Tampere University of Applied Sciences

Blue-Green Algae Detection Method for Rusko Laboratory

Topi Tanttu

BACHELOR'S THESIS
June 2020

Energy and Environmental Engineering

ABSTRACT

Tampereen ammattikorkeakoulu
Tampere University of Applied Sciences
Energy and Environmental Engineering

Topi Tanttu:
Blue Green Algae Detection Method for Rusko Laboratory

Bachelor's thesis 24 pages, appendices 8 pages
June 2020

In the past three decades, satellite imagery shows a clear increase in toxic blue-green algae bloom intensities all around the world. This increase can be explained through increased effect of climate change and the sewage and farming leachate released to water. This clear increase in toxin producing algae makes increased monitoring of blue-green algae blooms more important than ever, especially since the health effects of recreational use and the consumption of water contaminated with cyanobacterial toxins could be severe. Therefore, the increased surveillance of cyanobacterial blooms is becoming increasingly important.

The aim of the thesis is to find a method for detecting species and evaluating mass of blue-green algae species for Tampere's water company laboratory in Rusko for increased self-monitoring and make a general guide for using the proposed detection method.
The thesis is carried out as a literature review, due to the global health crisis going on during the thesis process, making access to the laboratory impossible.

Several different suitable methods were found in the research, but the most suitable for Tampere Waters needs was deemed to be sample concentration by sedimentation and microscopy, as it does not require many workhours to perform and is a quite straight forward process.

Key words: Cyanobacteria, blue-green algae, detection

CONTENTS

1 INTRODUCTION

The health effects of recreational use and consumption of water contaminated with toxins caused by Cyanobacterial blooms can cause severe health effects not only to humans, but also to pets and other mammals, (Dittman, Fewer & Neilan 2013) and have negative environmental effects such as oxygen depletion in the blooming waterbodies. (Cyanobacteria 2020)

Therefore, Finnish legislation requires certain amounts of testing from raw water intake sources and currently for Tampere Water, these tests are done by a outside company during the algae growth season.

In this thesis methods to measure the amount of cyanobacteria and detect the species are assessed, and one selected to be suggested to be taken into use in Tampere Water's Rusko laboratory. With this detection method the raw water quality could be measured more often as self-monitoring and tests could be done in non-growth season of blue-green algae.

As a consequence of the current worldwide covid-19 epidemic, this study is made as a literature review, since access to the laboratories is extremely limited.

2 THEORY

2.1 Blue green algae

Blue green algae or cyanobacteria are photosynthetic bacteria, that often look green or blue at the end of the lifespan of the algae. (CISRO, 2020) Unlike actual algae, blue-green algae are not generally eaten by other species, so it is not as important part of the food chain, as algae. (Blue-Green Algae, 2020) Blue green algae is often thought of as a contaminant as it can become so prevalent (bloom) in the water. It can affect the taste and the smell of the water, as well as deplete the oxygen in the water, negatively affecting other animal populations in the water body. (Cyanobacteria 2020)

Some of the blue algae species are also able to produce toxins, preventing its use for drinking, cooking and recreational use, some can even produce several harmful toxins at the same time. These toxins are categorized into three main groups based on their effects, neurotoxins, hepatotoxins and dermatoxins. More than 50 toxin producing species have been found to be harmful to mammals. (Dittman, Fewer & Neilan 2013)

The development of cyanobacteria requires a combination of factors for a significant bloom to occur. Nutrients are required for the growth and sustain of the cyanobacteria, but also the eutrophication, mainly caused by nitrogen and phosphorus, positively affects the cyanobacterial growth. These can be released from sediments in anoxic environment or from fertilizer / sewage runoff caused by humans. (What causes algal Blooms 2019)

In particular, the availability of phosphorus in the form of phosphate (PO_4^3) is the limiting factor in the bloom formulation. Nitrogen does not play such a large part, and in fact cyanobacteria usually dominate in conditions where nitrogen is limited, because of cyanobacteria's N_2- fixing capability gives it a distinct advantage over phytoplankton's in a such environment. (Parrish 2014)

Another aspect of cyanobacterial growth is temperature and light availability. In warmer months cyanobacteria has advantage over competing algae, as cyanobacteria's optimal water temperature for growth is around 25 °C while other algae's optimal is much lower, around 15 °C. (What causes algal blooms, 2019) Even though cyanobacteria benefit from warmer waters, too much direct high light intensity reduces the populations. The optimal situation for growth would be periodical exposure to light. (Toxic cyanobacteria in water 1999, 36-38)

Since the warm water benefits the growth of cyanobacteria, also stable conditions in other words, slow moving water benefits growth. With little wind and flow, there is no mixing of the water causing the cold water to sink to the bottom and warm water stay in upper layers. When the waterbody does not mix well enough, the bottom layer might also face anoxia, leading to nutrients being released for the bacteria's consumption. (Sivonen 2009, 304)

Eutrophication, hydrologic change of the surface waters and the increasing effect of climate change have made cyanobacterial blooms common in freshwater bodies all around the world. Therefore, increased monitoring and guideline levels of safe bacteria amounts have been developed by The World Health Organization. These guidelines have been put to use in most western countries as a framework for national drinking water legislations and as safety guidelines for recreational use of water bodies. (Otten & Paerl 2015)

Cyanobacteria are especially problematic when it comes to drinking water. Some of the bacteria are not easy to remove and for example simple boiling or common water treatment method of sand filtration will not remove species like microcystins (Otten & Paerl 2015), which are also found in the Tampere Water's freshwater intake sources.

For recreational user, the avoidance of blue green algae blooms is relatively easy, as stated above, the blooms can be easily seen and causes larger water discoloration with high enough concentration. There has even been recent invention to quickly test, for the presence of any blue green algae in the water in under 20 minutes, which can be used by anyone who might be uncertain whether or not water is safe for recreational use. (BlueGreenTest Sinilevätesti 2019)

2.1.1 Health

Effects of cyanobacteria on humans are respiratory problems (neurotoxins), skin or eye irritation, allergic reactions, and rashes (hepatotoxins), and gastroenteritis, diarrhoea, and headaches if ingested (dermatoxins). (Toxic cyanobacterial blooms 2016). Five species of cyanobacteria, *Anabaena, Aphanizomenon, Planktothrix, Microcystis and Woronichinia* were selected for closer examination, as these are the prevalent cyanobacteria species in Tampere Water's area of operation.

Microcystis release harmful toxin microcystin. Harmful levels depend heavily on individual in contact with the bacteria. Most common symptoms in recreational contact (swimming etc.) are irritation of skin and eyes, dizziness, hay fever like symptoms, fatigue, and gastroenteritis. 15000 cells/ml is considered being the limit of acceptable exposure, while 20 000 cells/ml causes water discoloration, it can be said that in every case discoloured water poses a health risk. Exposure to microcystins in drinking water could lead to more dangerous cases, such as kidney and liver damage as well as neurological damage. (Carmichael 1995, 7)

Anabaena produces a few toxins including microcystin and anatoxin, therefore the symptoms of microcystis can also be caused by anabaena blooms. Symptoms of anatoxin can be seen as numbness of lips, dizziness and tingling of extremities. If ingested anatoxin can cause diarrhea, vomiting and abdominal pain. (Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems 2014)

Aphanizomenon blooms cause the release of cylindrospermopsin and saxitoxin in freshwaters. Saxitoxin exposure can lead to similar symptoms as the previous toxins, such as numbness in the extremities and mouth / throat area, with the possibility of muscle paralysis and respiratory failure in extreme exposures. Unlike other toxins, cylindrospermopsin can affect other organs than liver, for example kidneys, thymus, and heart, with gastroenteritis and hepatitis if ingested orally. Although cylindrospermopsin can affect larger portion of the organs, it is slower

acting and less toxic than for example microcystin. (Lyon-Colbert, Su & Cude 2018)

Planktothrix blooms release microcystin and anatoxin, the health effects of planktothrix are similar to previous Anabaena and Microcystis, with irritation, dizziness and liver and kidney damage. (Kurmayer, Deng & Entfellner 2016)

Woronichinia also produces microcystin and anatoxin, but also microginin, a toxin affecting liver, like microcystin. (Colbert et al. 2018)

2.1.2 Toxin purification

Anatoxins degrade swiftly when exposed to sunlight at pH levels of 5,5 – 7 (Cheng et al. 2009), making UV light treatment optimal since the average pH of Finnish lakes is just a little under 7 (Kytölä 2019). Chlorine treatment of anatoxins proved to be unsatisfactory as in a test with various concentrations of chlorine, the maximum toxin removal was under 20%, whereas saxitoxin removal was highly effective while using chlorine. Especially at pH of 9 chlorine was reliable at removing saxitoxins. (Cheng et al. 2009)

Similar to saxitoxin Microcystin is reliably removed with chlorine through oxidation and cylindrospermopsin can also be effectively inactivated with ozone and chlorine, while other disinfectants were not effective enough for water purification standards. (Lahti et al. 2001)

2.2 Presence in lakes Roine and Näsijärvi

Tampere Water's water intake consists of multiple ground water pumping stations in lakes Roine and Näsijärvi. Both of these lakes have been identified to have five important blue-green algae species, which population needs to be monitored during the peak growth season in summer. These species are Anabaena, Aphanizomenon, Microcystis, Planktothrix and Woronichinia.

The cyanobacterial situation of Näsijärvi (Kauppi, Kämmenniemi and Polso) and Roine has been monitored for a long duration and the current situation can be seen from results from 2019 in the Figures 1-5. The data for the figures comes from unpublished measurements commissioned by Tampere Water and performed by Kokemäenjoen Vesistön Vesiensuojeluyhdistys ry (KVVY).

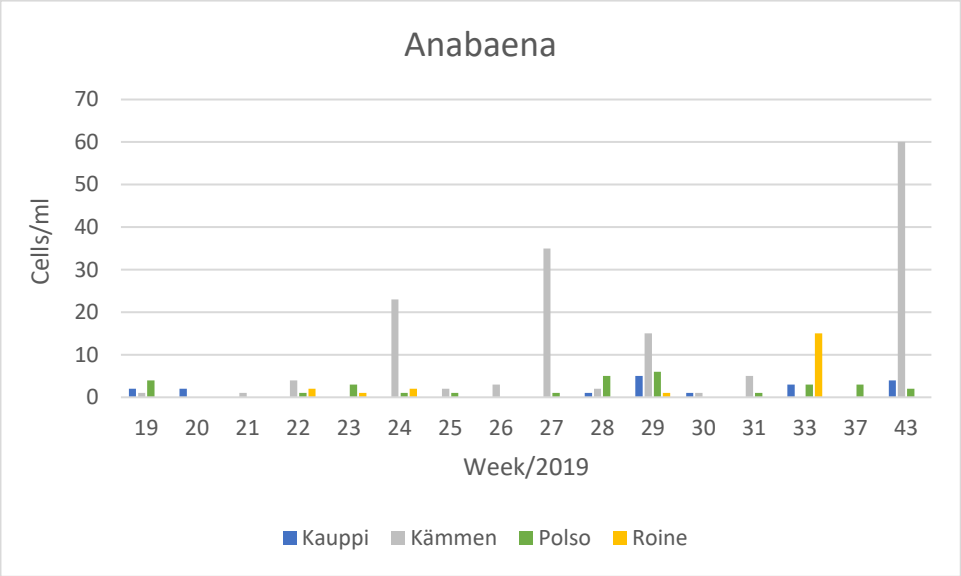


FIGURE 1. Observed amount of anabaena in Näsijärvi and Roine 2019

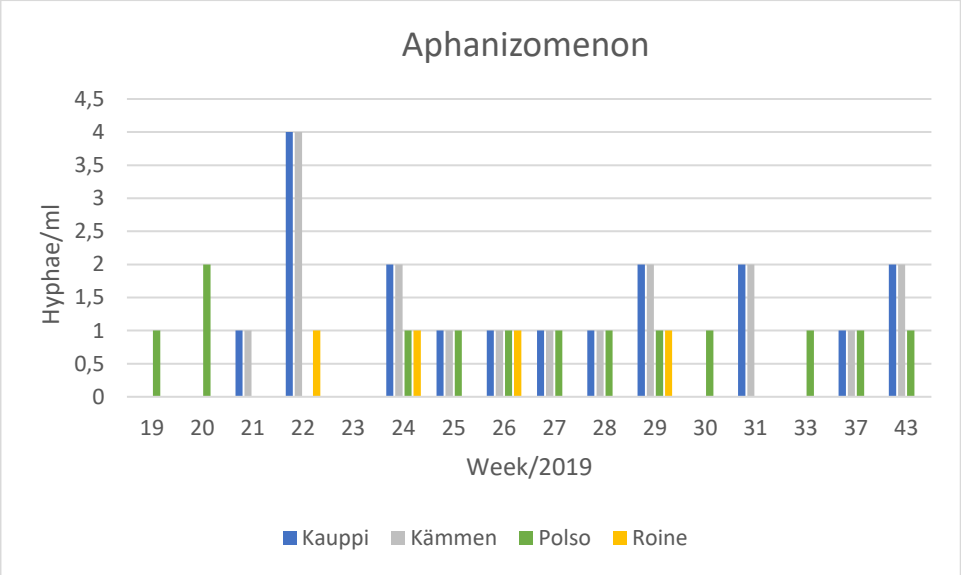


FIGURE 2. Observed amount of aphanizomenon in Näsijärvi and Roine 2019

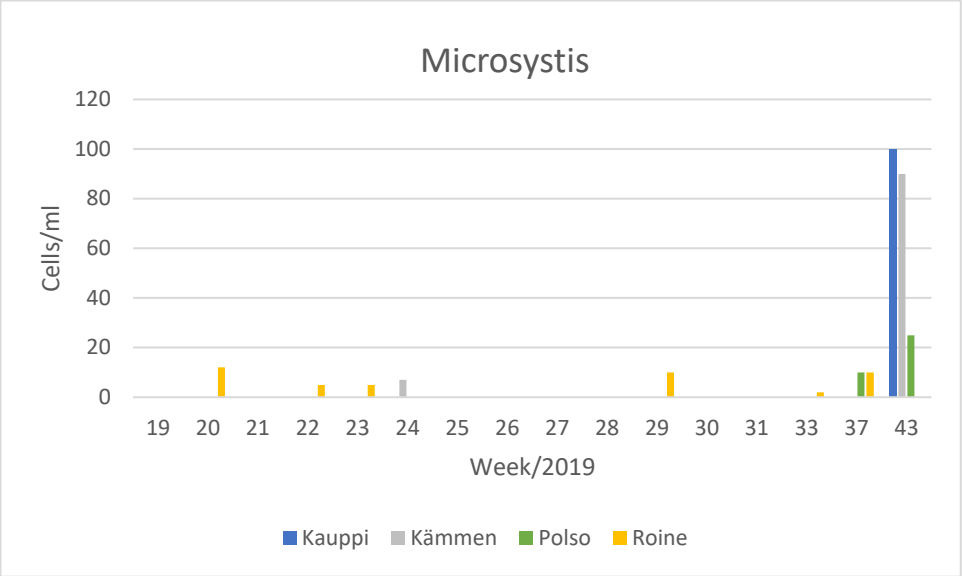


FIGURE 3. Observed amount of microcystis in Näsijärvi and Roine 2019

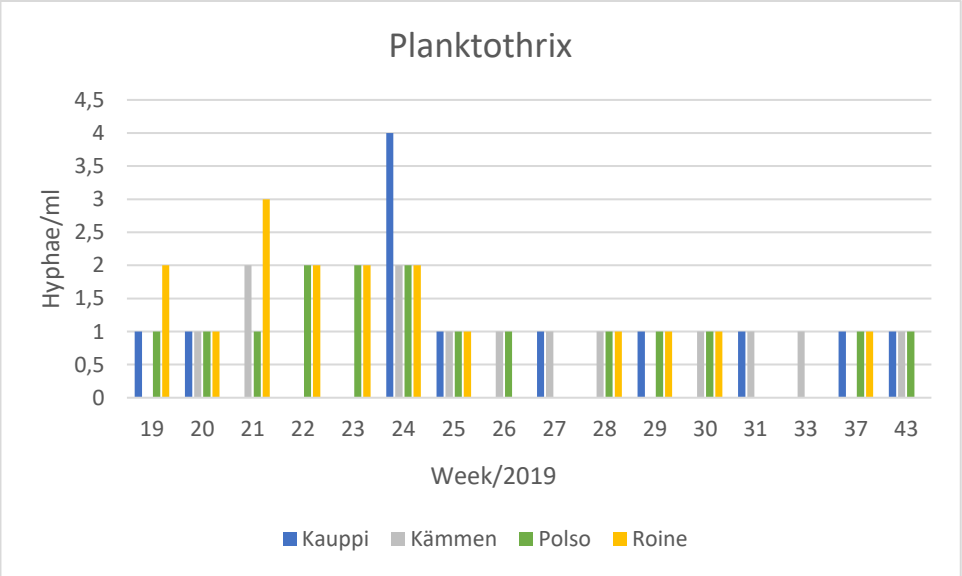


FIGURE 4. Observed amount of planktothrix in Näsijärvi and Roine 2019

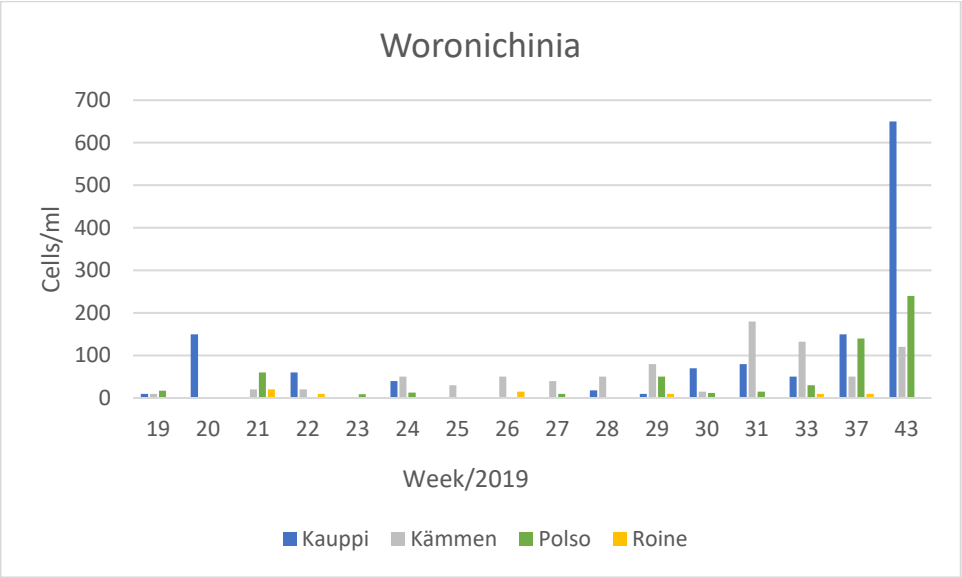


FIGURE 5. Observed amount of Woronichinia in Näsijärvi and Roine 2019

From these figures we can see that the lowest limit to act in the Finnish regulatory scale (Table 1) has been exceeded only once in the past year, resulting only in increased monitoring of the situation.

2.3 Finnish regulations

According to the National Supervisory Authority for Welfare and Health (Valvira) Finland uses the World Health Organizations recommendations for drinking water safety when it comes to the presence of cyanobacteria. Essentially this means that purified drinking water should not contain any toxins, but in reality, maximum concentration of microcystis is 1 µm/l and the presence of other toxins must be assessed case by case. (Toimintatavat talousveden laadun turvaamiseksi 2016)

Valvira also requires the assessment of the raw water sources ecological state for example, based on phytoplankton mass in the waterbody. For areas prone for algae blooming Valvira also recommends that sampling plan and calendar are made as well as contingency plan in case high number of cyanobacteria is found in the water. When it comes to exceeding recommended levels of toxins or cyanobacteria in the water Valvira uses the World Health Organizations procedural plan (Table 2). (Toimintatavat talousveden laadun turvaamiseksi 2016)

TABLE 1. Finnish regulations on cyanobacteria in water (Toimintatavat talousveden laadun turvaamiseksi 2016)

Water intake Threshold	**Procedure**	**Informing**
> 500 cells/ml > 0,1mg/l biomass	Increased monitoring	Prepare for inquiries
>5000 cells/ml > 1mg/l biomass	Identification of cyanobacteria / assessment of mass Health official assesses possibility of health hazard	Inform health officials
Toxin producing cells in intake water > 100 000 cells/ml > 20 mg/l biomass > 1ug/l microcystis	Changing water intake location or using alternative source Enhanced water treatment	Informing the public about water quality problems
Toxin producing cells in drinking water or < 1 ug/l microcystis in drinking water	Possible health hazard assessed by health officials Alternate water intake source Enhanced water treatment	Informing with the health officials about health effects and safe usage limits Informing of backup water supply
> 1ug/l microsystis in drinking water	If exceeded continuously prohibit the usage of water in cooking or drinking backup water supply is arranged	Informing the public about health effects and safe usage limits Informing of backup water supply
> 10ug/l microcystis in drinking water	Prohibit the water usage as household water Water can be used to flush toilets	Prohibit the usage of water Inform about alternate water source

The Finnish health regulations require that all water intake sources need to be evaluated based on the water intake quantity, minimum requirements seen in Table 2. For reference in 2018 the intake from Rusko freshwater plant was 12,7

million cubic meters of water, making it roughly 35 000 m^3/day. (Tampereen Vesi 2020)

TABLE 2. Minimum requirements of water sampling (Talousveden laatuvaatimukset 1352/2015)

Water quantity (m^3/day)	Samples per year	
	Continuous monitoring	Periodical monitoring
10–100	1	1 every other year
101–1 000	4	1
1 001–2 000	7	2
2 001–3 000	10	2
3 001–4 000	13	2
4 001–5 000	16	2
5 001–5 500	16	2
5 501–6 000	19	3
6 001–7 000	22	3
7 001–8 000	25	3
8 001–9 000	28	3
9 001–10 000	31	3
10 000–100 000	31 + 3 extra samples per 1 000 m^3/day exceeding 10 000 m^3/day	3 + 1 extra samples per 1 000 m^3/day exceeding 10 000 m^3/day
over 100 000	301 + 3 extra samples per 1 000 m^3/day exceeding 10 000 m^3/day	12 + 1 extra samples per 1 000 m^3/day exceeding 10 000 m^3/day

3 DETECTION METHODS

There are multitude of possible methods for cyanobacteria detection, differing in duration, complexity, and the process length. Four methods were chosen to be compared in multiple different areas. These methods have been gathered from World Health Organizations framework for cyanobacteria testing and chosen for their initial appearance to fit the criteria of Tampere Water. (Toxic cyanobacteria in water 1999, 345-360)

3.1 Sample concentration by sedimentation

Sedimentation is extremely simple way to get the samples concentrated enough to be assessed. No expensive equipment is needed, and the assessment can be done by using counting chamber and microscope.

Required equipment:
- Counting chamber with sedimentation tube
- Cyanobacterial identification key
- Sample preserved in Lugol's iodine solution
- Inverted microscope with 10x and 40x objectives

1. Leave the sample in room temperature to equilibrate, cold samples might cause air bubbles, making the process take longer

2. Mix the sample well by rotating the bottle around several times to ensure even mixing

3. Pour the sample into the sedimentation tube in place over the counting chamber.

4. Place the counting chamber somewhere, where it is not disturbed or exposed to sunlight

5. Wait for the sample to settle. Sedimentation time depends on the amount of water and the height of the tube. Settling time should be at least 3-4

hours per centimeter of liquid in the tube. Some cells might be buoyant and not settle, but the use of Lugol solution should correct the issue, as iodine uptake increases the weight of the cells

6. Remove the sedimentation tube carefully from the counting chamber. Now the density can be determined by counting the total number of cyanobacteria in the chamber or by counting subsections

3.2 Sample concentration by centrifugation

If sedimentation is not possible, the same results can be obtained through centrifugation, although the presence of buoyant cells requires extra steps to gain accurate results. Concentrated samples are again using counting chamber and microscope.

Equipment:
• Centrifuge
• Centrifuge tube,
• Syringe or bottle with cork, or plastic bottle with screw cap
• Microscope with 10x and 40x objectives
• Aluminum potassium sulphate, 1.0 g AIK(SO4)2.12H2O

1. Place 10-20 ml of sample in a centrifuge tube, seal using a cap, centrifuge at 360 × g for 15 minutes.

2. If the pelleting does not work as well as required, add 0.05 ml of aluminum potassium sulphate solution per 10 ml of sample.

3. Once the centrifuging is done, remove the supernatant carefully and re-suspend the pellet in known volume.

4. Now the density can be determined by counting the total number of cyanobacteria in the chamber or by counting subsections

3.3 Syringe filtration

Syringe filtration uses the same microscopic examination of the concentrated sample, but in case of small concentrations, it is much faster than regular sedimentation.

Equipment:
• Syringe, 10 ml
• Membrane filters, 13 mm diameter, 0.45 µm pore
• Membrane filter holder
• Glass microscope slides
• Microscope with 10x and 40x objectives
Reagents
• Immersion oil

1. Mix the water sample well.

2. Take 10ml of the sample into the syringe.

3. Put the filter in the holder and place the holder on to the end of the syringe.

4. Hold the filter in place and carefully push the sample through the filter by applying pressure on the piston.

5. Once all of the sample has passed through, take the filter out and place it on a glass slide with the captured cells on its top side.

.

6. Let the filter dry in room temperature and add couple of drops of immersion oil on the filter, making it transparent and allowing the observation of cyanobacteria trapped in the filter.

7. Lastly cover the filter surface with a glass cover and examine under the microscope.

5. Now the density can be determined by counting the total number of cyanobacteria in the chamber or by counting subsections and dividing with the amount of water filtered.

3.4 Determination of biomass using chlorophyll a analysis

Cyanobacterial presence can also be estimated by assessing biomass of chlorophyll a, although this method cannot be used to identify the species, it could be useful during cyanobacterial blooms, when most of the phytoplankton mass consists of cyanobacteria.

- Spectrophotometer suitable for readings up to 750 nm
- Glass cuvettes,
- Centrifuge
- 15 ml centrifuge tubes
- Heating device
- Glass fiber filters
- Filtration apparatus and vacuum pump
- Tissue grinder
- Pipette

1. Measure the initial volume of water and separate the cells and the water from each other through filtration. Do not let the filter of any sample to dry during the process. If the extraction cannot be done immediately after filtration, the filters can be stored in individual bags in the dark at -20°C.

2. Use the tissue grinder to grind the filters. Put the filter in place and add 2ml of boiling ethanol. Grind until the fibers are separated. Place the ground filter and ethanol into a centrifuge tube, clean the grinding tube with additional ethanol and pour this into the tube as well. Maximum of 10ml can be made into the centrifuge tube. Store in darkness at 20°C for 24-48 hours.

3. Centrifuge for 15 minutes at 3,000-5,000 g to clarify the samples. Transfer clear solution in clean receptacle and measure volume.

4. Blank Spectrophotometer with 90 percent ethanol solution at both wavelengths.

5. Place centrifuged sample in the cuvette and measure absorbance at 750 nm and 665 nm.

6. Add 0.01 ml of 1 mol l-1 HCl to sample in cuvette and mix for 1 minute. Record absorbance at 750 nm and 665 nm

4 RESULTS

Tampere Waters requirements for the method selected were to have it be comparable to the official measurements done by KVVY, be easy to use and to not require too many workhours to perform. In the table below the methods are assessed based on their usage, required materials, length, and accuracy.

TABLE 3. Comparison between the methods

Method	Complexity / equipment	Ease of use	Duration	Accuracy
Sedimentation	No expensive equipment needed	Easy to use, identification skills required	Depends on concentration, 4-48 hours	Fairly good, depends on the identification
Centrifugal sedimentation	Requires centrifuge	More technical process than sedimentation	1-2 hours	Fairly good, depends on the identification
Filtration	No expensive equipment needed	Easy to use, identification skills required	5-7 hours	Fairly good, depends on the identification
Chlorophyll-a	Requires multiple high-end machines	Knowledge of multiple procedures	2 – 5 hours	Quite accurate assessment of mass

As seen in the table the first method is quite easy and requires little to none effort from the employees to get the samples ready for microscopy, making it ideal process as the measurements are done more for self-interest, when the employees have time to do it. Even though in some cases the sedimentation process might take a long time, it does not require work input, whereas the microscopy takes the same time regardless of the method.

The second method is significantly faster than the first but requires much more work hours to get ready for the microscopy, requires more technical knowledge of the centrifugal process and it does need more chemicals in the process.
Syringe filtration (third method) is again a little faster than the first, but again requires more disposable equipment and work hours to get the samples ready for microscopical examination.

The last method requires the most knowledge of different technical machines, but also provides the most accurate results, as the results are not dependant on the accuracy of the microscope user. The disadvantage of this method is that although accurate mass results, it does not differentiate the cyanobacteria species from each other, making it unfit to serve the purpose of Tampere Water.

TABLE 4. Ranking the methods

Method	Complexity / equipment	Ease of use	Duration	Accuracy	TOTAL
Sedimentation	1	1	3	2	7
Centrifugal sedimentation	3	3	1	2	8
Filtration	2	2	2	2	8
Chlorophyll-a	4	4	2	1	11

In Table 4. all of the different methods were compared to the needs of Tampere Water, to find the most suitable way to measure cyanobacteria. 1 – 4 points given per section depending their suitability, and the lowest scoring method being considered the best.

5 DISCUSSION

The findings of this study might be a little lacking since the current covid-19 epidemic prevented any laboratory works, which would have given more accurate knowledge of the procedure's complexity, duration, and accuracy.

From the results gathered, the sedimentation and microscopic analysis seems to be the best fitting for the clients needs, as it does not require a lot of workhours, is quite simple to do and is easily compared to the findings of KVVY, who does the official measurements, but as seen in the Table 4. there are good alternate methods if the suggested method is not found suitable. The accuracy of the results gained by this method are hard to determine though, since the accuracy almost entirely depends on the person doing the microscopy, so most of the accuracy flaws from the method come from human error during the microscopy.

A guide for this method was made according to the World Health Organizations guidelines and delivered to Tampere Water (appendix 1.).

For further research, the actual laboratory comparison of these methods could provide better understanding of which the optimal method is to use in blue-green algae detection.

REFERENCES

Otten, T. Paerl, H. 2015. Health Effects of Toxic Cyanobacteria in U.S. Drinking and Recreational Waters: Our Current Understanding and Proposed Direction. Current Environmental Health Reports 2, 75-84.

Sivonen, K. 2009. Cyanobacterial Toxins. Encyclopedia of Microbiology. 290-307. Oxford: Elsevier.

Carmichael, W. 1996. Toxic Microcystis And the Environment. Toxic Microcystis. 1-12. Florida: CRC Press.

Lyon-Colbert, A. Su, S. Cude, C. 2018. A Systematic Literature Review for Evidence of Aphanizomenon flos-aquae Toxigenicity in Recreational Waters and Toxicity of Dietary Supplements: 2000–2017. Cyanobacteria and Cyanotoxins: New Advances and Future Challenges. 188-205.

Cheng, X. Shi, H. Adams, C. Timmons, T. Ma, Y. 2009. Effects of Oxidative and Physical Treatments on Inactivation of Cylindrospermopsis Raciborskii and Removal of Cylindrospermopsin. Water Science and Technology 60 (3). 689-697.

Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems. 2014. United States Environmental Protection Agency. Read on 02.06.2020. https://www.epa.gov/sites/production/files/2014-08/documents/cyanobacteria_factsheet.pdf

Lahti, K. Rapala, J. Kivimäki, A-L. Kukkonen, J. Niemelä, M. Sivonen, K. 2001. Occurrence of microcystins in raw water sources and treated drinking water of Finnish waterworks. Water Science and Technology 43 (12). 225-228.

Valvira. 2016. Toimintatavat talousveden laadun turvaamiseksi: Sinilevät ja niiden tuottamat toksiinit. Finnish National Supervisory Authority for Welfare and Health.

Sosiaali- ja terveysministeriön asetus talousveden laatuvaatimuksista ja valvontatutkimuksista. 1352/2015

World Health Organization. 1999. Toxic cyanobacteria in water. London: E & FN Spon.

Toxic Cyanobacterial Blooms. 2016. North American Lake Society. Read on 13.05.2020. https://www.nalms.org/nalms-position-papers/toxic-cyanobacterial-blooms/

Kytölä, A. 2019. Suomen suurten järvien veden laadun muutokset ja muutosten alueellinen samanaikaisuus 1960-luvulta tähän päivään. Jyväskylän Yliopisto.

Kurmayer, R. Deng, L. Entfellner, E. 2016. Role of toxic and bioactive secondary metabolites in colonization and bloom formation by filamentous cyanobacteria *Planktothrix*. Harmful Algae. Elsevier.

BlueGreenTest Sinilevätesti. 2019. Salofa Oy. Read on 29.05.2020. https://www.salofa.com/bluegreentest-sinilevatesti

What causes algal blooms. 2019. Indiana University. Read on 29.05.2020. https://cees.iupui.edu/research/algal-toxicology/bloomfactors

Cyanobacteria. 2020. Virginia Department of Health. Read on 01.06.2020. https://www.vdh.virginia.gov/environmental-epidemiology/waterborne-hazards-control-program/cyanobacteria/

Dittman, E. Fewer, D. Neilan, B. 2013. Cyanobacterial toxins: biosynthetic routes and evolutionary roots. FEMS Microbiology Reviews 37 (1). 23-43.

CISRO. 2020. What are blue-green algae. Commonwealth Scientific and Industrial Research Organization. Read on 20.05.2020. https://www.csiro.au/en/Research/Environment/Water/Blue-green-algae/What-are-blue-green-algae

Adrian, R. Wagner, C. 2009. Cyanobacteria dominance: Quantifying the effects of climate change. American Society of Limnology and Oceanography. Limnology and Oceanography 54 (6). 2460-2468

Blue-Green Algae. 2020. Wisconsin Department of Natural Resources. Read on 02.06.2020. https://dnr.wi.gov/lakes/bluegreenalgae/

Parrish, J. 2014. The Role of Nitrogen and Phosphorus in the Growth, Toxicity, and Distribution of the Toxic Cyanobacteria, Microcystis aeruginosa. University of San Francisco. Master's thesis.

Kokemäenjoen Vesistön Vesiensuojeluyhdistys ry. 2018–2019. Cyanobacteria measurement reports. Unpublished. In thesis authors' possession.

APPENDICES

Appendix 1. Detection guide for Tampere Water

MENETELMÄN SOVELTUVUUS

Menetelmä soveltuu levän tunnistamiseen järvi ja merinäytteistä.

PERIAATE

Menetelmän periaate on tiivistää vesinäytteestä levä laskentakammioon sedimentoimalla, jolloin sen mikroskopointi on mahdollista.
laitteet ja välineet

- Mikroskooppi 10x ja 40x objektiiveilla
- Laskentakammio ja jatkosylinteri 10, 25, 50 tai 100ml
- Sinilevän tunnistusohje
- Näyte säilöttynä Lugol liuoksessa, 2 tippaa per 25ml

SUORITUS

1. Anna näytteen lämmetä huoneenlämmössä, kylmää näytettä käytettäessä muodostuu kuplia, jotka vaikeuttavat sedimentaatiota
2. Sekoita näytepullo hyvin
3. Aseta jatkosylinteri putki laskentakammion päälle ja kaada näyte siihen, sylinterin koko riippuu näytteen levä konsentraatiosta.
4. Anna näytteen laskeutua 3-4 tuntia per senttimetri nestettä, suojassa suoralta auringonvalolta.

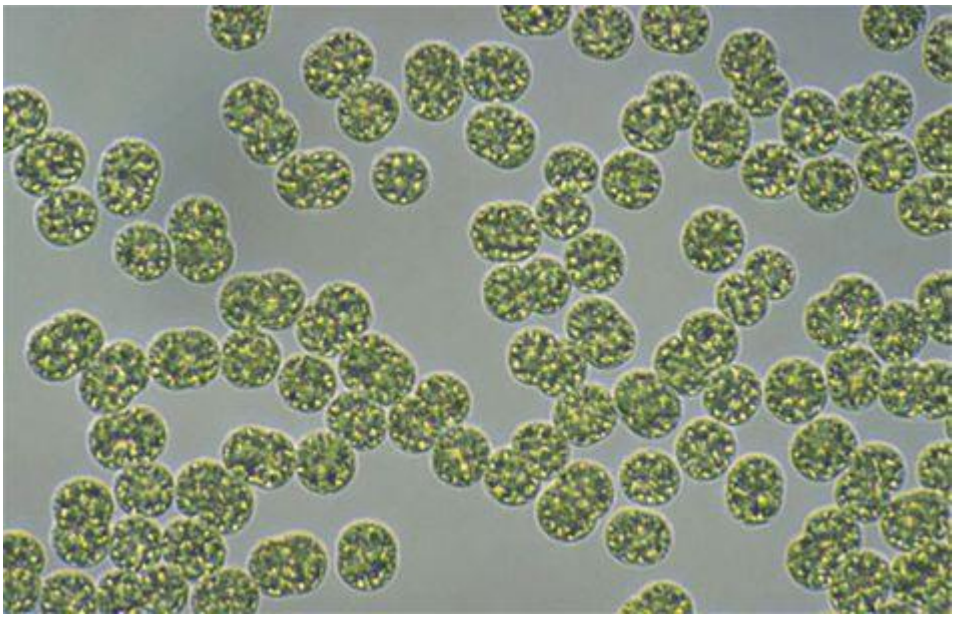
Yhteistilavuus (ml)	Jatkosylinterin korkeus (cm)	Laskeutusaika (h)
2-3	0,5 - 1	3
5	1	6
10	2	8
25	5	16
50	10	24
100	20	48

5.Jatkosylinteri poistetaan varovasti laskentakammion päältä häiritsemättä laskeutunutta näytettä ja kammion kansi peitetään lasilevyllä, jonka jälkeen näyte on valmis mikroskopoitavaksi

TUNNISTUS

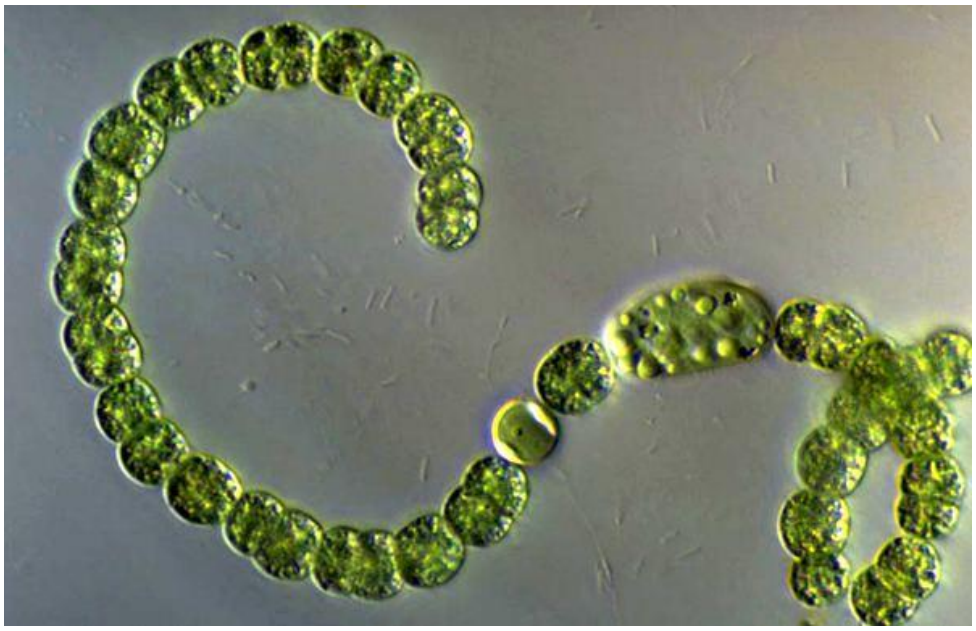
Mikäli levää on kovin vähän, voidaan tutkia koko kyvetin pohja, jos levää todetaan olevan liian paljon voidaan tutkia esimerkiksi 50 näkökenttää.

Näytteistä pyritään tunnistamaan viittä eri lajiketta

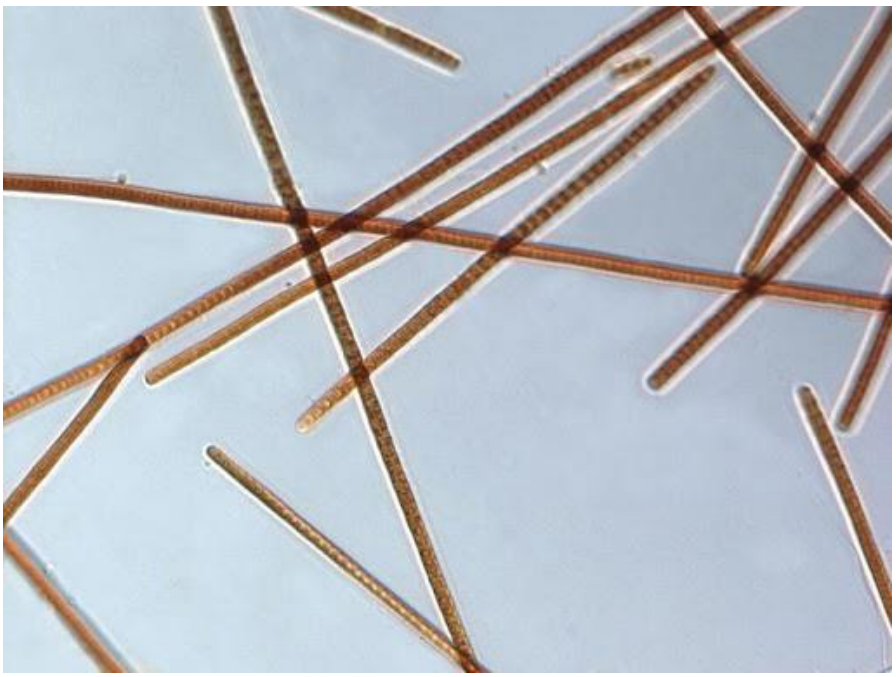


Microcystis, vaihtelevan muotoiset yhdyskunnat, solut tasossa tai kerroksittain. Lasketaan soluittain

Woronichinia, solut pitkänpyöreät tai munamaiset, ontto pallomainen yhdyskunta, lasketaan soluittain



Anabaena, tasapaksut rihmat, solut pyöreitä tai tynnyrimäisiä. Yksittäisiä rihmoja tai löysärakenteisissa kiemuroissa, lasketaan soluittain



Planktothrix, tasapaksut rihmat, kärjet voivat olla kaventuneita tai nuppimaisia. Lasketaan arvioimalla 100 µm osissa.

Aphanizomenon, päädyt voivat olla kapeita tai läpinäkyviä. Lasketaan arvioimalla 100 µm osissa.

VIITTEET

KVVY

Toxic cyanobacteria in water: A guide to public health consequences, monitoring and management. World Health Organization, E & FN Spon London UK. 1999

Field and Laboratory guide to freshwater cyanobacteria, United States Geological Service, USGS publishing network, Orland, USA, 2015

Appendix 2. KVVY cyanobacteria results

	Cells/ml	Hyphae (100 µm)/ml	Cells/ml	Hyphae (100 µm)/ml	Cells/ml
KAUPPI	*Ana-baena*	*Aphanizomenon*	*Micro-cystis*	*Planktothrix*	*Woronich-inia*
11.1.2018	1	1	0	0	38
20.2.2018	1	1	0	1	0
20.3.2018	1	1	0	1	37
17.4.2018	1	1	0	0	0
15.5.2018	130	0	0	0	0
23.5.2018	0	0	0	0	0
1.6.2018	0	2	0	0	75
7.6.2018	0	0	0	0	45
13.6.2018	8	1	0	1	0
19.6.2018	0	4	0	0	0
28.6.2018	10	0	0	0	0
3.7.2018	2	8	0	1	16
10.7.2018	2	1	0	1	84
19.7.2018	1	0	0	1	62
1.8.2018	1	0	0	0	84
15.8.2018	0	0	0	0	0
22.8.2018	1	0	0	0	71
29.8.2018	0	0	0	0	46
7.9.2018	1	1	0	0	218
19.9.2018	2	2	0	0	324
26.9.2018	2	1	112	0	174
3.10.2018	5	6	0	0	240
10.10.2018	2	4	80	1	242
18.10.2018	0	1	0	0	260
24.10.2018	2	10	157	0	224
8.5.2019	2	1	0	1	10
16.5.2019	2	1	0	1	150
29.5.2019	0	1	0	0	60
3.6.2019	0	0	0	0	0
14.6.2019	0	1	0	4	40
24.6.2019	0	1	0	1	0
2.7.2019	0	2	0	1	0
17.7.2019	5	1	0	1	10
24.7.2019	0	0	0	0	0
2.8.2019	1	2	0	1	80
8.8.2019	5	2	0	0	55
8.5.2019	2	1	0	1	10
16.5.2019	2	1	0	1	150
29.5.2019	0	1	0	0	60
3.6.2019	0	0	0	0	0

13.6.2019	0	1	0	4	40
19.6.2019	0	3	0	1	0
2.7.2019	0	2	0	1	0
10.7.2019	1	13	0	0	18
17.7.2019	5	1	0	1	10
24.7.2019	1	1	0	0	70
2.8.2019	1	2	0	1	80
14.8.2019	3	1	0	0	50
12.9.2019	0	2	0	1	150
21.10.2019	4	2	100	1	650

Näsijärvi, Kauppi intake plants cyanobacterial findings

	Cells/ml	Hyphae (100 µm)/ml	Cells/ml	Hyphae (100 µm)/ml	Cells/ml
KÄMMEN	*Ana-baena*	*Aphanizomenon*	*Micro-cystis*	*Planktothrix*	*Woronich-inia*
7.5.2019	1				10
15.5.2019	0	0	0	1	0
22.5.2019	1	1	0	2	20
28.5.2018	4	4	0	0	20
12.6.2019	23	2	7	2	50
24.6.2019	2	1	0	1	30
27.6.2019	3	1	0	1	50
3.7.2019	35	1	0	1	40
11.7.2019	2	1	0	1	50
16.7.2019	15	2	0	0	80
25.7.2019	1	0	0	1	15
30.7.2019	5	0	0	1	180
2.8.2019	1	2	0	1	80
14.8.2019	0	0	0	1	132
11.9.2019	0	1	0	0	50
17.10.2019	60	2	90	1	120

Näsijärvi, Kämmenniemi intake, cyanobacterial findings

	Cells /ml	Hyphae (100 µm)/ml	Cells /ml	Hyphae (100 µm)/ml	Cells /ml
POLSO	*Ana-baena*	*Aphanizomenon*	*Micro-cystis*	*Planktothrix*	*Woronich-inia*
7.5.2019	4	1	0	1	17
15.5.2019	0	2	0	1	0
22.5.2019	0	0	0	1	60
28.5.2019	1	0	0	2	0
5.6.2019	3	0	0	2	9
12.6.2019	1	1	0	2	13
24.6.2019	1	1	0	1	0
27.6.2019	0	1	0	1	0
3.7.2019	1	1	0	0	10
11.7.2019	5	1	0	1	0
16.7.2019	6	1	0	1	50
25.7.2019	0	1	0	1	12
29.7.2019	1	1	0	1	15
14.8.2019	3	1	0	0	30
11.9.2019	3	1	10	1	140
17.10.2019	2	1	25	1	240

Näsijärvi, Polso intake, cyanobacterial findings

	Cells/ml	Hyphae (100 µm)/ml	Cells/ml	Hyphae (100 µm)/ml	Cells/ml
ROINE	*Anabaena*	*Aphanizomenon*	*Micro-cystis*	*Planktothrix*	*Woronich-inia*
8.5.2019	0	0	0	2	0
15.5.2019	0	0	12	1	0
22.5.2019	0	0	0	3	20
28.5.2019	2	1	5	2	10
4.6.2019	1	0	5	2	0
12.6.2019	2	1	0	2	0
24.6.2019	0	0	0	1	0
27.6.2019	0	1	0	0	15
2.7.2019	0	0	0	0	0
10.7.2019	0	0	0	1	0
16.7.2019	1	1	10	1	10
24.7.2019	0	0	0	1	0
2.8.2019	0	0	0	0	0
13.8.2019	15	0	2	0	10
11.9.2019	0	0	10	1	10
21.10.2019	0	0	0	0	0

Roine intake, cyanobacterial findings