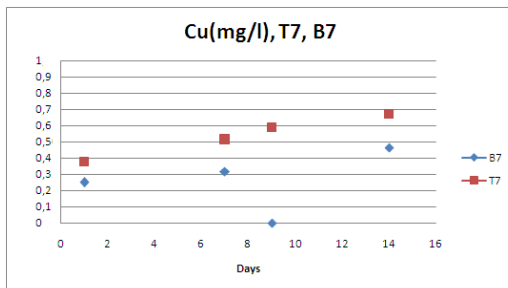


Figure 8: Cu concentration in T7, B7.

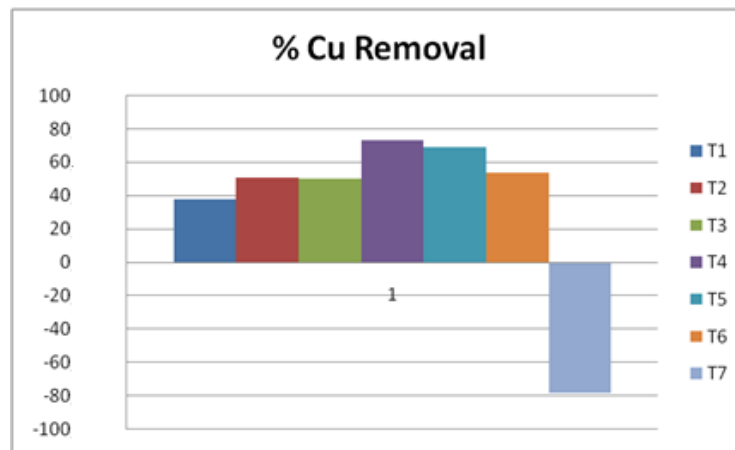


Figure 9: Percentage of copper removal in test samples.

The removal of Copper in the test bottles during the batch experiment are presented in Figure 9. Bottles 1, 2, 3 are mixture of algae, and the Bottles 4, 5, 6, 7 are single algae. On the one hand, in the bottles with Copper and mixture of algae the most efficient Copper removal was observed bottle 2, where the mixture contained the following the algae species: *Selenastrum capricornutum*, *Pediastrum simplex*, *Chlorophyta sp.*, *Haematococcus pluvialis*.

For single algae, the most efficiency specie is specie 4: *Anabaena Cylindrical*. For the second test we decided use a mixture of algae, bottle 2, because it represents more diversity, and that bottle contains *Pediastrum Simplex* the most efficient single algae. The species of *Chlorella*, *Anabaena inaequalis*, *Synechococcus sp.* tolerate heavy metals. However, several species of *Chlorella*, *Anabaena*, marine algae have been used for the-removal of heavy metals, (Dwivedi, 2012). In a previous work done in our laboratory it was found that the *Anabaena*'s percentage removal is 77,55%, this algae is very efficient (Benchraka, 2014).

4.2 Second Test Run in ATS (Algae Turf Scrubber)

The second test, the ATS respectively, was started on 09/05/2014 at the greenhouse in TAMK, this test was done like a continuous process, because a big amount of wastewater was needed.

A standard of Copper solution was prepared, with a concentration of 33,5mg/l (Benchraka, 2014).

In this test run we used the mixture of *Selenastrum capricornutum*, *Pediastrum simplex*, *Chlorophyta* sp., *Haematococcus pluvialis*(see B2, T2), since this proved to be an efficient Cu removing mixture in the first (batch) test run.

In this test run we used duplicate sample run and a blank, in the Table 11 are shown the initial amounts of wastewater, algae and Cu solutions in each tank.



Picture 4: Algae Turf Scrubber, semi-continuous process

| T1 | (ml) | T2 | (ml) | B1 | (ml) |
|-------------------|-------|-------------------|-------|-------------------|-------|
| Cu | 225,0 | Cu | 225,0 | Cu | 0,000 |
| Algae | 337,5 | Algae | 337,5 | Algae | 337,5 |
| Wastewater | 20000 | Wastewater | 20000 | Wastewater | 20000 |

Table11: Initial amounts of wastewater, selected algae mixture and Cu solution in ATS experiment

In this test AAS was used for analyse the Cu concentration, and the method was the same, but in this case was used other wavelength, 324 nm, because copper concentration in wastewater normally it is very low, so we used the lowest wavelength, because it works perfectly. The Cu concentrations measured in the ATSs in the first and second weeks of experiments are presented in Table12 this table shows the averages of copper concentration in ATS.

As can be seen from Table 14 and Figures 10 and 11, Cu concentration in the tanks was reduced during the first week of the experiment but increased after that .This happened

probably because of the exhaustion of nutrients in the system followed by algae death and dissolution of the Cu. After 10 days wastewater was added in each tank that added some nutrients to the system. As a result, algae started to grow again and within only two days the Copper concentration decreased significantly. This suggests that the Cu removal by algae happens in a relatively short time

| | T1(mg/l) | | T2(mg/l) | |
|--------------|------------|-------|------------|-------|
| Week1 | 09.05.2014 | 3,539 | 09.05.2014 | 1,632 |
| | 14.05.2014 | 1,625 | 14.05.2014 | 1,281 |
| Week2 | 20.05.2014 | 5,571 | 20.05.2014 | 5,228 |
| | 22.05.2014 | 2,957 | 22.05.2014 | 2,578 |

Table12: Copper concentration in ATS samples

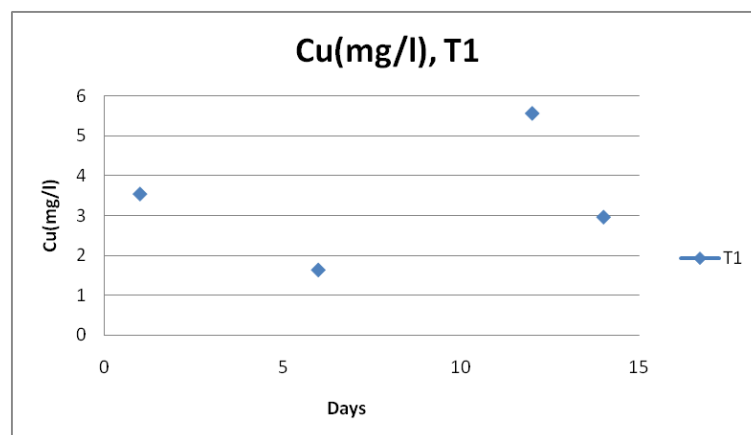


Figure 10: Copper concentration in Tank1.

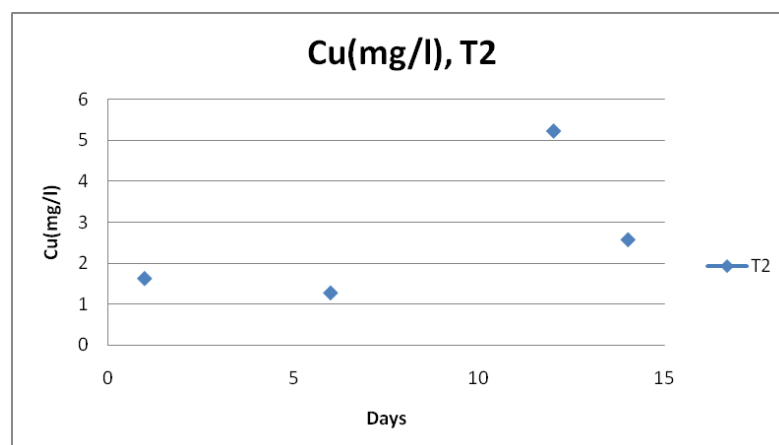


Figure 11: Copper concentration in Tank2.

The percentage of Cu removal in the first week of test run is given in Figure 12.

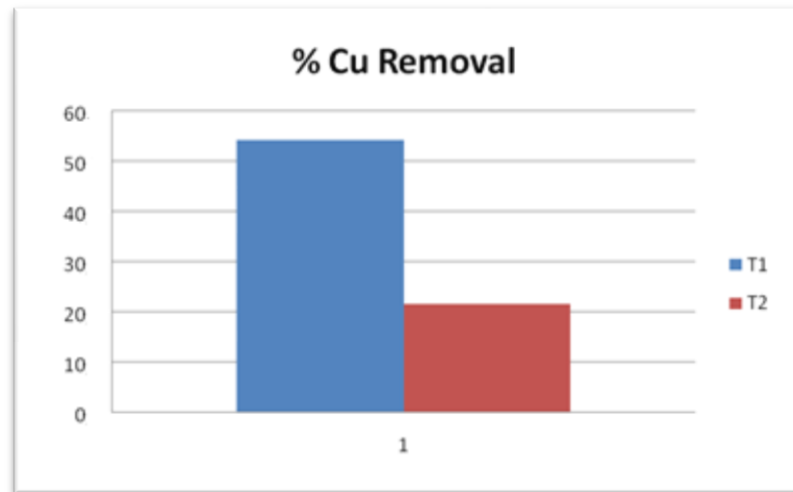


Figure 12: Percentage of Copper removal in ATSS

In the second test, we analyzed the mixture of algae, that it contained *Pediastrum Simplex*, bottle number 2, with the obtained results, could be appreciate, that, algae removed Cu, in the first days, after that, it is necessary to add more wastewater and copper for an efficient removal, because algae needs nutrients..

The difference in the results can be because of the evaporation and considering solutions and standards were made with some human errors, and there are mistakes in measurement.

Removal percentage is good, and it is expected, because we can find and we can find similar data in previous studies, bottle number two contains four algae species, the percentage of copper removal for *Pediastrum Simplex* is about 93,91 %, *Selenastrum Ca.* is 57,01%, *Scenedesmus sp.* is 81,20% and *Haematococcus* percentage removal is 82,75%, the initial Cu concentration for these algae species as 33,5 mg/l (Benchraka, 2014).

Cu is used to make photosynthetic enzyme found in electron transport chain, necessary for algae reproduction, Cu is found in plastocyanin and amine oxidase. (Lobban and Wynne, 1981)

5 CONCLUSION

Several algae species, *Selenastrum Capricornutum*, *Pediastrum Simplex*, *Synechococcus Spiriluna*, *Anabaena cylindrical*, *Scenedesmus Spiriluna*, *Chlorophyta Spiriluna*, *Purpurella Spiriluna*, *Haematococcus* and their mixtures, mixture one: all the species, mixture two: *Selenastrum Capricornutum*, *Pediastrum Simplex*, *Scenedesmus Spiriluna*, *Haematococcus*, and mixture three: *Anabaena cylindrical*, *Scenedesmus Spiriluna* removed efficiently the Cu from wastewater. The mixture of *Selenastrum Capricornutum*, *Pediastrum Simplex*, *Scenedesmus Spiriluna*, *Haematococcus* used in the semi-continuous ATS the Copper with a 50-55% in this study Copper concentration decreased in the first week of the ATS experiment, but increased after that. Wastewater addition after four days from the starting of the experiment caused algae to grow again and Copper concentration decreased as a consequence of algal growth. Algal growth and the most efficient Cu removal took place within two days from the addition of wastewater. These results suggest that in the ATS it is necessary to add new wastewater in spans of 2-3 days to provide algae the necessary nutrients and to assure efficient Cu removal.

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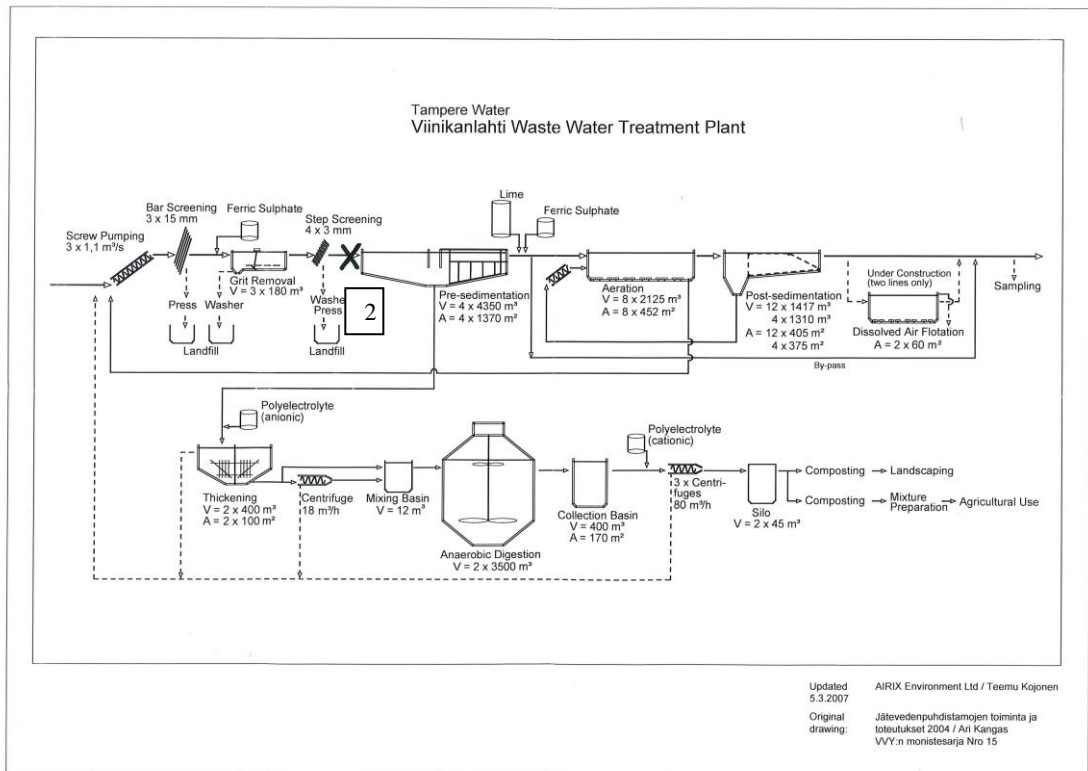
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APPENDICES

Appendix 1. Wastewater Treatment Plant Diagram.



Appendix 2. AAS Data Blanks Samples.

-First Test Run.

| | B1(mg/l) | B2(mg/l) | B3(mg/l) | B4(mg/l) | B5(mg/l) | B6(mg/l) | B7(mg/l) |
|--------------|----------|----------|----------|----------|----------|----------|----------|
| Week1 | 0,466 | 0,301 | 0,434 | 0,182 | 0,343 | 0,609 | 0,264 |
| | 0,365 | 0,464 | 0,375 | 0,439 | 0,231 | 0,284 | 0,284 |
| | 0,303 | 0,487 | 0,654 | 0,455 | 0,382 | 0,258 | 0,258 |
| Week2 | 0,441 | 0,33 | 0,402 | 0,392 | 0,411 | 0,28 | 0,326 |
| | 0,31 | 0,233 | 0,267 | 0,418 | 0,176 | 0,141 | 0,421 |
| | 0,383 | 0,065 | 0,151 | 0,334 | 0,331 | 0,313 | 0,206 |
| Week3 | 0,206 | 0,205 | 0,000 | 0,353 | 0,159 | 0,032 | 0,000 |
| | 0,233 | 0,044 | 0,000 | 0,202 | 0,214 | 0,000 | 0,091 |
| | 0,25 | 0,154 | 0,000 | 0,117 | 0,341 | 0,192 | 0,000 |
| Week3 | 0,386 | 0,154 | 0,337 | 0,141 | 0,342 | 0,32 | 0,425 |
| | 0,358 | 0,278 | 0,165 | 0,429 | 0,208 | 0,223 | 0,467 |
| | 0,291 | 0,333 | 0,163 | 0,526 | 0,441 | 0,419 | 0,499 |

-Second Test Run.

| Week1 | B1(mg/l) |
|--------------|-----------------|
| | 1,145 |
| | 1,099 |
| | 1,07 |
| | 1,435 |
| | 1,449 |
| | 1,249 |
| Week2 | 5,611 |
| | 6,384 |
| | 6,587 |
| | 2,378 |
| | 2,420 |
| | 2,444 |

Appendix 3. AAS Data Test Samples.

-First Test Run.

| | T1(mg/l) | T2(mg/l) | T3(mg/l) | T4(mg/l) | T5(mg/l) | T6(mg/l) | T7(mg/l) |
|--------------|----------|----------|----------|----------|----------|----------|----------|
| Week1 | 0,906 | 0,81 | 0,685 | 0,476 | 0,929 | 0,798 | 0,344 |
| | 0,764 | 0,611 | 0,873 | 0,781 | 0,911 | 0,84 | 0,365 |
| | 0,84 | 0,861 | 0,858 | 0,65 | 0,748 | 0,654 | 0,422 |
| Week2 | 0,274 | 0,262 | 0,553 | 0,267 | 0,285 | 0,344 | 0,433 |
| | 0,318 | 0,191 | 0,509 | 0,431 | 0,466 | 0,285 | 0,483 |
| | 0,52 | 0,237 | 0,36 | 0,449 | 0,416 | 0,339 | 0,629 |
| Week3 | 0,417 | 0,132 | 0,55 | 0 | 0,479 | 0,379 | 0,794 |
| | 0,626 | 0,512 | 0,344 | 0,162 | 0,266 | 0,178 | 0,477 |
| | 0,691 | 0,351 | 0,315 | 0,053 | 0,274 | 0,358 | 0,494 |
| Week3 | 0,608 | 0,412 | 0,531 | 0,156 | 0,237 | 0,345 | 0,767 |
| | 0,556 | 0,394 | 0,446 | 0,19 | 0,259 | 0,277 | 0,459 |
| | 0,407 | 0,314 | 0,226 | 0,173 | 0,313 | 0,445 | 0,788 |

-Second Test Run.

| | T1(mg/l) | T2(mg/l) |
|--------------|----------|----------|
| Week1 | 4,603 | 1,798 |
| | 3,3 | 1,692 |
| | 2,715 | 1,632 |
| Week2 | 1,74 | 1,317 |
| | 1,605 | 1,276 |
| | 1,531 | 1,249 |
| Week2 | 7,238 | 4,708 |
| | 5,039 | 5,063 |
| | 4,436 | 5,914 |
| Week2 | 3,978 | 2,551 |
| | 2,607 | 2,705 |
| | 2,277 | 2,478 |

Appendix 4. Calibration Data AAS.

-First Test Run.

| Week1 | | |
|--------------|-----------------------|-----------------------|
| Repl | StndConc(mg/l) | BlkCoor Signal |
| 1 | 5 | 0,122 |
| 2 | 5 | 0,121 |
| 3 | 5 | 0,121 |
| Mean | 0,121 | |
| SD: | 0,001 | |
| %RSD | 0,810 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,02427 |

| Week1 | | |
|--------------|-----------------------|-----------------------|
| Repl | StndConc(mg/l) | BlkCoor Signal |
| 1 | 7 | 0,174 |
| 2 | 7 | 0,168 |
| 3 | 7 | 0,176 |
| Mean | 0,173 | |
| SD: | 0,0044 | |
| %RSD | 2,560 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,02454 |

| Week1 | | |
|--------------|-----------------------|-----------------------|
| Repl | StndConc(mg/l) | BlkCoor Signal |
| 1 | 10 | 0,235 |
| 2 | 10 | 0,241 |
| 3 | 10 | 0,239 |
| Mean | 0,239 | |
| SD: | 0,0031 | |
| %RSD | 1,290 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,02416 |

| Week1 | | |
|--------------|-----------------------|-----------------------|
| Repl | StndConc(mg/l) | BlkCoor Signal |
| 1 | 13 | 0,355 |
| 2 | 13 | 0,359 |
| 3 | 13 | 0,357 |
| Mean | 0,357 | |
| SD: | 0,0018 | |
| %RSD | 0,510 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,980 |
| Slope | 0,02589 |

| Week1 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 15 | 0,359 |
| 2 | 15 | 0,357 |
| 3 | 15 | 0,355 |
| Mean | 0,357 | |
| SD: | 0,0021 | |
| %RSD | 0,580 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,02391 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 5 | 0,116 |
| 2 | 5 | 0,11 |
| 3 | 5 | 0,106 |
| Mean | 0,111 | |
| SD: | 0,0048 | |
| %RSD | 4,360 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,02215 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 7 | 0,156 |
| 2 | 7 | 0,161 |
| 3 | 7 | 0,158 |
| Mean | 0,161 | |
| SD: | 0,0036 | |
| %RSD | 2,220 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9933 |
| Slope | 0,02276 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 10 | 0,238 |
| 2 | 10 | 0,239 |
| 3 | 10 | 0,24 |
| Mean | 0,239 | |
| SD: | 0,0009 | |
| %RSD | 0,360 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9949 |
| Slope | 0,02343 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 13 | 0,3 |
| 2 | 13 | 0,307 |
| 3 | 13 | 0,304 |
| Mean | 0,304 | |
| SD: | 0,0033 | |
| %RSD | 1,080 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9982 |
| Slope | 0,0234 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 15 | 0,346 |
| 2 | 15 | 0,349 |
| 3 | 15 | 0,347 |
| Mean | 0,347 | |
| SD: | 0,0017 | |
| %RSD | 0,490 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9982 |
| Slope | 0,0233 |

| Week2b | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 5 | 0,101 |
| 2 | 5 | 0,101 |
| 3 | 5 | 0,1 |
| Mean | 0,101 | |
| SD: | 0,006 | |
| %RSD | 0,560 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,02016 |

| Week2b | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 7 | 0,156 |
| 2 | 7 | 0,16 |
| 3 | 7 | 0,157 |
| Mean | 0,158 | |
| SD: | 0,0023 | |
| %RSD | 1,470 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9497 |
| Slope | 0,02178 |

| Week2b | | | | |
|---------------|-----------------------|------------------------|------------------------|---------|
| Repl | StndConc(mg/l) | BlnkCoor Signal | | |
| 1 | 10 | 0,23 | | |
| 2 | 10 | 0,235 | | |
| 3 | 10 | 0,239 | | |
| Mean | 0,234 | | | |
| SD: | 0,0042 | | | |
| %RSD | 1,800 | | | |
| | | | CorrelationCoef | 0,9831 |
| | | | Slope | 0,02277 |

| Week2b | | | | |
|---------------|-----------------------|------------------------|------------------------|---------|
| Repl | StndConc(mg/l) | BlnkCoor Signal | | |
| 1 | 13 | 0,302 | | |
| 2 | 13 | 0,306 | | |
| 3 | 13 | 0,303 | | |
| Mean | 0,304 | | | |
| SD: | 0,0023 | | | |
| %RSD | 0,760 | | | |
| | | | CorrelationCoef | 0,9934 |
| | | | Slope | 0,02307 |

| Week2b | | | | |
|---------------|-----------------------|------------------------|------------------------|--------|
| Repl | StndConc(mg/l) | BlnkCoor Signal | | |
| 1 | 15 | 0,353 | | |
| 2 | 15 | 0,352 | | |
| 3 | 15 | 0,361 | | |
| Mean | 0,356 | | | |
| SD: | 0,0052 | | | |
| %RSD | 0,760 | | | |
| | | | CorrelationCoef | 0,9957 |
| | | | Slope | 0,0233 |

| Week3 | | | | |
|--------------|-----------------------|------------------------|------------------------|--------|
| Repl | StndConc(mg/l) | BlnkCoor Signal | | |
| 1 | 5 | 0,099 | | |
| 2 | 5 | 0,102 | | |
| 3 | 5 | 0,1 | | |
| Mean | 0,101 | | | |
| SD: | 0,0012 | | | |
| %RSD | 1,160 | | | |
| | | | CorrelationCoef | 1,00 |
| | | | Slope | 0,0201 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 7 | 0,154 |
| 2 | 7 | 0,153 |
| 3 | 7 | 0,152 |
| Mean | 0,153 | |
| SD: | 0,0011 | |
| %RSD | 0,700 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9729 |
| Slope | 0,02227 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 10 | 0,227 |
| 2 | 10 | 0,232 |
| 3 | 10 | 0,229 |
| Mean | 0,23 | |
| SD: | 0,0024 | |
| %RSD | 1,700 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9859 |
| Slope | 0,02227 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 13 | 0,3 |
| 2 | 13 | 0,298 |
| 3 | 13 | 0,303 |
| Mean | 0,3 | |
| SD: | 0,0021 | |
| %RSD | 0,710 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9935 |
| Slope | 0,02269 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 15 | 0,353 |
| 2 | 15 | 0,342 |
| 3 | 15 | 0,346 |
| Mean | 0,347 | |
| SD: | 0,0054 | |
| %RSD | 1,560 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9961 |
| Slope | 0,02286 |

-SecondTestRun.

| Week1 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 1 | 0,01 |
| 2 | 1 | 0,01 |
| 3 | 1 | 0,01 |
| Mean | 0,01 | |
| SD: | 0,0003 | |
| %RSD | 3,230 | |

| | |
|------------------------|--------|
| CorrelationCoef | 1,00 |
| Slope | 0,9989 |

| Week1 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 2 | 0,02 |
| 2 | 2 | 0,021 |
| 3 | 2 | 0,021 |
| Mean | 0,021 | |
| SD: | 0,0002 | |
| %RSD | 0,860 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,01024 |

| Week1 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 5 | 0,061 |
| 2 | 5 | 0,062 |
| 3 | 5 | 0,062 |
| Mean | 0,062 | |
| SD: | 0,0009 | |
| %RSD | 1,470 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,99 |
| Slope | 0,01202 |

| Week1 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 7 | 0,088 |
| 2 | 7 | 0,089 |
| 3 | 7 | 0,091 |
| Mean | 0,09 | |
| SD: | 0,0013 | |
| %RSD | 1,450 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,996 |
| Slope | 0,01252 |

| Week1 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 10 | 0,136 |
| 2 | 10 | 0,136 |
| 3 | 10 | 0,137 |
| Mean | 0,137 | |
| SD: | 0,0007 | |
| %RSD | 0,550 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9951 |
| Slope | 0,013 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 1 | 0,01 |
| 2 | 1 | 0,01 |
| 3 | 1 | 0,01 |
| Mean | 0,01 | |
| SD: | 0 | |
| %RSD | 0,430 | |

| | |
|------------------------|--------|
| CorrelationCoef | 1,00 |
| Slope | 0,0096 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 2 | 0,021 |
| 2 | 2 | 0,021 |
| 3 | 2 | 0,021 |
| Mean | 0,021 | |
| SD: | 0,0001 | |
| %RSD | 0,570 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9946 |
| Slope | 0,01 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 5 | 0,055 |
| 2 | 5 | 0,055 |
| 3 | 5 | 0,056 |
| Mean | 0,055 | |
| SD: | 0,0006 | |
| %RSD | 1,070 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9984 |
| Slope | 0,01 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 7 | 0,086 |
| 2 | 7 | 0,086 |
| 3 | 7 | 0,087 |
| Mean | 0,086 | |
| SD: | 0,0006 | |
| %RSD | 1,070 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9938 |
| Slope | 0,011 |

| Week2a | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 10 | 0,130 |
| 2 | 10 | 0,131 |
| 3 | 10 | 0,130 |
| Mean | 0,130 | |
| SD: | 0,0004 | |
| %RSD | 0,300 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9936 |
| Slope | 0,012 |

| Week2b | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 1 | 0,001 |
| 2 | 1 | 0 |
| 3 | 1 | 0 |
| Mean | 0 | |
| SD: | 0,0005 | |
| %RSD | 155,430 | |

| | |
|------------------------|---------|
| CorrelationCoef | 1,00 |
| Slope | 0,00034 |

| Week2b | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 2 | 0,002 |
| 2 | 2 | 0,002 |
| 3 | 2 | 0,001 |
| Mean | 0,001 | |
| SD: | 0,0002 | |
| %RSD | 13,230 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9990 |
| Slope | 0,00079 |

| Week2b | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 5 | 0,002 |
| 2 | 5 | 0,003 |
| 3 | 5 | 0,003 |
| Mean | 0,003 | |
| SD: | 0,0005 | |
| %RSD | 21,560 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9364 |
| Slope | 0,00057 |

| Week2b | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 7 | 0,004 |
| 2 | 7 | 0,004 |
| 3 | 7 | 0,003 |
| Mean | 0,003 | |
| SD: | 0,0005 | |
| %RSD | 14,620 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9630 |
| Slope | 0,00052 |

| Week2b | | |
|---------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 10 | 0,005 |
| 2 | 10 | 0,005 |
| 3 | 10 | 0,005 |
| Mean | 0,005 | |
| SD: | 0,0002 | |
| %RSD | 4,070 | |

| | |
|------------------------|---------|
| CorrelationCoef | 0,9840 |
| Slope | 0,00051 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 1 | 0,01 |
| 2 | 1 | 0,01 |
| 3 | 1 | 0,01 |
| Mean | 0,01 | |
| SD: | 0,0001 | |
| %RSD | 1,350 | |

| | |
|------------------------|------|
| CorrelationCoef | 1,00 |
| Slope | 0,01 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 2 | 0,021 |
| 2 | 2 | 0,021 |
| 3 | 2 | 0,021 |
| Mean | 0,021 | |
| SD: | 0,001 | |
| %RSD | 0,480 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9990 |
| Slope | 0,01 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 5 | 0,052 |
| 2 | 5 | 0,052 |
| 3 | 5 | 0,052 |
| Mean | 0,052 | |
| SD: | 0,0008 | |
| %RSD | 1,590 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9990 |
| Slope | 0,01 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 7 | 0,081 |
| 2 | 7 | 0,082 |
| 3 | 7 | 0,082 |
| Mean | 0,082 | |
| SD: | 0,0005 | |
| %RSD | 0,640 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9958 |
| Slope | 0,011 |

| Week3 | | |
|--------------|-----------------------|------------------------|
| Repl | StndConc(mg/l) | BlnkCoor Signal |
| 1 | 10 | 0,117 |
| 2 | 10 | 0,129 |
| 3 | 10 | 0,131 |
| Mean | 0,125 | |
| SD: | 0,0077 | |
| %RSD | 6,110 | |

| | |
|------------------------|--------|
| CorrelationCoef | 0,9934 |
| Slope | 0,012 |